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Endotrack

Tracking the endocytic routes of growth factor receptor complexes and their modulatory role on signalling

Integrated Project

Integrating and strengthening the European Research area

Publishable Final Activity Report

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the Consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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1. Project Summary

The EndoTrack (Tracking the endocytic routes of growth factor receptor complexes and their modulatory role on signalling) project has involved 11 research institutes and enterprises throughout Europe and USA. It was funded with 11 million Euros by the European Commission's Sixth Framework Program for Research and Development (FP6) and had a final duration of 4,5 years.

The EndoTrack project aimed at gaining conceptual advance into the signaling function of growth factors (GFs) from an ***unconventional*** perspective, namely by **exploring the role of endocytic trafficking in the modulation of GF signaling**. It further aimed at translating the generated basic knowledge into novel opportunities for the development of a new generation of tools to combat diseases like cancer and neurodegenerative diseases. To achieve this ambitious goal, a multidisciplinary action plan carried out by a consortium of academic groups and SMEs defined the intracellular routes of GFR complexes, identified selective regulators of trafficking, and provided mechanistic insights into the contribution of endocytic trafficking to the signaling outcome. The approach utilized *in vitro*, *ex-vivo* and *in vivo* systems both in large scale screening as well as in more focused screening settings. The multidisciplinary strategy delivered the proof of principle that it is possible to qualitatively and quantitatively modify the signal transduction output of a variety of GFs via the modulation of endocytic routes, with predictable consequences at the patho-physiological level.

2. Project Execution

2.1. Introduction

The EndoTrack project aimed at gaining basic knowledge of the relationship between the endocytic transport and signaling activity of GFRs. EndoTrack combined leading European multidisciplinary research teams both from academia and biotech industry to pursue five interrelated major goals. The EndoTrack multidisciplinary approach based on the extensive functional integration and bi-directional interactions among scientific work packages (WPs) corresponding to the following major objectives (Figure 1):

1. To define the trafficking routes of various GFR complexes in cultured cells with an unprecedented degree of precision, combining high-throughput microscopy, electrochemiluminescence technology and automated image analysis.
2. To define the molecular machinery responsible for this transport, using proteomics and functional genomics approaches, and generate proof of concept that trafficking contributes to GF signaling activity in cultured cells.
3. To integrate the information from cultured cells with in vivo studies on animal model systems, in particular Drosophila, zebrafish, Xenopus and mouse.
4. To test the relevance of the modulation of endocytic trafficking on signal transduction in disease model systems.
5. Within the 4,5 years, the use of knockdown approaches, reporter cell lines and animals, combined with target validation proprietary technology, has provided a new generation of assays to measure GFR trafficking and signaling. The EndoTrack partnership of leading research institutions and biotech SMEs has delivered a novel technology platform for high content and high throughput functional genomics studies based on RNAi and quantitative multiparametric image analysis. These assays supported the identification of novel key regulatory components, and therefore, a new generation of diagnostic markers and potential targets for modulation of GF signaling and for the treatment of human diseases. The very stringent statistical control of quantitative data analysis has introduced a new quality standard in the research files of GF signaling and trafficking.

EndoTrack's translational research will thus have a strong impact on the innovation potential of basic research as well as European biotech and pharmaceutical industry.

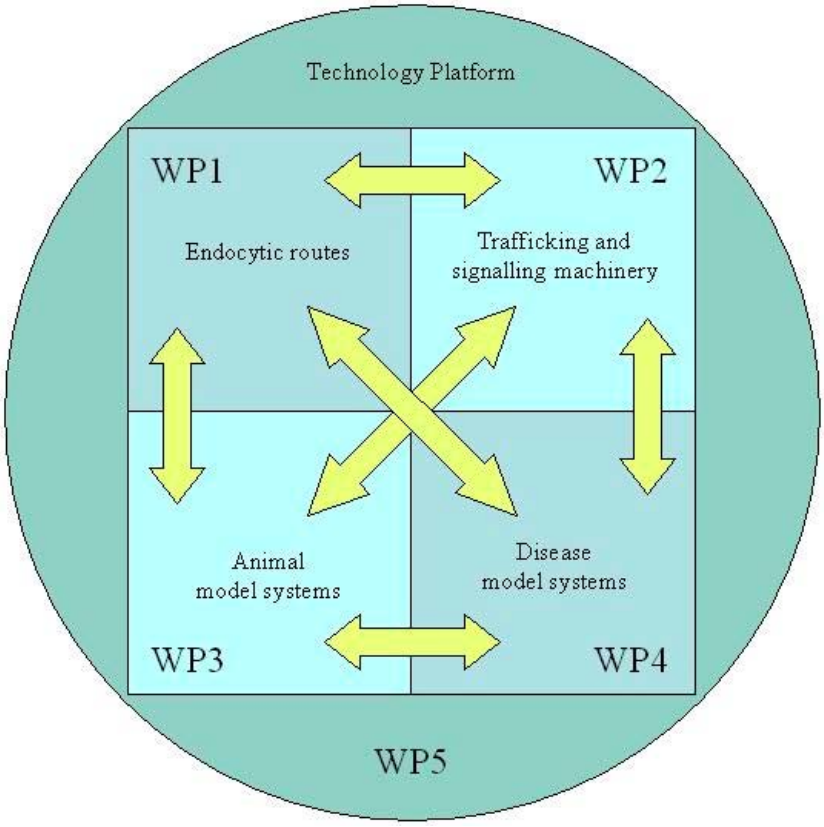

Figure 1. EndoTrack multidisciplinary approach and scientific WPs of the project. The diagram illustrates the extensive functional integration and bi-directional interactions among WPs. The entire R&D plan was supported by a state-of-the-art hi-tech platform described in WP5, with exclusive and innovative proprietary technologies. The activities in WP1 were tightly connected with those in WP2 and aimed at defining the endocytic routes of GF-GFR complexes and corresponding regulatory mechanisms. WP1 and WP2 provided the basis for functional studies in animal model systems (WP3) and for the proof of principle that trafficking regulators can modulate patho-physiological processes (WP4). The studies *in vivo* established feedback on the role of the endocytic and signalling regulators and their partners analysed in WP1-2.

In the following chapter are described the specific objectives and main results in each of the ENDOTRACK research areas after 4.5 years. It is followed by the progress beyond the state-of-the-art made in EndoTrack and the impact of project results on the pharmaceutical industry and the scientific community.

2.2. Specific objectives and main achievements

EndoTrack pursued a multidisciplinary strategy based on a set of ambitious objectives integrated together in a comprehensive plan aiming at developing new knowledge of the molecular mechanisms that underlie GF signaling in the context of their trafficking along the endocytic pathway. The technological advances necessary for this aim (i) resulted in a new generation of assays that allow to selectively measure the endocytic routes of various GFR complexes, (ii) led to the identification of specific trafficking regulators of each route and the signaling components associated with each route, and (iii) provided a proof of principle for the therapeutic potential of these regulators for the treatment of human diseases.
To achieve maximum efficiency and effectiveness of resource utilization EndoTrack focused on representative classes of GFs selected on the basis of key criteria such as mode of action, availability of experimental systems, and therapeutic potential. The **combination of *in vitro* and *in vivo* studies** allowed for a correlation between endocytosis and GF signaling. To this end, EndoTrack has established a complete technology development pipeline involving **an interdisciplinary research team** with expertise in cell biology (intracellular trafficking, endocytosis), biochemistry (signal transduction, target gene identification and validation), developmental biology (animal models, cell proliferation, survival, differentiation, adhesion and migration), molecular genetics (genome-wide screening) and medicine (human disease). All partners worked on various aspects of GF signaling and/or intracellular trafficking and **expanded their available key competences with novel and forward looking cell biological and signal transduction studies**, in order to integrate this knowledge in this project Europe-wide at an unprecedented scale.
Cell biology and disease models were used to address the function of novel trafficking and signaling modulators and the proof of principle for their effectiveness for the treatment of representative diseases. EndoTrack employed a combination of models ranging from cell cultures with clear advantages in terms of handling, optical resolution of intracellular structures, and accessibility to kinetics measurements, to animal models for validation and further analysis in a *bona fide* more physiological context.
Keeping with the combination of *in vitro* and *in vivo* approaches in this project and considering the assessment of the spatio-temporal regulation of GF signaling in physiological and disease state, the EndoTrack consortium aimed **at five main objectives** as described below.

2.2.1. Endocytic routes

The main objective of this research field was to define the intracellular trafficking routes of selected classes of GFR complexes **in a systematic way**. In order to optimally integrate endocytosis research and GF signaling research, the EndoTrack consortium has analysed the following of GF systems: the EGF, TGFb/activin/BMP, FGF, PDGF, Wnt, and Ephrin pathways in details.
Generation of tools required to study GF-GFR endocytic trafficking and signaling in cellular systems. In preparation for the analysis of trafficking and signaling components along the endocytic routes and the kinetics of GF-GFR transport the first step was to generate the required specific tools for each GF system (described in details in the technology platform section, 2.2.5).

Monitoring the trafficking of GF-GFRs along different endocytic routes
The trafficking routes of ligand-receptor complexes were visualized using a combination of organelle markers in high-resolution microscopy-based assays developed by Zerial in collaboration with the HT-TDS (MPI-CBG). The localization of GF-GFR complexes was monitored i) along the various endocytic routes (see Figure 2) and ii) also within sub-compartments of endocytic organelles, such as Rab-domains. Selected key components of the trafficking machinery were detected either by using specific antibodies or GFP-tagged recombinant proteins, transiently or permanently expressed from cDNA in stable cell lines. The following markers were used:

- AP2 for the clathrin-coated vesicle-mediated pathway
- GFP-Caveolin1 for caveolae and caveosomes
- GFP-GPI as general markers for lipid rafts, including non-clathrin and non-caveolar structures

- GFP-Rab5 for the early endocytic pathway
- EEA1, Rabenosyn-5 for early endosomes
- Rabankyrin-5 for macropinosomes
- APPL1 for the recently described signaling compartment
- GFP-Rab4 and Rab11 for the recycling pathway
- GFP-Rab7 for the late endocytic pathway.

In addition to studies on trafficking of wild type receptors, also mutated GFRs (activin receptor ALK3 and its co-trafficking with receptors BMPRII, ActRII(A) and ActRII(B)) were analysed with relevance for the selected disease models in the EndoTrack project. The first comprehensive analysis of all GF-GFRs was performed using HeLa cells. Validation of the obtained results was carried out in physiologically appropriate systems such as: primary neuron cultures (e.g. hippocampal and cortial neurons from wild type and genetically modified mice), human fibroblasts, MEFs, and in cells derived from selected animal models (e.g. zebrafish fibroblast cell line and primary cultures of gastrulating ectodermal and mesendodermal cells).

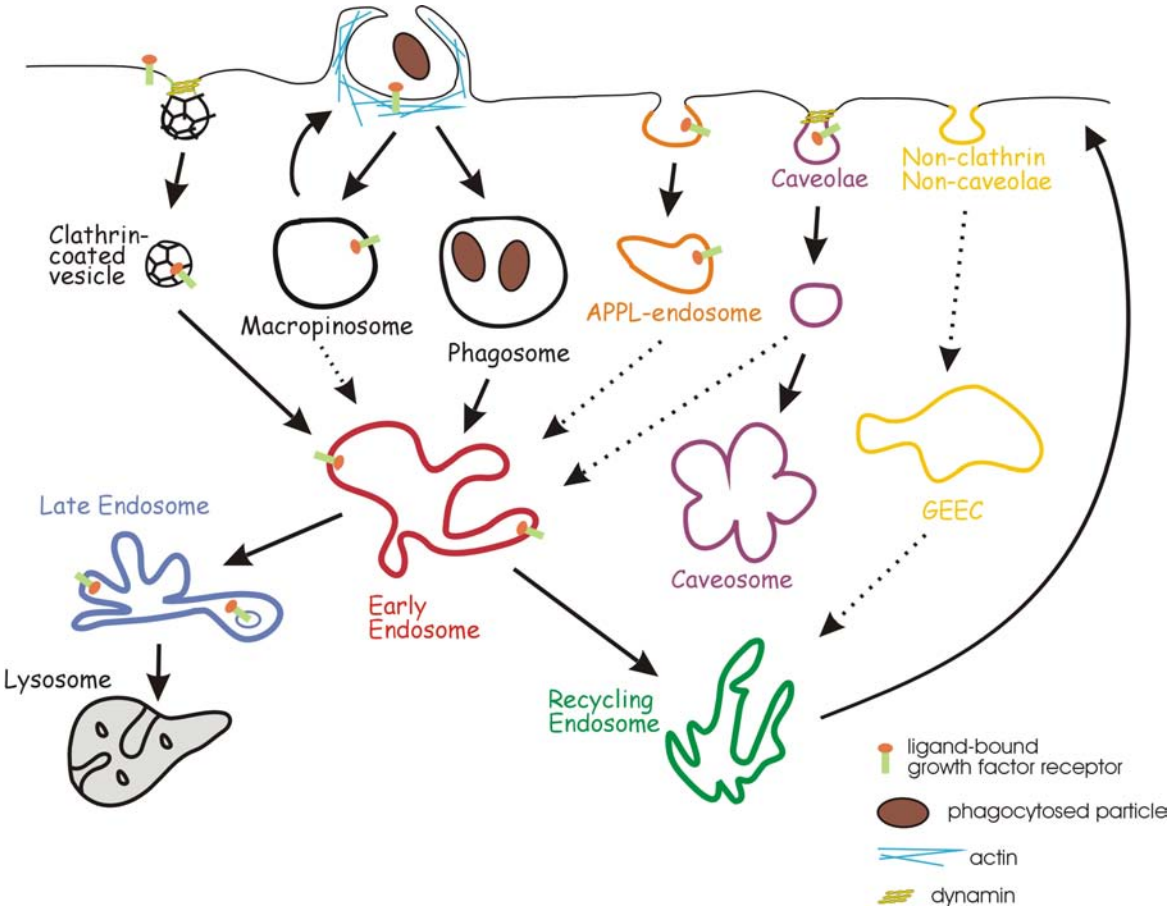

Figure 2. The different endocytic routes analysed in the EndoTrack project

EGF

A comprehensive analysis of intracellular trafficking of fluorescently labeled-EGF in HeLa cells has been carried out, using a pulse-chase protocol. This analysis was performed in the presence of fluorescently labelled-transferrin to compare the trafficking of a GF (EGF), which exemplifies ligand-mediated internalization with another cargo molecule, which undergoes constitutive endocytosis. Trafficking of EGF through clathrin-coated vesicles, early endosomes and APPL-containing endosomes has been quantitatively assessed by measuring EGF (and transferrin) colocalization with clathrin heavy chain, EEA1 and APPL1 proteins, respectively. The quantitative analysis of microscopical images was performed employing the MotionTracking software further developed for this purpose. This analysis has quantitatively estimated the fraction and kinetics of cargo that enters canonical EEA1-positive early endosomes vs. the newly described APPL endosomes. Their studies further demonstrate that sorting of EGF occurs independently and with different kinetics than that of transferrin, although both types of cargo are trafficked through the canonical EEA1- positive and APPL-harboring endosomes.

Activin A
The localization of the fluorescently tagged Alk4 and ActRIIB receptors has been tested using several organelle markers.

Wnt
The sub-cellular distribution of rab5-Y/CFP, cellubrevin-Y/CFP and rab11-Y/CFP in zebrafish gastrulating cells in vivo was specifically found in endocytic structures located both in the peri-nuclear space and close to the plasma membrane.

Ephrin
EphB2 receptor uptake by cells contacting neurons has been analysed in mouse hippocampal neuronal cultures expressing an EphB2-enhanced yellow fluorescent protein (YFP) fusion protein using time-lapse microscopy. 16-day old neurons in high-density cultures containing glial cells, often displayed filopodia-like protrusions at the tips of growth cones and along neurite shafts. These protrusions appeared to form contacts with other neuronal and non-neuronal cells of the culture. Fluorescent clusters have been observed to be released from the neuron or pinched off by another cell and transported away from the filopodia of the transfected neuron. Since the YFP-tag was inserted into the cytoplasmic domain of EphB2, the presence of fluorescent clusters in neighboring cells indicated that the entire EphB2 molecule (including parts of the plasma membrane) was transported towards the encountered cell and not shed from the cell surface. It is very likely that the EphB2-expressing neuron is contacted by a nearby glial cell which expresses ephrinBs; this contact triggers bi-directional signalling events resulting in the uptake of the EphB2-containing vesicle by the opposing cell in a process that resembles the trans-endocytosis seen in immature neuronal growth cones. The release of fluorescent clusters requires neuronal expression of EphB2. Upon transfection of neuronal cultures with expression constructs coding for fluorescently tagged ephrinB1 or ephrinB2 (YFP-ephrinB1/B2), the release of ephrinBs towards opposing cells in culture there only rarely observed. These findings indicated that the Eph/ephrin system is not equally active in both directions. Furthemore, the EphB2-expressing cell is more efficient in releasing clusters than the ephrinB-expressing cell, or that the pulling force exerted by the ephrinB-expressing cell is stronger than the EphB-expressing cells (Lauterbach and Klein, Journal of Neuroscience, 2006).

The role of Eph receptor clustering for endocytosis. Stimulation of Ephs by soluble ephrins causes Eph clustering, activates kinase signalling and endocytosis. Eph-GFP and ephrin-GFP fusion proteins were generated endowed with target sequences for an artificial “dimerizer”, a derivative of FK506, within their cytoplasmic tails. Application of the membrane-permeable dimerizer to cells expressing these GFP fusion proteins caused oligomerization of Ephs in the absence of their ephrin ligands.

1. Tracking of growth factors in physiologically relevant model systems

Mouse hippocampal neurons and astrocytes: Ephrin
High- and low-density cultures of dissociated mouse hippocampal neurons and astrocytes have been established and transfected with expression plasmids encoding YFP fusion proteins to follow the trafficking of YFP-containing vesicles by time-lapse imaging (Lauterbach and Klein, 2006).

Measurements of the kinetics of GF-GFR endocytosis, recycling and degradation.
The *kinetics* of (1) endocytosis, (2) recycling and (3) degradation was determined for selected GFs by using a combination of fluorescent analysis by high-resolution light microscopy-based assays and biochemical measurements using electrochemiluminescence (ECL) technology. The transport kinetics of unliganded versus liganded GFRs and mutated forms of GFRs was compared as a prerequisite for the analysis of endocytic trafficking under non-stimulatory and signaling conditions. There was a special emphasis taken to examine the trafficking routes of GF-GFR complexes at the *lowest* possible concentration of ligand, i.e. at *sub*maximal reporter response, in order to avoid or at least minimize the possibility of saturation of the pathways, and non-physiological responses. The following GF-GFR/co-receptor complexes were analysed: PDGF and EGF as examples of GFs using receptor tyrosine kinases, as well as BMP and Activin A for serin/threonine kinases. Furthermore, endocytosis and degradation rates of Wnt3a/Wingless, LRP5/6/Arrow and Frizzled family members was measured in human 293 and *Drosophila* cells.

Intracellular localization of signaling components downstream of GF-GFRs.
The GF-GFR localization studies were extended to known GFR signaling effectors along the endocytic routes. The following pathways were analysed: EGF, TGF-beta, FGF, Wnt and Eph.

Tyrosin Kinase Receptor pathway

The precise, quantitative characterization of endocytic trafficking of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) after their internalization in cultured mammalian cells has been achieved. The realization of these goals was possible due to the combined application of novel internalization protocols, high-quality confocal microscopy imaging and quantitative image analysis, in collaboration with several EndoTrack partners. Moreover, tracking of PDGF internalized in cells was possible for the first time thanks to the generation of a novel tool (labeled PDGF) for microscopy-based detection in cells. These data demonstrated that multiple internalization routes can be employed for the internalization of growth factors and depending on the uptake route, the activation of certain signalling effectors is changed.

EGF

To determine the precise intracellular routing of internalized EGF. EGF is one of the first growth factors, which have been visualized in cells after internalization. Although it has been reported that EGF undergoes both degradation and recycling after internalization, no precise characterization of its trafficking through subsequent endosomal compartments has been previously achieved. The objective was fully achieved, due to the combination of innovative internalization protocols, high-quality imaging and quantitative image analysis.

Characterization of EGF trafficking via endosomes, including APPL endosomes. Quantitative kinetic analyses of EGF transport demonstrated that when given to cells in the form of a short pulse, EGF is internalized via clathrin-mediated endocytosis into two subpopulations of early endosomes: the canonical ones marked by the presence of EEA1 and the APPL endosomes positive for APPL1 protein. Inhibition of clathrin-dependent internalization inhibits EGF endocytosis under such experimental conditions. Moreover, the analysis of EGF transport allowed for a detailed characterization of APPL endosomes as a distinct subpopulation of early endosomal compartments. APPL endosomes are involved in early trafficking of cargo internalized via clathrin-mediated endocytosis and destined for recycling or degradation. APPL endosomes exhibit cargo- and clathrin-independent biogenesis, abilities to sort cargo and undergo fission, fusion and tubulation, while their temporal stability resembles other early endosomes.

PDGF

To determine the precise intracellular routing of internalized (PDGF), along various endocytic routes and to determine their impact on PDGF signaling. Although PDGF is an important growth factor with multiple roles in cell physiology and developmental processes, its intracellular routing after internalization remained largely unknown, mainly due to the lack of appropriate tools to track the internalized ligand. Therefore, the efforts within EndoTrack concentrated initially to create an appropriately labeled ligand and then perform a detailed characterization of PDGF endocytosis and signaling. The objective was fully achieved, due to the creation of a novel tool to track internalized PDGF in cells, high-quality imaging and quantitative image analysis

Characterization of endocytic trafficking of PDGF and its impact on signaling. The basal routes of intracellular transport of internalized PDGF were determined. Using various internalization protocols, labelling of endosomal compartments with appropriate markers and quantitative image analysis tools, a precise description of PDGF internalization and trafficking was provided. By modulation of endocytic routes using RNAi or pharmacological approaches, it was demonstrated that PDGF can be internalized into cells both in clathrin-dependent and -independent manners. Importantly, depending on the uptake route, the activation of certain signalling effectors is changed. Moreover, it appears that the kinetics of PDGF transport can be perturbed upon silencing of certain endocytic proteins and such perturbations result in the changed kinetics of signalling events, measured as the level of activation of signal transduction kinases. These studies confirm that changes in endocytic trafficking of PDGF have impact on the signalling machinery.

Identification of a novel set of proteins interacting with PDGFR-beta. By the use of synthetic peptides corresponding to all autophosphorylation sites in the receptor the goal was to identify binding proteins. Synthetic peptides corresponding to all autophosphorylation sites in the receptor were made either tyrosine phosphorylated or not. Immobilized peptides were then incubated with cell lysates and interacting proteins were separated by SDS-PAGE, silver stained and interacting proteins identified by MALDI-TOF mass spectroscopy. It was possible to purify and identify 14 interaction proteins; Fes,

Pacsin3, Pacsin1, Grb2, p85, p110, RasGAP, centaurin d2, Vav2, G3BP, PARP1, NICE4, SHP2 and PLCg. Out of these, 3 might be of particular interest in regard to PDGFR-B downregulation, i.e. Pacsin which is a member of a family of proteins that regulate intracellular vesicle trafficking possibly through their ability to regulate the cytoskeleton, centaurin proteins that also regulate cytoskeleton and vesicular trafficking, and NICE4, a ubiquitin associated protein.

To study the function of Alix and HD-PTP in PDGFR downregulation. To address whether Alix and HD-PTP are involved in PDGFR downregulation cell lines were engineered to overexpress Alix or to downregulate HD-PTP and Alix, and studied PDGFR ubiquitination, internalization and degradation. It was found that both Alix and HD-PTP are implicated in PDGFR downregulation. The data suggest that Alix has a role in removal of receptor from the cell surface, while HD-PTP is involved in intracellular sorting toward degradation. By the use of cells with elevated expression of Alix or cells depleted of HD-PTP expression it was shown that: 1) Alix overexpression resulted in decreased PDGFR ubiquitination and removal from cell surface but no significant effect on receptor degradation, 2) depletion of HD-PTP did not influence the rate of receptor clearance from the cell surface but did reduce the rate of degradation.

A further finding was that loss of the **RasGAP binding site** on the PDGF beta-receptor induced receptor recycling, and therefore Ras activation might regulate receptor trafficking. It was shown that cell transformation with oncogenic H-Ras (H-RasG12V) induced dynamin-independent internalization of the PDGF beta-receptor. This was accompanied by increased and prolonged receptor phosphorylation and downstream signaling. The increased PDGF B-receptor activation contributed to H-Ras transformation, and PDGF receptor kinase inhibition decreased the ability of transformed cells to grow in soft agar. These findings suggest that H-Ras transformation is augmented by the increased PDGF beta-receptor signaling, induced by H-Ras.

Ephrin

Rin1 regulated ligand-stimulated endocytosis of EphA4 receptors.

Using avidin pull-down assays followed by immunoblotting for EphA4 it has been shown that in the absence of overexpressed Rin1, ephrinB3-Fc stimulation leads to a significant increase in biotinylated, internalized EphA4. In the presence of overexpressed Rin1, ephrinB3-Fc stimulation resulted in a further increase of biotinylated, internalized EphA4 compared to ephrinB3-Fc stimulation in the absence of exogenous Rin1. Similar results were obtained when the number of internalized EphA4 receptor clusters has been quantified using immunofluorescence.

In the presence of dominant-negative Rin1 (catalytically-inactive, GEF domain was deleted) the increase in EphA4 internalization induced by ephrinB3-Fc stimulation was suppressed. Cells expressing dominant-negative Rin1 showed internalization levels comparable to control Fc-stimulated cells.

APPL pathway

Elucidating the roles of APPL endosomes and of APPL proteins endocytosis and in signal transduction. Cumulatively, the data obtained within EndoTrack support a model in which APPL endosomes represent a distinct and stable compartment involved in cargo trafficking and regulation of signal transduction, acting in parallel to and in connection with canonical EEA1 endosomes.

APPL signalling endosome APPL endosomes have been characterized as a distinct and stable compartment involved in trafficking of cargo, including growth factors, and in the regulation of signal transduction. APPL endosomes appear to act in parallel to and in connection with canonical EEA1 endosomes. In addition to being markers of APPL endosomes, APPL1 and APPL2 proteins perform additional roles in signaling processes, including their interactions with the nuclear proteins. APPL proteins were identified as novel activators of β-catenin/TCF-mediated transcription in the canonical Wnt signaling pathway. Both APPL proteins interact directly with Reptin, a transcriptional repressor binding to β-catenin and histone deacetylase 1 (HDAC1). Overexpression of either APPL protein relieves Reptin-dependent transcriptional repression and correlates with the reduced amounts of HDACs and β-catenin associated with Reptin, as well as with the lower levels of Reptin and HDAC1 on the promoters of β-catenin target genes.

In addition, APPL proteins interact with the nuclear co-repressor complex NuRD, containing nucleosome remodeling and histone deacetylase activities, and this interaction regulates the nucleocytoplasmic distribution of APPL1.

Tracking/characterization of APPL1 compartments in primary neuronal cultures

APPL localization was analyzed by immunofluorescence in mouse primary cortical and hippocampal neurons using rabbit anti-APPL1 and 2 antibodies produced in EndoTrack. APPL was found in axons, dendrites, cell body and nuclei of cells. APPL endosomes were divergent form EEA1 endosomes, as previously described (Miaczynska et al., 2004), confirming that they were also a distinct endosome

population in neurons. EEA1 endosomes were found in dendrites and cell bodies. The lack of EEA1 in axons in contrast with the presence of APPL suggests a role of APPL as a main Rab5 effector in axons.

Wnt pathway
Wingless
EndoTrack has made major contributions that further our understanding of the trafficking mechanisms that enable the Wingless morphogen to form a concentration gradient. A regulatory mechanism was also uncovered that contribute to the interpretation of the Wingless gradient.
Wingless endocytosis. The objective to identify components that regulate the trafficking of the Wingless receptors has been challenging. The main focus was on Arrow, which appears to be ubiquitinated (as shown by Western blot analysis). Through genetic screening, two ubiquitin modifying enzymes have been identified that had a mild effect on Wingless signalling. However, no effect on the Wingless gradient was detected. These studies are currently ongoing using mass spec to identify the ubiquitinated residues and also to identify proteins that bind to Arrow in the endocytic pathway.
Wingless secretion. A candidate gene approach has been taken to the problem of Wingless secretion. In particular, the function of Drosophila VPS35, a retromer component that had been shown to be required for Wnt gradient formation in C. elegans was characterized. This approach led to the discovery that Wingless secretion requires retromer-dependent retrograde transport of Wls/Evi, a multipass transmembrane protein that transports Wnts from the Golgi to the plasma membrane. In the course of further studies on Wingless secretion, two new components of the metazoan secretion pathway have also been identified.
Interpretation of the Wingless gradient. While assessing the role of Arrow and Frizzled2 in Wingless degradation, the unexpected observation has been made that some aspects of wing development can occur in the absence of Wingless. This led to the identification of a novel feedback mechanism that ensures robust interpretation of the Wingless gradient. Specifically, it was shown that cells influence each other's response to Wingless through at least two modes of lateral inhibition one is acting at short range and the other over several cell diameters. The short range interaction remains largely mysterious but it was possible to show that medium range inhibition is mediated by Notum, a secreted GPI-specific phospholipase shown previously by others to act as a secreted feedback inhibitor of Wingless signaling.

Wnt (zebrafish)
The analysis of the sub-cellular distribution of Fz7-Y/CFP and Flamingo-Y/CFP in zebrafish gastrulating cells in vivo showed that both Fz7 as well as Flamingo co-localized with Rab5-, Cellubrevin- and Rab11-positive endocytic structures.

LRP6 trafficking

LRP6 is a Wnt co-receptor essential for the transmission in the beta-catenin pathway. In order to study the dynamics of LRP6 trafficking after Wnt ligand binding and to characterize its significance for signaling live cell imaging and confocal microscopy techniques have been used (Bilic et al., Science, 2007).
In the EndoTrack project it was shown that the phosphorylation by CKI gamma is triggered by formation of signalosomes, endocytic vesicles harbouring clustered LRP6 receptor in association with other components of the Wnt signalling pathway. Thus, phosphorylation of LRP6 and endocytosis are intimately linked. This was an unexpected finding and a new concept for Wnt signal transmission.

Fgf pathway
Fgf8
The EndoTrack project focused on the influence of endocytosis and endocytic trafficking on Fgf signaling. In particular it was investigated how endocytosis of Fgf8 through its receptors and subsequent trafficking of its signaling complexes influences the formation and interpretation of the Fgf8 morphogen gradient during zebrafish gastrulation. Since there were no available appropriate tools for monitoring Fgf pathway molecules the following tools have been developed: fluorescently labeled recombinant zebrafish Fgf8 as well as constructs coding for fusions of Fgf8 and Fgf receptors with fluorescent proteins of both mammalian and zebrafish molecules. Also, for studies in zebrafish it was still necessary to clone established components and regulators of the endocytic pathway (e.g. rabenosyn5, rififylin, hrs, stam and amsh) and fluorescently labeled markers of endocytic compartments (e.g. Rab5, 7, 11, Caveolin). In parallel tissue culture based and in vivo uptake assays for Fgf8 were also established. Using the in vivo assays it was shown that on the one hand Fgf8 endocytosis determines the half-life of extracellular Fgf8 protein and thus the shape of the extracellular Fgf8 morphogen gradient. On the other hand the endocytic sorting towards the lysosomes regulates the half-life of the intracellular Fgf8-FgfR signaling

complexes and thus the interpretation of the morphogen gradient. Thus, the EndoTrack studies suggest that endocytosis has two independent roles in morphogen formation and interpretation.

TGFbeta Pathway

ActivinA

EndoTrack has investigated the role of trafficking in the regulation of ActivinA signalling and delivered the following main results:
Successful mapping of the endocytic routes of ActivinA receptors. At the beginning of ENDOTRACK the endocytic route followed by ActivinA receptors was largely unknown and poorly characterised. During the course of ENDOTRACK the trafficking route of the receptors in the absence and presence of ligand has been mapped. The endocytic route followed by the TypeI and II ActivinA receptors minus and plus ligand has been completed. Furthermore, localisation of signalling components downstream of the ActivinA receptors has been completed. The trafficking of ActivinA ligand has been delayed due to the inability to see the ligand following internalisation due to low receptor numbers. Therefore P11 has generated 2 tagged recombinant adenoviruses, one expressing Alk4 and the second expressing ActRIIB, both viruses express well and are now being used to monitor ligand internalisation. Receptor internalisation, degradation and recycling are also being addressed using the viruses. This work will go beyond EndoTrack as a basic part of our attempt to understand the trafficking machinery involved in ActivinA signalling. In summary the main achievement was the quantitative map of the receptors trafficking routes in the absence and presence of ligand.

Study endocytosis of Activin in the early Xenopus embryo. By using a labelled form of activin its endocytic path was followed in animal pole cells, thereby providing some information about how it signals and how it exerts long-range effects. This involved finding a form that was both labelled and active.
The EndoTrack work has made substantial contributions to our understanding of endocytosis in long-range signalling in the *Xenopus* embryo and has highlighted differences in the strategies employed by different embryo species at different stages. The main results have been published in an important paper in *Development* (Rab5-mediated endocytosis of activin is not required for gene activation or long-range signalling in *Xenopus*; *Development* **136**, 2803-2813, 2009), and this paper represents an important milestone in the careers of two young scientists: Anja Hagemann and Xin Xu. Importantly, the work has also introduced a cell biological aspect to our studies of early vertebrate development, including new approaches to embryonic cell culture, new imaging methods, and real-time imaging of cells and embryos. The main conclusions are that labelled activin travels exclusively through the extracellular space and that its range is influenced by numbers of type II activin receptors on responding cells. Inhibition of endocytosis, by means of a dominant-negative form of Rab5, blocks internalisation of labelled activin, but does not affect the ability of cells to respond to activin and does not significantly influence signalling range. Together, the EndoTrack data indicate that long- range signalling in the early *Xenopus* embryo, in contrast to some other developmental systems, occurs through extracellular movement of ligand. Signalling range is influenced by numbers of cognate receptors on the surfaces of responding cells.
Importantly, the work does not stop here, because the EndoTrack experiments have inspired new approaches to the study of BMP signalling in *Xenopus* addressing the role of endocytosis in BMP signalling in the early embryo.

TGF-beta
To study the intracellular localization of a TRAF- and Alk/Smad-interacting protein TTRAP, they have made (i) various N-terminally tagged TTRAP proteins and (ii) a panel of monoclonal antibodies (15 in total). The data showed that overproduced TTRAP localized in the nucleus and when co-produced with TRAFs (TRAFS were initially chosen because TTRAP was isolated as a CD40 and TNFR-interacting protein) TTRAP was found in expanded vesicles, possibly early endosomes.
SARA has been found to be partially colocalised with rab5, rab7, rab4, rab11, rabankyrin5, EEA1, rhoD, rabenosyn5, caveolin1, but APPL1, consistent with the absence of PI(3)P on this endosomal compartment (Miaczynska et al., 2004).

2.2.2. Trafficking and signaling machinery

Identification of the molecular machinery responsible for the intracellular trafficking in relation to signaling of GFR complexes

In the second research area of EndoTrack the main objective was to define components of the molecular machinery responsible for the intracellular trafficking of GFR complexes, and to determine the consequences of selectively manipulating the endocytic routes, via targeting their specific transport regulators, on the signaling output of the studied receptors. In order to *systematically* identify regulators of GFR trafficking **genome-wide and focused cell-based image analysis screens as well as the proteomics of organelles** have been established. EndoTrack considered regulatory proteins as (1) regulators of vesicular trafficking and membrane-cytoskeleton interactions, (2) effector proteins of the signaling system itself, and (3) of the co-receptors or receptor-associated cytoplasmic proteins that physically integrate different GF systems. This task involved the development and use of cell lines (or animals, see below) transgenic for a reporter gene specific for each different class of GFs (described in the technology platform section). Through this activities, **an extensive and unique collection of endocytic and signaling regulators as well as assays** were generated, which provides a unique European resource of wide utility in the molecular cell biology field.

Modulation of the endocytic routes by established regulatory components.
EndoTrack performed a systematic analysis of the endocytic pathway by identifying regulatory components of each endocytic route. The five endocytic routes (Figure 2) were affected by interfering with the established regulators of transport by 1) their ablation by RNAi, 2) the loss-of-function via the use of dominant negative inhibitors, 3) the gain-of-function either via overexpression or the use of dominant active mutants (e.g. Rab5Q79L) or 4) the use of selective inhibitors [aptamers developed by **Imaxio**, see technology platform, 52.2.5]. Experimental conditions and reagents for this task have been developed to cross-compare the activity of these regulatory molecules with respect to the trafficking of the selected GF and GFRs (EGF, Activin A, FGF, PDGF, Wnt) along the various endocytic routes.
Various techniques (gene knock down using siRNA, stable cell lines, overexpression studies) have been applied already in the first 12 months of EndoTrack to modulate expression of endocytosis regulators.

EGF
Different established regulators of endocytosis (modulators of Rab5 and other regulators of transport, APPL1 and APPL2, clathrin heavy chain, EEA1, selected Rab5 effectors) have been used to knock down gene expression in HeLa cells. The cells were analysed by quantitative image analysis (developed in EndoTrack) for their ability to internalize cargo via the clathrin-coated endocytic pathway using transferrin and EGF as cargo. A number of hits has been obtained that resulted in interesting phenotypes altering the intracellular trafficking of EGF, affecting its internalization, degradation or redirecting its transport between various pathways.

PDGF
The analysis of Hrs phosphorylation upon PDGF b-receptor activation has led to the conclusion that tyrosine phosphorylation of Hrs is very likely not a major determinant for PDGF receptor sorting.
The recycling of the PDGF b-receptor in TC-PTP knock-out MEFs was shown to be critically dependent on Rab4 activity.
Downregulation of classical and novel protein kinase C isoforms was shown to inhibit PDGF b-receptor recycling in TC-PTP knock-out MEFs. Preactivation of PKC induced recycling of the PDGF b-receptor in wt MEFs. These data indicated that PKC activation was involved in the sorting of this receptor.

Activin A
The siRNA approach has been used against known trafficking regulators: Clathrin heavy chain and EPS15 for the clathrin pathway, Dynamin1 for the clathrin and caveolar pathways, Rab34 for macropinocytosis, Filamin for caveolae-mediated Endocytosis, Cdc42 for the non-clathrin, non-caveolae-mediated route, RhoD as a regulator of membrane-cytoskeleton interactions, ERBIN as a binding partner of SARA, Arf6 as a binding partner for SMAD4, Caveolin1, and the 78 Rab5 effectors. The role of Arf6 and ERBIN proteins has been confirmed in Activin signaling.

Ephrin

Endocytosis induced by Eph/ephrin interaction is bi-directional and therefore rather complex. In order to characterize endocytosis pathways used by Eph receptors and ephrins, they simplified the system such that endocytosis happens uni-directionally. To study ‘forward endocytosis’ (in the Eph-expressing cell), cells expressing full-length Eph (mostly EphB2) were co-cultured with cells expressing C-terminally truncated ephrin (mostly ephrinB2) lacking the intracellular domain. Under these conditions all Eph-

ephrin clusters trafficked into the EphB2-expressing cell. To study 'reverse endocytosis' (in the ephrin-expressing cell), they co-cultured cells expressing full-length ephrinB2 with cells expressing a truncated EphB2 receptor. Under these conditions all Eph-ephrin clusters trafficked into the ephrinB2-expressing cell.
1) Forward and reverse endocytic vesicles co-localized within the Rab5 compartment when transfected with the constitutively active Rab5Q79L-GFP and both types of endocytic events were strongly inhibited when they transfected the dominant negative Rab5-GFP construct Rab5S34N in the cells that express the full-length EphB2 or full-length ephrinB1.
2) Both forward and reverse endocytosis were strongly reduced by Cytochalasin D. Forward endocytosis was strongly inhibited by the two PI3-Kinase inhibitors, wortmannin and LY294002, whereas the inhibition of reverse endocytosis was weaker.
3) The kinase activity of the Eph receptor was necessary for forward endocytosis in co-culture experiments, but did not seem to be required for endocytosis of soluble pre-clustered ephrinB2 ligand.

Identification of novel trafficking regulators using functional genomics.

Human genome-wide screening for endocytosis (in collaboration with the HT-TDS (MPI-CBG) and the Center for High-Performance Computing of the TUD, Dresden).

Understanding how endocytosis is regulated and integrated with other cellular processes required not only a comprehensive analysis of its molecular constituents but also the detailed knowledge of the general design principles underlying the endocytic system.
In addition to an exclusive collection of regulatory components, EndoTrack has developed a unique technology platform dedicated to **HT assay development and genome-wide RNAi screening** (MPI-CBG in collaboration with the **HT-TDS) (**Collinet et al., Nature, 2010). The technology pipeline integrated the RNAi technology using human siRNA libraries; automated cell transfection, microscopy and image analysis; as well as high performance computing (collaboration with the University of Technology, Dresden). To reduce the risk and to set new standards for the quality control of RNAi off-target effects the genome wide functional genomics screen has been performed in HeLa cells using three independent genome-wide siRNA libraries (Ambion, Qiagen and an esiRNA library, with at least 4 siRNAs/gene). For hit validation and secondary screens with a limited number of candidates, other relevant cell systems, not amenable to HT analysis such as primary cells, has been also established. Based on the quantitative multiparametric image analysis developed in EndoTrack the genome-wide screen has simultaneously provided information on the endocytic trafficking in relation to general signaling parameters such as cell proliferation and apoptosis.

As a first step towards the systems analysis of endocytosis, a new strategy to accurately and quantitatively profile the activity of human genes with respect to Transferrin (Tfn) and Epidermal Growth Factor (EGF) endocytosis was developed. RNAi phenotypes were described by a set of 58 parameters that quantitatively describe specific endocytic features. Therefore, rather than assigning functions to genes with a single, semi-quantitative parameter value as usually done in screens, EndoTrack performed a multi-dimensional analysis to achieve a more detailed and thorough description of phenotypes. This platform proved effective at identifying several novel components of endocytosis and endosome trafficking, including many genes implicated in human diseases. The regulatory activity of signalling pathways such as Wnt, Integrin, TGF-β, and Notch was also uncovered as well as new genes regulating the sorting of cargo to a specialized subset of early endosomes functioning as intracellular signalling platforms. Using the quantitative data set a systems analysis by Bayesian networks was performed, that revealed new mechanisms regulating organelle (in this case endosomes) number, size as well as concentration of cargo and intracellular position of endosomes. Therefore, the screen also uncovered novel properties of the endocytic system that strongly argue in favour of an unexpected regulation of signalling properties through the modulation of the endocytic transport system.
The screen was performed using the High-Content assay looking at trafficking regulators of the EGF and Tfn endocytic trafficking and therefore of Clathrin mediated endocytosis. Therefore conducting the genome-wide screen in such a way, it was possible to analyse three different endocytic routes and endosomes: clathrin dependent-endocytosis, Appl-containing endosomes, macropinocytosis and/or clathrin-independent endocytosis. After several round of robust statistical analysis and hit validations there is a list of 4600 gene hits providing a comprehensive collection of endocytosis regulators for further focused screens and analysis.

Identification of novel signalling regulators using functional genomics.

Identification of novel signaling regulators of the Smad-dependent activin A signaling pathway
Two approaches have been undertaken to set up the conditions for the siRNA library screen:

- Screen based on chemiluminescence: an adenovirus expressing the CAGA-luciferase reporter was generated to infect cells the screening procedure. Activin A was used to activate the reporter construct – readout is luciferase relative light units.
- The high throughput RNAi screen of a Phosphatase and Kinase library on ActivinA signalling has yielded many interesting regulators of ActivinA signaling. Secondary validation screens are underway. The main achievement was the implication of several kinases and phosphatases (previously unkown) in ActivinA signalling.

Modulation of the known endocytic regulators has allowed a dissection of the endocytic regulation on ActivinA signaling. Furthermore, the role of Rab5 effectors on ActivinA signalling has been addressed and is ongoing.

Wnt pathway

Identification of genes required for non-canonical Wnt signaling to induce the endocytosis and recycling of Cadherins - Frizzled 7
The aim of EndoTrack project was the identification and functional characterization of candidate genes that modulate the activity of Wnt signals in regulating the endocytosis and/or recycling of the Wnt receptor Frizzled 7 (Fz7) and the cell-cell adhesion molecule E-cadherin (E-cad). To this end they worked on establishing a genome-wide RNAi screen for genes involved in Wnt induced Fz7 and E-cad endocytosis and/or recycling. Various human cell lines, Wnt ligands, Frizzled receptors, and Cadherin cell-cell adhesion molecules were tested in order to establish a reliable assay system. None of the tested assay systems was functioning sufficiently well in order to start a high-throughput genome-wide RNAi screen. The EndoTrack approach was modified and several candidate molecules, such as Ryk and Arrestin, were tested for their capacity to modulate Wnt11 mediated endocytosis of Fz7. Ryk and Arrestin were found to cooperate with Fz7 to promote Wnt11 endocytosis. The function of Rab5, Rab11, and Cellubrevin in Wnt11 mediated Fz7 endocytosis was also tested and it was show that a significant proportion of intracellular Fz7 is localized to Rab5, Rab11 and Cellubrevin positive vesicles, suggesting that Wnt triggers Fz7 endocytosis and/or recycling.

Using S2 cells to investigate the potential trafficking roles of genes known to modulate Wingless signaling - Arrow

The objective to identify trafficking regulators that modulate the Wingless gradient was achieved. While assessing the role of Arrow and Frizzled2 in Wingless degradation, the unexpected observation was made that some aspects of wing development can occur in the absence of Wingless. This led to the identification of a novel feedback mechanism that ensures robust interpretation of the Wingless gradient. Specifically, it was shown that cells influence each other's response to Wingless through at least two modes of lateral inhibition, one acting at short range and the other over several cell diameters. The short range interaction remains largely mysterious but we have been able to show that medium range inhibition is mediated by Notum, a secreted GPI-specific phospholipase shown previously by others to act as a secreted feedback inhibitor of Wingless signaling.
The next task was to devise genetic screens for new regulators of Wingless. Through genetic screening, two ubiquitin modifying enzymes were identified that have a mild effect on Wingless signalling but no effect on the Wingless gradient. It is ongoing work using mass spec to identify the ubiquitinated residues and also to identify proteins that bind to Arrow in the endocytic pathway.
The role of Arrow in Wingless trafficking was addressed by the characterization of interaction partners and post-translational modifications of Arrow. This work is still in progress and will continue beyond EndoTrack.

In order to identify new regulators of Wnt secretion, a genome-wide cell-based screen was devised and found two new components of the secretory pathway. A firefly Luciferase-Wingless fusion protein was expressed in S2 cells and luminescence of the conditioned medium was measured. This assay turned out to be inappropriate for high throughput screening. At high level of expression, the production of Luciferase-Wingless was insensitive to a general block in secretion (e.g. with *syntaxin5* RNAi), while at low level, secretion of Luciferase-Wingless could be blocked but too little protein was produced for

reliable detection. Therefore an alternative approach was utilized to capitalize on the screen. As part of its original design, a 'control screen' for general modulators of secretion (secretion of firefly Luciferase) was performed. This was completed and led to the identification of two novel components that were validated and characterised in yeast, Drosophila and mammalian cells. The results were published jointly with the lab of Sean Munro at the LMB in Cambridge.

EndoTrack took a candidate gene approach to the problem of Wingless secretion and demonstrated that Wnt secretion requires retrograde transport of Wls/Evi. In particular, the function of Drosophila VPS35, a retromer component that had been shown to be required for Wnt gradient formation in C. elegans was characterized. This led to the discovery that Wingless secretion requires retromer-dependent retrograde transport of Wls/Evi, a multipass transmembrane protein that transports Wnts from the Golgi to the plasma membrane.

Mass spec analysis of Wnt containing exosomes. In collaboration with the group of Roland Leborgne (University of Rennes), the conditioned medium from Wingless-secreting cells was characterized. Following density gradient centrifugation, Wingless is found in a fraction that has the expected density of exosomes. One of the aims was to define the protein composition of this fraction with the hope of identifying key components that can be knocked down in vivo for functional tests. The preliminary mass spec analysis identified several proteins of interest. However, the number of proteins identified by mass spec is large and it is clear that more stringent purification is required. This will be pursued beyond the life of EndoTrack.

LRP6

In an attempt to identify further components involved in receptor phosphorylation the Drosophila Cyclin-dependent kinase (CDK) L63 has been identified (Davidson et al., DEV CELL 2009). L63 and its vertebrate homolog PFTK are regulated by the membrane tethered G2/M Cyclin, Cyclin Y, which mediates binding to and phosphorylation of LRP6. As a consequence, LRP6 phosphorylation and Wnt/beta-catenin signaling are under cell cycle control and peak at G2/M phase; knockdown of the mitotic regulator CDC25/string, which results in G2/M arrest, enhances Wnt signaling in a Cyclin Y-dependent manner. In Xenopus embryos, Cyclin Y is required in vivo for LRP6 phosphorylation, maternal Wnt signaling, and Wnt-dependent anteroposterior embryonic patterning. G2/M priming of LRP6 by a Cyclin/CDK complex introduces an unexpected new layer of regulation of Wnt signaling.

Fgf8 pathway

Modulation of the endocytic routes by established regulatory components. To interfere with Fgf8 trafficking mRNAs coding for the zebrafish wild-type Crk or a dominant negative version of c-Cbl into zebrafish embryos were injected and the width of sprouty4 expression as a measure of Fgf8 signaling range was monitored. Fgf8 and FgfR1 endocytic trafficking was also quantitated under these conditions.

Identification of novel trafficking and signaling regulators using proteomics.

Proteomics of PDGFbeta-receptor complex. After ligand binding, the PDGFb-R undergoes dimerization and autophosphorylation on specific tyrosine residues. EndoTrack intended to identify the autophosphorylated tyrosine residues that are important for receptor recycling, by use of phospho-specific antisera established against the autophosphorylation sites in the receptor. Affinity chromatography was used to identify proteins binding to these phosphorylation sites with the help of the corresponding phospho-peptides immobilized on Sepharose beads.

Proteomics of FGF-FGFR complex.
FGF-FGFR
The work performed has produced two major outcomes – a functional categorization of direct and indirect FGF signalling trafficking partners and a 'target list' of proteins with further functional investigation with a special focus on cytoskeletal and signalling regulators (eg kinases, kinase binding proteins). A central database (http://msdm.bham.ac.uk) and bioinformatics tools for data analysis has been established.

Two proteomics screens were conducted for proteins, which are directly tyrosine phosphorylated, or associated with tyrosine phosphorylated proteins in FGF stimulated MEFs using anti P-Tyr monoclonal affinity capture. The aim of the screens was to (i) determine the 'global' functional categories of pathways

regulated by FGF stimulation and (ii) to identify candidate targets for further validation and functional genomic analysis.

A total of 664 proteins were recovered in this screen as statistically confident hits and many were recovered in both experiments further increasing confidence in their functional involvement in FGF signalling. Using bioinformatics tools these hits were functionally categorized into subclasses: cytoskeleton, signalling, molecular chaperones, RNA binding, nuclear proteins, protein synthesis and degradation and unclassified. Amongst the proteins identified were many known tyrosine phosphorylated proteins – or proteins known to interact with tyrosine phosphorylated proteins. Interrogation on protein interaction databases allowed for identification of members of known protein complexes (e.g. Arp2/3 complex ERM complex). This analysis increased the confidence in the functional validity of the obtained data. Further interrogation of the data identified a small number of proteins that on the basis of literature evidence had potential involvement in FGF signalling and trafficking (Flii, G3BP, NTKL, IQGAP and LASP1 and Rap1).

A second screen has been developed for direct purification of phosphopeptides in FGF stimulated cells using immobilization on Titanium oxide supports. Using this approach known tyrosine phosphorylated targets (eg FRS2, Paxillin) and candidate novel kinase targets (eg EPLIN, ankcorbin, phosphatidylserine binding protein) have been identified.

A third screen has involved affinity capture of phospho-peptide binding proteins using a trial peptide set from FRS2 and identified EBP1 (an Erb2 biding partner) as a novel FRS2 interactor.

The ALK4 receptor complex proteomics has been performed and the functional characterisation of SARA interacting proteins ERBIN and RNF11has been completed. Due to degradation of the expressed recombinant Alk4 in E.coli adenoviruses and lentiviruses have been generated to allow identification of SARA interacting proteins. In a yeast 2 hybrid screen 24 Alk4 interacting proteins have been identified and validated by gst pulldowns and using co-immunoprecipitation with SARA. The role of these proteins regarding the trafficking and signaling properties of SARA was analysed in loss/gain-of-function studies, including Aptamers (Imaxio) using trafficking and signaling read-outs.
Interacting proteins both from the proteomics and the yeast 2 hybrid have enabled us to generate a detailed “interaction map” of both the receptors and the SARA protein. This has been successful and has allowed new conclusions regarding the regulation of signalling and also trafficking and their interplay.

Manipulation of trafficking and assessment of signaling read-out.
EndoTrack has acquired new knowledge of trafficking and signaling components assessed the impact of regulators of trafficking on the signaling output. Components selected on the basis of the phenotypic and biochemical analysis were evaluated with respect to their ability to affect the signaling response upon their overexpression, ablation by RNAi or inhibition by **Aptamers**. To this end several biochemical and cell-based assays have been established and used.

2.2.3. Functional studies in animal model systems

The third major objective of EndoTrack was to validate the role of the established and newly identified regulators of GFR signaling and trafficking in selected and physiologically relevant cell culture models (cultures of primary neurons) and in animals (*Xenopus*, zebrafish, mouse, and with immediate access to *Drosophila*). **This part of the project mainly based on loss-of-function approaches in animal models and cells to document the defects at the organism and cellular level caused by the absence or down-regulation of the selected components.** The EndoTrack project has successfully delivered the **proof of principle** that modulation of trafficking can modulate the signaling output of GFs.

Establish mouse models for selected number of key regulators to obtain in vivo evidence of the relevance of the tight connection between trafficking and signalling
EndoTrack produced via the ES cell route conditional knockout mice that can be used in future studies beyond EndoTrack and by the scientific community. Knockout mice (for Rin1, EphA4 and ephrinA3, respectively) have been used to study a mediator (Rin1) of Eph (EphA4) endocytosis, and for documenting EphA4-mediated ephrin reverse signaling, in postnatal amygdala neurons and in the hippocampus, respectively, within the context of synaptic plasticity and long-term potentiation. Conditional knockout mice have also been established for the genes Hgs/Hrs, both Appl genes (1 and 2),

and Ttrap, as planned, and another gene that encodes an endosome-associated protein that modulates BMP signaling. In addition, ES cells with a floxed allele for a 2nd such gene are now available, and ES cells are underway for a 3rd gene.
In addition, sixteen Cre-expressing mouse strains have been made available to EndoTrack.

Gene knock-down approaches using TILLING in Drosophila and zebrafish (see 2.2.5) EndoTrack has generated mutations in 9 zebrafish genes (APPL1 and 2, Fzd7a and b, Fgf8, FfgR1, FgfR2, FgfR4, FgfBp) 7 of which lead to premature termination of the open reading frames (knock outs) and 3 Drosophila genes (Sara, Alb23B, Arf6).

Genetic screen for modulators of Wingless signalling and trafficking in *Drosophila*.
The analysis of *Drosophila* mutants in VPS35, a component of the retromer complex demonstrated a clear link between endocytosis and Wnt secretion (published in *Nat Cell Biol*). This work has been extended to assess the role of Wls/Sprinter endocytosis in the formation of the Wg gradient. The hypothesis was tested whether Wg may be internalized by Wls and targeted to an endocytic compartment where it would be packaged for long-range spread.
Furthermore an AP2 binding site has been identified in Wls and it was shown that AP2 RNAi inhibits Wg gradient formation.
Exosomes have been prepared and shown to contain both Wg and Evi/Wls. Mass spec analysis was performed and gave a list of hits, suggesting that further purification is needed. There has been a recent progress by using anti-Wingless to immunopurify Wingless-containing exosomes.

Documentation of the spatio-temporal expression pattern of genes encoding the trafficking regulators and signaling regulators during embryogenesis in animal models. The gene expression patterns of various regulators of GFR trafficking and signaling (known, candidate and novel ones) has been identified with **HT *in situ* hybridisation** in embryos (fish, frog, mouse; whole-mount, sections) for monitoring the expression domain of many candidate genes of the endocytic machinery. EndoTrack has successfully introduced the Ventana platform for automated semi/high-throughput *in situ* hybridization and immunohistochemistry in sections of post-gastrulation mouse embryos, sections of embryonic and post-natal brain (for more details see Deliverables reports).

In the *Xenopus* Image Search Engine 20,834 images from five image collections have been aggregated into a single virtual database to allow the expression patterns of relevant genes to be retrieved speedily and conveniently.

Wnt in Xenopus
A small pool cDNA expression screen for covalent modifiers of the Wnt receptor LRP6 has been previously carried out with the aim to identify genes involved in regulating trafficking, maturation, recycling, processing of the receptor. More than 30 genes were identified that affect LRP6 mobility on SDS gels, and following further testing 25 genes were retained. In situ hybridizations for the more promising candidates have started to be carried out, to characterize their spatial expression pattern during Xenopus development. Most of the genes are rather ubiquitously expressed (not shown) but some show a differential expression pattern (e.g. In neurula stages, beta 1,3-N-acetylglucosaminyltransferase is expressed in the notochord, hatching protease in the hatching gland precursors, presenilin stabilization factor is expressed in the CNS with strong anterior enrichment, as is the case for sialyltransferase, a Ser/Thr Kinase with ankyrin repeats is expressed in the embryo posterior while Gadd45 is expressed in the somite anlage).

Generation of phenotypes by manipulation of trafficking and/or signaling regulators *in vivo*.
The aim was to document the defects at the organism level in animal models caused by the absence or downregulation, either globally or in a tissue/cell type restricted manner, of the identified novel trafficking and/or signaling regulators. EndoTrack has provided two representative examples of trafficking and/or signalling regulators: **APPL** proteins and **TTRAP in zebrafish**.

APPL
During development of multicellular organisms, cells respond to extracellular cues through nonlinear signal transduction cascades whose principal components have been identified. The molecular mechanisms underlying specificity of cellular responses remain poorly understood. Spatial distribution of signaling proteins may contribute to signaling specificity. The main goal of the study was to test this hypothesis by investigating the role of the Rab5 effector Appl1, an endosomal protein that interacts with transmembrane receptors and Akt.

Studies in cultured cells have shown that APPL proteins, residing on specialized endosomes, are required for cell proliferation and their signalling pathway is triggered by various GFs.
Functional characterization of APPL proteins in zebrafish. Morpholinos targeting APPL, as well as dominant-negative and wild type forms of APPL1/2 proteins were used for the analysis of a panel of loss- and gain-of-function-induced phenotypes (Schenk et al, 2008).
It was shown that in zebrafish, Appl1 regulated Akt activity and substrate specificity, controlling GSK-3b but not TSC2. Consistent with this pattern, Appl1 was selectively required for cell survival, most critically in highly expressing tissues. Remarkably, Appl1 function required its endosomal localization. Indeed, Akt and GSK-3b, but not TSC2, dynamically associated with Appl1 endosomes upon growth factor stimulation. The results suggested that partitioning of Akt and selected effectors onto endosomal compartments represents a key mechanism contributing to the specificity of signal transduction in vertebrate development.

Ttrap in zebrafish
EndoTrack has obtained important new results on the signalling activity of Ttrap by using a Ttrap knockdown in zebrafish embryos. In combination with biochemical analyses the interaction of Ttrap with Alk receptors and with Smads was demonstrated. In addition, Alk4 phosphorylates Ttrap at two amino acid residues in the N-terminal domain *in vitro* and *in vivo*. Characterization of the defects in Ttrap morpholino-injected embryos and dorsal forerunner cell morpholino-injected (DFCMO) embryos suggests a function of Ttrap in early nodal signalling based on the visible defects in gastrulation movement and left-right axis determination. The two phosphorylation sites in Ttrap have to be intact in order to rescue these defects. Further analysis suggests that Ttrap modulates NodalAlk4Smad3 signalling. Defects resulting from knockdown of Ttrap are comparable to Smad3b overactivation phenotypes (one read-out is the expression of the gene *bonny and clyde*, encoding a transcription factor of the Mix family). It was shown that this overactivation of Smad3b (in DFCMO-injected embryos) on its turn results in a strong downregulation of cdh1 (E-cadherin) mRNA levels in the node via upregulation of the transcription factor snail, which has been shown to bind to and repress *cdh1* gene transcription (Esguerra et al., 2007).

Studies of GF spreading and signaling in well characterized systems of development, patterning and morphogenesis.
EndoTrack has monitored how GFs spread through embryonic tissues and establish the correct and dosed cellular responses, critical for their correct fate and behaviour, respectively. The Wnt/Wingless, Fgf8 and TGFb pathways was analysed.

Modifiers of Wnt/Wingless signaling (*Xenopus*, *Drosophila*):

Wnt in Xenopus
Wnt reporter assays with the majority of the candidates from the LRP6 modification screen were carried out using TOP-FLASH luciferase reporter in HEK 293 cells. Cells were transfected with candidate plasmid of interest and cells were stimulated by co-transfection with Wnt8, Wnt8 + Fz, Wnt8 + LRP6, Wnt + Rspondin, LRP6-Delta E1-4 (const. active), dvl or beta-catenin. This procedure allowed classifying the genes of interest. The majority of the candidate genes were found to be inhibitory in one or more of the luciferase assays. Two genes activated the reporter assays, namely Casein kinase 1 gamma and a WD40 domain containing gene.

Wingless in Drosophila
Transgenic flies expressing mutated forms of Arrow (DPPSP, Dlys) have been generated and assessed for degradation and signalling activity. It was shown that removal of key phosphorylation sites makes Arrow degradation incompetent.

LRP6
A phenotypic screen in the fruit fly has identified a number of components, which should now be tested as modulators of Wingless stability, secretion and/or signaling, or its gradient formation. In this signaling system, the study of phosphorylation of the co-receptor LRP6/Arrow has identified a novel LRP6 kinase.

Functional characterization of genes required for non-canonical Wnt signaling to induce the endocytosis and recycling of Cadherins (zebrafish).
The aim of the project was the identification and functional characterization of candidate genes that modulate the activity of Wnt signals in regulating the endocytosis and/or recycling of the Wnt receptor Frizzled 7 (Fz7) and the cell-cell adhesion molecule E-cadherin (E-cad). Towards this they have tried to set up an assay system, with which they would be able perform a genome-wide RNAi screen for genes

involved in Wnt induced Fz7 and E-cad endocytosis and/or recycling. They have tested various human cell lines, Wnt ligands, Frizzled receptors, and Cadherin cell-cell adhesion molecules in order to establish a reliable assay system, but none of the tested assay systems was functioning sufficiently well in order to start a high-throughput genome-wide RNAi screen. The approach was modified and they tested several candidate molecules, such as Ryk and Arrestin, in their capacity to modulate Wnt11 mediated endocytosis of Fz7. We found that Ryk and Arrestin cooperate with Fz7 to promote Wnt11 endocytosis. They also tested the function of Rab5, Rab11, and Cellubrevin in Wnt11 mediated Fz7 endocytosis and could show that a significant proportion of intracellular Fz7 is localized to Rab5, Rab11 and Cellubrevin positive vesicles, suggesting that Wnt triggers Fz7 endocytosis and/or recycling.

Manipulation of the regulators of TGFb endocytosis and their effects on TGFb gradient formation (*Xenopus*).
An inhibitor of endocytosis was used to answer the question whether cultured *Xenopus* animal pole cells expressing such a reagent can transmit a morphogen and, if so, whether the shape of the morphogen gradient is normal. This made use of state-of-the-art imaging techniques and bimolecular fluorescence complementation to visualise the cellular response to induction. The work has emphasised the fact that in the early Xenopus embryo long-range signalling occurs through an extracellular route, and therefore that different embryos and different tissues use different mechanisms to establish positional information.

Manipulation of the regulators of TGFb endocytosis and their effects on TGFb signal transduction (*Xenopus*): The *Xenopus* animal cap assay and an endocytosis inhibitor was used to analyse the effects of endocytosis on quantitative and temporal aspects of TGFb signaling. Different genes are activated by different concentrations of TGFb family members (activin). The work involved experimental embryology, cell biology, bimolecular fluorescence complementation and real-time PCR to monitor gene activation.

Activin
The role of regulators of endocytosis, such as Rab5 has been investigated in the formation and interpretation of traceable Activin in its short and long-range signaling in *Xenopus* embryos.
Regarding the Activin signaling two main objectives were tackled:
Inhibit endocytosis for its role in long-range signaling A suitable inhibitor of endocytosis that is effective in *Xenopus* animal pole tissue was selected and it was analysed whether cells expressing that a reagent can transmit a morphogen and whether the shape of the morphogen gradient is normal. This was achieved by the use of state-of-the-art imaging techniques and bimolecular fluorescence complementation to visualise the cellular response to induction.
Inhibit endocytosis for its role in the response to induction. The same inhibitor of endocytosis was used to analyse whether cells expressing it can respond to activin or other members of the TGF-beta family. The work involved experimental embryology, cell biology, bimolecular fluorescence complementation and real-time PCR to monitor gene activation. Further experiments are now ongoing using BMP and nodal family members as well as activin

Interference with Fgf8 and Wnt8 trafficking for signaling and gradient formation (zebrafish). The cell biological mechanisms controlling Fgf8 and Wnt8 trafficking from the producing cell into the receiving tissue have been analysed in the intact embryo, where target cells express target genes in a GF concentration dependent manner. Using morpholino and TILLING technology (see technology platform) to create knockouts of genes of interest, they focused initially on the regulation of signaling processes at the level of the receptor/ligand complex, and how these are dealt with in the embryo in space and time in relation to the endomembrane system.
To follow the endocytic routes taken by Fgf8 and its receptor within embryonic tissue they have generated FP-tagged versions of a wild type zebrafish Fgf receptor 1 (-eGFP, -mRFP) and a truncated dominant negative version of the FgfR1 that blocks FgfR internalization as a negative control.

Both FgfR1-FP and Fgf8-FP have been tested for their localization in specific endocytic compartments within cells of the developing embryo. As markers of the zebrafish endomembrane system FP-tagged versions of Rab5, Rab7 and Rab11 have been used. In addition FP-tagged zebrafish caveolin-1 was generated. Experiments where Fgf8-FP expressing cells are transplanted into host embryos expressing the respective FgfR1-FP or marker-FP molecule to analyze Fgf8 localization in host cells have been performed.
To interfere with Fgf8 trafficking mRNAs coding for the zebrafish wild-type Crk or a dominant negative versions of c-Cbl were injected into one cell stage embryos and monitored the width of *sprouty4* expression as a measure of Fgf8 signalling range. In both cases the *sprouty4* expression domain was found to be enlarged. The localization of Fgf8 within endocytic compartments of cells expressing Crk or

dominant negative c-Cbl and the Fgf8 diffusion range has been compared with the wild type situation. Using the in vivo assays it was shown that on the one hand Fgf8 endocytosis determines the half-life of extracellular Fgf8 protein and thus the shape of the extracellular Fgf8 morphogen gradient. On the other hand the endocytic sorting towards the lysosomes regulates the half-life of the intracellular Fgf8-FgfR signaling complexes and thus the interpretation of the morphogen gradient. Thus, the EndoTrack studies suggest that endocytosis has two independent roles in morphogen formation and interpretation.

Eph receptor trafficking in neurons (mouse).
The focus was on the mechanisms of **axon pathfinding** and **neuronal plasticity**. The Klein group has developed an alleleic series of *ephA4* mutants in the mouse with variable phenotypes. The following main questions were investigetad both in vitro and in vivo: the mapping of endocytic pathways of transmembrane ephrinB and EphB receptors in cell-cell communication between transfected cells and primary neurons; the regulation of clustering of ephrinB and EphB and the importance for endocytosis and repulsive guidance; and the analysis of conditional EphA4 knockout mice.

2.2.4. Studies in disease model systems

Cell-based and mouse systems with pathological alterations in selected diseases

The fourth major objective was to explore whether perturbations of the intracellular trafficking of signal transduction proteins are involved in human disease (cancer and neurodegenerative diseases).

The EndoTrack approach targeted the role of PDGF trafficking and sorting in causing malignant transformation for which a tumor model has been established. In a second approach the function of the APPL trafficking pathway has been investigated. APPL1 and APPL2 proteins are novel effectors of Rab5 that reside on a new endosome devoted to signalling between the plasma membrane and the nucleus. To test their functional importance, APPL1 and 2 have been knocked out in zebrafish and mice, and the associated components of the APPL trafficking pathway such as Akt have been downregulated using the Aptamer technology. With regards to neurodegenerative disease, genetic models in Drosophila and mouse have been used to investigate the importance of glial cell-line derived growth factor (GDNF) and its receptor Ret for the survival of midbrain dopaminergic neurons, with a specific focus on the importance of Ret internalization and trafficking for the survival effect.

Cancer

The EndoTrack strategy built on discoveries from the previous research areas regarding mechanisms regulating intracellular trafficking of signal transduction proteins. It is well established that enhancement of growth stimulatory signals or suppression of growth inhibitory signals can cause cell transformation. The general aim of EndoTrack was to investigate whether perturbations of regulatory components and mechanisms, which control intracellular trafficking of signal transduction proteins, can promote tumorigenesis. In a first set of experiments it was analysed whether the overexpression and/or elimination of proteins involved in regulation of trafficking has transforming activity in vitro. Further on peptide aptamers were selected to allow evaluation of the effect of binding a ligand to the target. The peptide aptamers served as surrogate ligands to define the druggability of the proteins under study.

PDGF receptor turnover

To investigate if perturbations in trafficking and sorting mechanisms can cause malignant transformation, overexpression and knock down studies have been conducted to identify components involved in sorting of PDGF receptors after activation and internalization induced by ligand binding. Alix, PKC, Appl and HD-PTP as potential candidates have been identified.

PDGF beta-receptor overactivation has been shown to transform cells, but it was not known if changes in receptor trafficking would affect its transforming ability. A model system, NIH3T3 cells transformed by the v-sis oncogene, which corresponds to the PDGF-B gene, has been established. Additionally, H-Ras-transformed human fibroblasts have been used. With these model systems, it was investigated how altered receptor recycling affects tumor cell transformation.
Cell transformation with oncogenic H-Ras (H-RasG12V) induced dynamin-independent internalization of the PDGF beta-receptor. This was accompanied by increased and prolonged receptor phosphorylation and downstream signaling. The increased PDGF beta-receptor activation contributed to H-Ras

transformation, and PDGF receptor kinase inhibition decreased the ability of transformed cells to grow in soft agar. It is currently ongoing study to investigate the internalization and trafficking of the PDGF beta-receptor and EGF in H-Ras-transformed fibroblasts using the MotionTracking software developed in EndoTrack. In summary, H-Ras transformation was found to alter the PDGF beta-receptor trafficking and signaling. This promoted H-Ras transformation.

The involvement of the APPL pathway in tumorigenesis.
The strategy was to explore the role of regulators of the APPL endocytic and signalling pathway in mice, including established GFR-dependent cancer model systems (one of which is based on autocrine production of PDGF or activating mutations of the PDGFRs). A focused approach was taken to perform a quantitative analysis on the expression levels of APPL1 and APPL2 in mammary gland carcinomas at both the protein and RNA levels. There was a highly significant difference found in APPL1 expression when the lowest grade of adenocarcinoma was compared to the higher two grades. There was an increase in APPL1 expression in the higher two grades of tumor suggesting a potential role of APPL1 in later stages of mammary adenocarcinoma.
Additionally, a small subset of colon adenocarcinoma samples was also analyzed for their expression of APPL1, APPL2 and the control MIB1 using antibody staining where APPL1 has shown an altered expression pattern.
Primary normal mammary epithelial cells have been used in order to determine the role of APPL1 in proliferation and survival of these cells utilizing both overexpression and knockdown experiments.

Furthermore, certain signaling pathways such as integrin-associated signaling play a role in causing cells to be resistant to anticancer agents like ionizing radiation. Since APPL1 has a role in signaling and proliferation, they hypothesized that this protein may be a potential therapeutic target in these cases. To test this, the knockdown of APPL1 was first performed to examine if and then how broad is the role of APPL1 in the following cell lines: A172-gliobastoma; A549-lung adenoma; DLD1-colon carcinoma; MiaPaCa2-pancreatic carcinoma; MCF7- mammary carcinoma; and UTSCC15-head and neck squamous cell carcinoma. These cells were then tested for the 1) expression of APPL1, 2) phosphorylation of Akt, GSK3beta and FAK, and 3) radiosensitivity compared to control cells. The MCF7 and MiaPaCa2 cell lines showed significant radiosensitization upon APPL1 knockdown relative to the controls. This correlated with a decrease in GSK3beta phosphorylation in both cell lines along with a decrease in Akt Phosphorylation (S473) only in the MiaPaCa2 cells. These data suggested the involvement of APPL1 in the radiation response of pancreas and mammary carcinoma cells.

Neurodegenerative diseases

Dysfunctions in the signalling activity of neurotrophic factors represent one of the key pathogenic events that trigger neurodegeneration in the brain. To exert their functions, neurotrophic factors must be internalized from the pre-synaptic terminal and, via the long-range movement of endosomal carriers along microtubules, be transported to the cell body. To objective was to test the mechanisms underlying endocytosis of neurotrophic factors with a special focus on the APPL, Ephrin and GDNF pathways both in neuronal and glial cells.

Neurotrophic factors
GDNF
With regard to neurogenerative disease, genetic models in Drosophila and mouse were used to investigate the importance of glial cell-line derived growth factor (GDNF) and its receptor Ret for the survival of midbrain dopaminergic neurons, with a specific focus on the importance of Ret internalization and trafficking for the survival effect.
The Ret receptor is specifically required for long-term target innervations and cell survival of a significant fraction of substantia nigra pars compacta (SNpc) DA neurons. DJ-1/PARK7 deletion in mice resulted in hypolocomotion, whereas the effects on target innervation and cell survival were very subtle. To investigate the combined effects of Ret and DJ-1 deletions, double mutant mice were generated lacking expression of Ret in midbrain dopaminergic neurons and DJ-1 in all cells of the body (DAT-Cre;Retlx/lx;DJ-1-/- mice, in short Ret;DJ-1 mutant mice). At 18 and 24 month of age mutant mice suffered from marked loss (40%) of dopaminergic neurons, but no additional loss of target innervation. These results indicated that DJ-1 was required for survival of midbrain dopaminergic neurons, which suffer from partial trophic deprivation. These findings also suggested that DJ-1 played no or a minor role in maintaining target innervation.

Investigation of the role of endocytosis in Ret function using fly genetics

Mutant flies overexpressing constitutive active forms of Ret (MEN2A and MEN2B) in the eye using the eye specific promoter GMR (GMR-dRetMEN2A and GMR-dRetMEN2B), display a rough eye phenotype due to excessive proliferation, increased cell size, patterning defects and apoptosis. Analysis of sections of adult eyes stained with toluidine blue revealed that the remaining ommatidia were increased in size. The stereotyped regular organization of ommatidia seen in control eyes is absent in GMR-dRetMEN2B eyes, which display random ommatidial rotations. Combined overexpression of RetMEN2B and haploinsufficiency for either DJ-1A or DJ-1B, or complete loss of DJ-1A rescued the rough eye phenotype of GMR-dRetMEN2B flies. The total eye area, ommatidia and rhabdomere sizes, as well as overall eye organization were rescued by loss of DJ-1. These results provide evidence that Ret and DJ-1 genetically interact in the fly. This work was published (Aron L, Klein P, Pham TT, Kramer ER, Wurst W, Klein R. (2010). Pro-survival role for Parkinson's associated gene DJ-1 revealed in trophically impaired dopaminergic neurons. *PLoS Biology* 8(4): e1000349. doi:10.1371/journal.pbio.1000349). Other aspects of this analysis of genetic interaction between Ret and DJ-1 in Drosophila are still ongoing. P1b confirmed that Ret gain-of-function phenotypes are rescued by DJ-1 loss-of-function and enhanced by DJ-1 gain-of-function alleles. They are currently testing if Ret gain-of-function can rescue fly viability, which is compromised by DJ-1 loss-of-function. Similar experiments are being done in neuronal cells.

Evaluation of the relevance of the revealed mechanism for whole HD pathology *in vivo*
HAP40

A transgenic mouse line bearing a BAC construct for inducible HAP40 overexpression has been generated. The transgene is under control of the Tetracycline responsive element (TRE) CMV promoter that requires binding of the transactivator protein (tTA) for expression. Competition of Tetracycline/Doxycycline (Dox) for binding with tTA allows to suppress HAP40 expression at high Dox concentration (Tet Off system). Rederivation of all three founder lines (TRE HAP40 KIN) lines by embryo transfer has been completed. The induction of HAP40 in TRE HAP40 mice is very robust upon addition of doxycycline in vitro and mimics faithfully the natural upregulation of HAP40 seen in HD models.
GFP-Rab5 dynamics was studied by IF video microscopy on neurons prepared from inducible TRE HAP40 mice in comparison to all HD and WT lines. By tracking all GFP-Rab5-positive early endosomes with the Motion Tracking software in a quantitative fashion, it was demonstrated that the fast, processive, long-distance transport was compromised in all HD lines upon HAP40 upregulation. Remarkably, these perturbations were faithfully mimicked through artificial induction of HAP40 in TRE HAP40 neurons. Moreover, the failure of HD neurons to internalize BDNF-GFP biochemically upon HAP40 upregulation was also mimicked in induced TRE HAP40 neurons. Conversely, uninduced TRE HAP40 neurons did not show any defects in Rab5 dynamics and BDNF-GFP uptake, consistent with all WT control lines. Collectively, the in vitro data so far obtained on TRE HAP40 mice have confirmed the causative key role of HAP40 in compromising Rab5-dependent neurotrophin trafficking events in HD.
The generation of the following mouse lines is in the pipeline:
HAP40 cKO: neo-deleted and heterozygous 'floxed' HAP40 cKO mice were obtained and subjected to colony expansion, further breeding to homozygosity, crossing with HD models and Nestin Cre deleter mice to excise the HAP40 alleles.
HAP40 shRNAi: the establishment of a colony and first induction tests to score for reduced HAP40 expression using already established protocols for Western blotting and qRT-PCR is ongoing.

Tracking/characterization of APPL1 compartments in primary neuronal cultures

APPL localization was analyzed by immunofluorescence in mouse primary cortical and hippocampal neurons using rabbit anti-APPL1 and 2 antibodies produced in EndoTrack. APPL was found in axons, dendrites, cell body and nuclei of cells. APPL endosomes are divergent form EEA1 endosomes, as previously described (Miaczynska et al., 2004), confirming that they represent a distinct endosome population in neurons. EEA1 endosomes are found in the dendrites and in the cell body. The lack of EEA1 in axons in contrast to APPL suggests the specific role of APPL as a main Rab5 effector in axons.

The endocytic machinery of glial cells has also been implicated in neurodegeneration. Whereas glial cells play an active role in axon pruning processes during development, they may phagocytose axon processes in neurodegenerative disorders that manifest selective axon loss (Broadie, 2004). Glial phagocytosis of hippocampal neurites in culture has been observed after overexpression of EphB2 receptors, and ephrin-induced phagocytosis may be an extrinsic mechanism for axon degeneration. The potential of ephrin-Eph signalling required for the communication between degenerating axons and phagocytosing glia has been explored (Lauterbach and Klein, Journal of Neuroscience 2006).

Neural crest specific ablation of endocytic machinery components

Neural crest stem cells are subject to Wnt and TGFbeta/BMP signalling in the early embryo and the EndoTrack consortium has accumulated experience with the analysis of neural crest defects using a combination of in situ hybridization and immunohistochemistry, in particular in the process of induction of and migration of cephalic neural crest and cranio-facial development, the induction of trunk neural crest, the analysis of neuronal precursor subtypes and satellite glial cells in dorsal root ganglia and the generation of its transient stem cell compartment (the boundary cap stem cells), the analysis of the migration and generation of the enteric nervous system during embryogenesis, and the analysis of the subcutaneous sensory system.

Ligands and receptors and intracellular effectors of the Tgfb/Bmp, Wnt and Fgf (and Shh, Notch) families of growth factors control embryonic development, including of the central nervous system (CNS). During the EndoTrack project components of the Tgfb/Bmp signaling pathway including a long list of Smad-interacting proteins has been isolated. **Sip1** was used within EndoTrack in conditional knockout mice addressing its function in neural crest and in CNS. This work has led to the establishment of important protocols for functional studies of candidate genes (in particular Appl1 and Appl2) within EndoTrack in embryonic brain and obtaining either embryonic phenotypes or early post-natal defects, which include (for Sip1 in GABAergic interneurons of the forebrain) new epilepsy models.

The work on the Sip1 conditional knockout mouse in the embryonic cortex has been completed and published (Seuntjens et al. (2009) *Nature Neurosci* 12:1373-80). Four different mouse models for studying Sip1's functions in GABAergic interneuron generation and cell fate in the basal ganglia, and tangential migration into the cortex, have been established, and important progress has been made.

The Cre-dependent R26-based Sip1 overexpression mouse model has been established (with J. Haigh, Gent). Tissue/cell type-specific rescue with this mouse model has been obtained in the corresponding knockout background. This was executed first as part of a study of Sip1 in embryonic hematopoietic cell differentiation, and was recently submitted for publication (Goossens et al., *submitted*).

Protocols for studying Sip1-deficient and Sip1 knock-in ES cells *in vitro* in pluripotency, and in neural commitment and differentiation have been established. Knock-in Sip1 ES cell clones for assessing Cre-dependent rescue by Sip1 wild-type and domain mutants of Sip1 have been obtained, both in the Sip1 locus itself (Sip1 wt) but also in the R26 locus (Sip1 wt, Sip1 w/o Smad-binding domain, Sip1 w/o NuRD-interaction motif). The mouse ES cell adherent monoculture system also serves now at P2 in an esiRNA-screen (with technical input from P1a Zerial and F. Buchholz, MPI-CBG, Dresden).

Wnt

The characteristic anteriorisation observed upon blocking Wnt signalling was observed in the LRP6 morphant Xenopus embryos, indicating that they functionally knock-down the receptor. Analysis of its role in neural crest development is underway.

2.2.5. Technology Platform

To support the various research activities, EndoTrack has identified a number of technological areas that had to be further developed and combined in order to provide unique assets for meeting the ambitious objectives and to increase the competitiveness of European research in this emerging field:
1) EndoTrack developed a range of ***in vitro* models of increasing complexity**, i.e. from **standard cell lines** to **primary cell cultures** from various **animal model systems**. Experimental conditions were established in simple and easily accessible cellular systems and then transferred to more physiological, but complex, experimental systems for validation purposes.
2) EndoTrack developed **cell–based image analysis assays amenable to High Throughput (HT) and High Content (HC) screening**. The assays were designed to allow **quantitative measurements** of the flow of GF-GFR complexes along the various compartments and sub-compartments of the endocytic routes and **time-lapse video microscopy assays**, where the behaviour of endocytic vesicles is automatically analysed using *ad hoc* developed software (MotionTracking, a quantitative multiparametric image analysis (QMPIA) software, Yannis Kalaidzidis, Zerial Group, MPI-CBG).
3) EndoTrack applied the **ECL-based detection** technology to develop quantitative measurements of GF-GFR complexes trafficking as well as cell-based and cell-free assays that recapitulate trafficking and signalling events. Importantly, the ECL-based technology platform allowed the up-scaling of such assays for HT.
4) The functional genomics approaches were complemented by quantitative **proteomics** methods applied to the search for interacting partners of established GF-GFR signalling complexes. The peptide **aptamer** technology (Imaxio) was aplied to assess the impact of selectively blocking the key GF-GFR signalling complex protein-protein interactions thus permitting stringent evaluation of the potential of the interactions as targets for disease intervention. Advanced MS technologies such as the Fourier Transform Ion cyclotron resonance (FT-ICR) and Electron capture dissociation (ECD) were applied for the analysis of **complex mixtures of proteins** and their **post-translational modifications**, respectively. The ECL technology was utilized for the fast and sensitive measurements of post-translational modifications (e.g. phosphorylation) of known trafficking and signalling components.
5) Three approaches were used to assess the functional role of candidate regulatory components of endocytic trafficking and their impact on GF-GFR complexes signalling in cellular and animal systems. First, focused libraries of siRNAs were assembled to silence genes encoding key regulators of endocytic trafficking and signalling. Second, specific Aptamers were selected to block the function of these factors or prevent their interactions. The latter approach was tightly linked to a protein-protein interaction map. Third, **TILLING** (Targeting Induced Local Lesions in Genomes) was established to enable the generation of conditional or domain specific mutations in specific target genes of interest, allowing a more detailed genetic analysis in animal model systems.
In summary, a substantial program of instrumentation, methods and software development has produced innovative tools for precise analysis of molecular trafficking dynamics in high throughput format. Using siRNA screens a wide variety of novel participants in the Epidermal Growth Factor receptor endocytosis and signaling pathways have been discovered which are being taken forward for more detailed scrutiny in model systems developed by the EndoTrack consortium. This involves the application of analytical tools developed in the program to quantitatively detect specific proteins in the endocytosis pathway with high precision.

2.2.5.1. Tool development

Development of new cell culture models and stable cell lines
The EndoTrack consortium developed the following models:
1. Development of primary cell cultures from animal models (neurons, neuronal progenitor cells, neural crest stem cells, primary cultures of gastrulating ectodermal and mesendodermal cells from zebrafish.
2. Development of permanent cell cultures from animal models (spontaneous, i.e. ES cells and fibroblasts; immortalization, endothelial cells)
3. The use of explants from animal models: Xenopus animal caps, and Drosophila wing imaginal disks**.**
4. Generation of stable GFP-reporter cell lines: ES cell lines were obtained via collaborations, from public repositories and generated in **EndoTrack**. Primary cell lines were generated from fibroblasts, neurons and neural crest stem cells. Cell lines were immortalised with tsSV40 and curated. Floxed mutant alleles were replaced by Cre-mediated incision following transfection with viral vectors. These cell lines were

used alone or in multiplex formats with RNAi mediated gene knockdown approaches. Cell lines expressing shRNAs proved to be unstable.

Cell cultures models developed in EndoTrack

- primary Mouse embryonic fibroblast (MEF) cell lines have been derived an evaluated: from Sprouty null mice, from BAC transgenic mice harboring a range of FGR activating mutations, from mouse embryos harboring shMIR transgenes to inhibit expression of FRS2.
- Stably transfected HeLa cell lines have been derived expressing shMir constructs, which ablate the expression of Rab5 and FRS2. HeLa cell line with a stable knockdown of the T-cell protein tyrosine phosphatase.
- stable HEK 293 cells expressing LRP6, Dkk1 and DKK1 have been generated to study Wnt endocytosis
- primary cultures of gastrula-stage embryonic ectodermal, mesendodermal, mesodermal and endodermal cells have been established. These cultures have been used to monitor dynamic changes in the sub-cellular distribution of cellubrevin-positive endocytic structures accompanying cell polarization and process formation.
- primary cell cultures of mouse hippocampal neurons have been established.
- long term (20 passage +) mouse embryonic fibroblasts cultures from FGFR1 hetreozygous mice have been established using low oxygen culture methods.
- endocytosis and signalling assays have been developed using Xenopus animal pole regions and dorsal marginal zone organotypic culture systems to investigate the role of endocytosis in developmental signalling.
- Xenopus Animal cap explant assay has been developed to study LRP6 intracellular localization using antibody staining.
- Drosophila imaginal Wing disk explant system as been developed for Wingless endocytosis and degradation assay.
- Rab5 and FRS2 knockdown HeLa cell lines have been generated using commercially available shMIR vectors.
- Cell lines harbouring stable GFP fusion reporter clones: A431 Rab5-GFP; A431 Rab11-GFP; Hela Rab11-GFP. The BAC transgene techniques were used to derive reporter HeLa cell lines for the following genes: Rab5, Rab7, Rab11-GFP, GalNuc, VLP16, Cytochrom C, Nurin. The advantage of the BAC technology lies in the fact that the tagged Rab protein is expressed under the control of the endogenous promoter, thus avoiding potential artifacts due to overexpression of the fluorescent protein.
- Constructs/expression vectors: BAC construct for GFP and YFP-Rab5. Lentiviral expression vector with Rab4, 5, 7, 11 with GFP- and alternative red fluorescent tags (Cherry).

Pathway specific tools

EGF
Different preparations of fluorescently labeled EGF have been generated (direct labeling with Alexa dyes, biotinylation of EGF and labeling with Alexa- or quantum dots-conjugated streptavidin).

PDGF
Alexa-labeled PDGF, biotinylated PDGF using a cleavable cross-linker arm
PDGF-BB labelled with Alexa546 did not retain proper biological activity
MEFs with stable knocked down of T-cell protein tyrosine phosphatase (TC-PTP) (in which the PDGF b-receptor, but not the PDGF a-receptor, recycles)
HeLa cell lines with stable knocked down of TC-PTP; Wt HeLa & TC-PTP KD Hela cell lines, stably expressing PDGFb-receptor
Constructs with Y/F point mutations in the autophosphorylation sites of the PDGF b-receptor, as well as C-terminal deletion mutants have been generated. These constructs are stably expressed in PAE cells. PAE cells stably expressing a GFP-tagged PDGF b-receptor is available.

APPL
New anti-APPL1 and anti-APPL2 monoclonal and polyclonal antibodies.

TGB-β pathway
BMP
cDNA clones of various type I receptors for BMP, i.e. human and mouse Alk3 and Alk6, and BMPRII.

Dual Myc-His tagged sequences (for the detection of receptor synthesis in cells transiently transfected with receptor-encoding expression vectors - Hek293T and BMP-responsive MC3T3-E1 cells).
Stably transformed cell lines (HeLa) for Alk3 – BMPRII combinations, both in tagged but also in non-tagged form.
A panel of BMP receptors tagged HA or with fluorescent tags to document the colocalization of these receptors with markers for various endocytic routes.

TGB-β signalling in Xenopus
GFP tagged forms of activin, derrière and Xnr2 (Xenopus). Alexa 488-labeled activin was contributed by the collaborator Dr Marko Hyvonen (Department of Biochemistry, Cambridge University) for studies of endocytosis and long-range signalling.

Tagged Activin A receptors
Alk4-GFP, CFP, YFP, mcherry, c-myc and HA: ActRIIB2- GFP, CFP, YFP and mcherry; ActRIIa- GFP, CFP, YFP and mcherry
ALK4 monoclonal antibodies have been purified and labeled using Molecular Probes Alexa488 and 567 protein labelling kits.
Biologically active chimeric receptors consisting of the ligand binding domain of the granulocyte/macrophage colony stimulating factor (GM-CSF) a or b receptors fused to the transmembrane and cytoplasmic domains of the type I and type II Activin A receptors.

Fluorescently tagged TGB-β receptors
Alk5- GFP, CFP, YFP and mcherry, TGF-bRII- GFP, CFP, YFP and mcherry

Fluorescently tagged Activin and TGB-β ligands
Baculovirus expression system for:
Activin A mature form –gst fusion in pFastbac, Activin A mature form –his fusion in pFastbac, TGF-b1 mature form –gst fusion in pFastbac, TGF-b1 mature form –his fusion in pFastbac, Activin A full length untagged in pFastbac, Furin in pFastbac, TEV protease-his tagged in pFastbac, Precision-protease-gst tagged in pFastbac, Purification of recombinant activin A using the second follistatin domain of follistatin-related gene (FLRG) his tagged.
WACII cells stably transfected with Activin A to purify ligand from conditioned medium.

Wnt
Plasmids: Fluorescently (GFP)-tagged LRP6, axin, Casein kinase1 gamma, and deletion variants thereof; luciferase-tagged LRP6.

Antibodies: Polyclonal Abs were raised against LRP6 and specific phosphorylated forms of the receptor

Wingless
Plasmids for expression in S2 cells: GFP-Wg *, His-Wg, HA-Wg *, BirA_Target-Wg , HA-Arrow *, FLAG-Frizzled2 (for *, also the cell line was generated)

FGF signalling
Several tagged versions of a wild type human Fgf receptor 1 (-eGFP, -mRFP, -V5) and a truncated dominant negative version of the FgfR1 that cannot be internalized as negative control. These constructs are available in a CMV promoter driven version for transient expression and are currently generated in an EF1a promoter driven version for the generation of stable cell lines.
To visualize Fgf8: Fgf8 purified from bacteria and chemically labeled with fluorophores, tagged versions of Fgf8 to be expressed and purified from mammalian cells to monitor its localization in live (-eGFP) or fixed (-HA, -HRP) cells.
The zebrafish cDNAs have been cloned for rabenosyn-5, rififylin, hrs, stam and amsh to test their influence on Fgf8 trafficking in cellular systems.

Biotinylated FGF2 (BioFGF2) was generated that retains full biological activity and indicate that the preparation is suitable for monitoring FGF trafficking.

Ephrin:
GFP-tagged Eph receptors and ephrins; EphB2-tagged with enhanced yellow fluorescent protein (YFP)

Mouse models

For conditional knockout and knockout animal models generated in the EndoTrack project see Deliverables reports.

TILLING

TILLING (Targeting Induced Local Lesions IN Genomes) technology is based on a combination of forward and reverse genetic tools, for which libraries of mutagenized genomes are generated that are screened by Cel-1 mediated detection or direct sequencing for mutations in specific genes (amplicons) of interest. By using TILLING EndoTrack successfully identified several mutants in genes encoding components of the endocytic machinery in both zebrafish and Drosophila.
ENU based new zebrafish and Drosophila TILLING libraries have been established with optimized mutation rates for isolating mutations in specific target genes.
Mutations have been isolated for the following zebrafish genes: APPL1 and 2; Fzd7a and b; Fgf8, FfgR1, FgfR2, FgfR4. For the following genes missense or splice site mutations were found: APPL1 and 2, FGFR1, FgfBp, FGFR3 and pk1b. In addition to the zebrafish work we have isolated mutations in three in Drosophila genes: Sara, Alb23B, Arf6.

Monoclonal antibody development

The EndoTrack SME member, MSD has generated a panel of mouse monoclonal antibodies. To this end the EndoTrack Steering Committee had put together a list of interesting targets, and has prioritized them. In practice it however turned out that it was not possible to produce antibodies in order of priority, mainly because there were significant delays in delivery of antigens from some partners. Monoclonal antibodies were produced in collaboration with the Antibody Facility at MPI-CBG. MSD has established protocols that allow immunization of a single mouse with multiple antigens, which then can be screened for in parallel using MSD multi-array plates coated with the different antigens as well as with control proteins or peptides. This is especially interesting and efficient for the generation of antibodies that recognize post-translational modifications such as phosphorylation. For partners located at MPI-CBG MSD was able to complete a project (from immunization to delivery of mg quantities of purified antibody) in less than four months.
Antibodies were raised both against peptides and recombinant proteins. Monoclonal antibodies were obtained for 16 out of 20 targets. This can be considered a high success rate made possible by the close interaction of MSD with the Antibody Facility at MPI-CBG. All antibodies from the successful 16 targets were then tested in the respective partner laboratories in their specific applications. Out of these 6 proved to work well, and another 6 are still under evaluation.

Development of inhibitors of protein-protein interactions - Aptamers
Using its proprietary technology, the EndoTrack SME partner, **Imaxio** delivered according to the following objectives:

1. Providing ENDOTRACK partners with new loss/gain of function tools for exploring signaling pathways (the APPL pathway): IMAXIO successfully performed the aptamers screening against APPL1, getting 3 highly specific aptamers against APPL1. Besides, IMAXIO performed the cloning steps and the characterization study for the APPL2 target. IMAXIO's experiment demonstrated the APPL2 expression in yeast made unfeasible the APPL2 aptamers screening, inducing unexpected signals (false positives with and without expression of a prey). IMAXIO collaborated with ZERIAL's laboratory for the cloning steps.
2. IMAXIO successfully performed the aptamers screening against SARA, getting 4 highly specific aptamers against SARA. IMAXIO did the 4 steps (cloning, 2H assay for characterization, aptamers screenings and specificity matrix, and cloning in mammalian vectors). IMAXIO collaborated with the MURPHY's laboratory for the cloning steps.
3. Peptide aptamers have been selected against targets involved in tumorigenesis representing 3 different families (kinase, signalling adaptor and anti-apoptotic proteins) and hits were identified after HTS against kinases (Akt1, Cdk2), signaling adaptor protein (RasGap) and anti-apoptotic proteins (BIRC 7). These discoveries could support the study/validation of new targets and may contribute to the development of new drugs in the field of oncology in the next years (Figure 3).

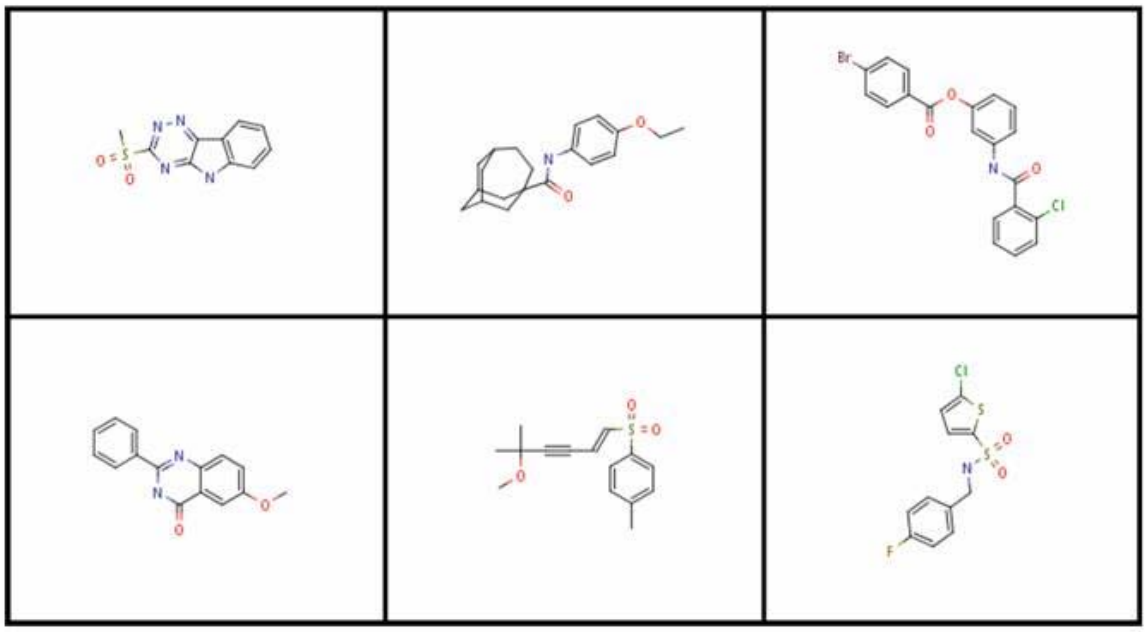

Figure 3. Representation of the 6 aptamer molecules inhibiting interaction between Akt1 and Ak6

2.2.5.2. Assay development

EndoTrack has developed cell based assays amenable to High-Throughput screening. These assays provide quantitative measurements of the flow of growth factor- growth factor receptor complexes along the various compartments of the endocytic routes. Microscopy based assays have been developed to visualize and measure endocytic compartments. These assays allow the tracking of fluorescently marked cargo and endosomes within the cell, the quantitation of vesicle movements and the tracking of endosomes in 3D over time.
Various RNAi-based endocytosis screens have been established thereby aiming at using forefront technology and increasing the quality standards in functional genomics applied to signalling and endocytosis. A quantitative image analysis assay has been used to monitor clathrin-mediated endocytosis by monitoring fluorescent transferrin and EGF endocytosis.
The hits identified by these screens have been further analysed using secondary assays. EndoTrack established medium-throughput and high-content secondary assays in order to provide further characterization and biological interpretation of the data produced by the primary screen (i.e. primary hits).
The ECL-technology of MSD is being used and further developed for HT to quantify the complex formation and trafficking of EGF, Activin A, Wnt and PDGF and their receptors.
The Multi-Array technology for the discovery of binding partners for specific phosphorylation sites on proteins is applied to screen for protein binding interactions, e.g. PDGFR binding partners, for the GF-GFR systems researched by the EndoTrack partners.
EndoTrack has also used a Mass spectrometry (MS) technology platform for characterizing protein and metabolite modifications. The FTICR mass spectrometer has been used to identify protein complexes formed as a result of FGF signalling.

Multiparametric assay development for high throughput and high content screening based on microscopy
The main aim was to develop robust and reproducible assays for the visualization of intracellular routing of GFs and GFRs. Cell-based assays were designed on the main pathways of endocytosis (see Figure 2) using automated microscopy-based multi-parameter assays for primary and secondary assays. The individual bench-scale experiments were scaled towards HT screening standards, and the high-content screens were performed by applying focused or genome-wide RNAi libraries.

EndoTrack has set up an automated work flow for cell-based assays using microscopy and image analysis in high throughput. Tissue culture cells are grown and transfected with siRNA in 384-well plates.

Labeling of the endocytic compartments can be performed using immunostaining and labeled endocytic cargo molecules. High throughput and high resolution imaging is performed using the fully automated spinning disk confocal platform (OPERA, Evotec/Perkin Elmer technologies). Images are subsequently quantified using the custom designed image analysis solution (MotionTracking, Yannis Kalaidzidis, MPI-CBG) on a linux based cluster computer (2500 processors) in the TU-Dresden.
This pipeline has been applied to the Genome-wide screen performed in the EndoTrack project (Collinet et al., Nature, 2010). The assay performed for this particular screen has been the simultaneous uptake of fluorescently labeled EGF and Tfn for 10 min before fixation of the cells with standard PFA.

Software development for quantitative image analysis

New software has been developed by the Zerial group to allow the identification and quantitative description of cellular as well as subcellular parameters, in order to further characterize cell shape in a fully automated and high throughput manner. The algorithms have been parallelized in order to work on a cluster of more than 2000 CPUs present in the High Performance Computing Center (ZIH) in Dresden (Collinet et al., Nature, 2010).

The quantification of cell morphological features has been achieved by the design, implementation and testing of new descriptors (Figure 4). Several statistical descriptors have been implemented for describing cell morphology/shape and endosome localization within the cell. The new statistics has been embedded in the in-house developed MotionTracking software, a very powerful tool for detecting vesicles and describing the features of the endosomal system in terms of endosome morphology and positioning. The cell parameters, together with the vesicular ones, allow a more precise estimation of the subcellular localization as well as they offer the possibility of performing a population analysis on different subsets of cells (Collinet et al., Nature, 2010).

The new descriptors allow identifying the cell physiological state and add a phenotypic dimension to the multi-parametric analysis of the screening; this could further elucidate the relationship between signaling and endocytosis, and how the different cellular cues are integrated into or regulated by the endocytic system. Furthermore the newly developed parameters have been tested to assess the reproducibility of the whole experimental procedure, going from cell plating to automatic image acquisition and processing. A mathematical descriptor for this is the Pearson correlation coefficient between different runs of the experiment and different oligonucleotides performing the knock-down of the same gene: these two descriptors together allow the estimation of experimental and biological noise in our assay.
The cell detection has been performed on the entire genome wide screening on the trafficking of EGF and Transferrin, and the statistical parameters describing cell shape, cell context and subcellular cell position are in the process of being calculated, together with the per cell information. The presence of this data will further require the development of new methodologies for phenotype description and extraction, for cell population analysis and for models of phenotype emergence under gene knock-down perturbation. Only by combining the development of new statistical and data-mining methods and information technology for handling, processing and presenting huge amounts of data, we can successfully extract all the information that these systematic studies provide us, and therefore lead to new biological discoveries.

Quantitation of vesicle movements in 3D over time using fully automated vesicle tracing and tracking software. The quantification of endosomal properties in a 3D fashion was a very important and challenging task. Especially for more physiological cell systems, it was not possible to neglect the third dimension by only quantifying a single focal plane.
For this task it was needed to develop new algorithms that dealt with the high complex and dynamical nature of the endosomal system. They have developed a very robust vesicle tracking algorithm for 2D movies using the MotionTracking software, and by overimposing different 2D slices in a maximum projection image they can already track vesicles that move across few different focal planes.
The challenge was to identify and describe in a quantitative manner the 3D distribution of endosome properties, such as size, cargo content and subcellular position. They have establisheda collaboration with the company Definiens and they have achieved significant progress in segmenting 3D stacks of images.

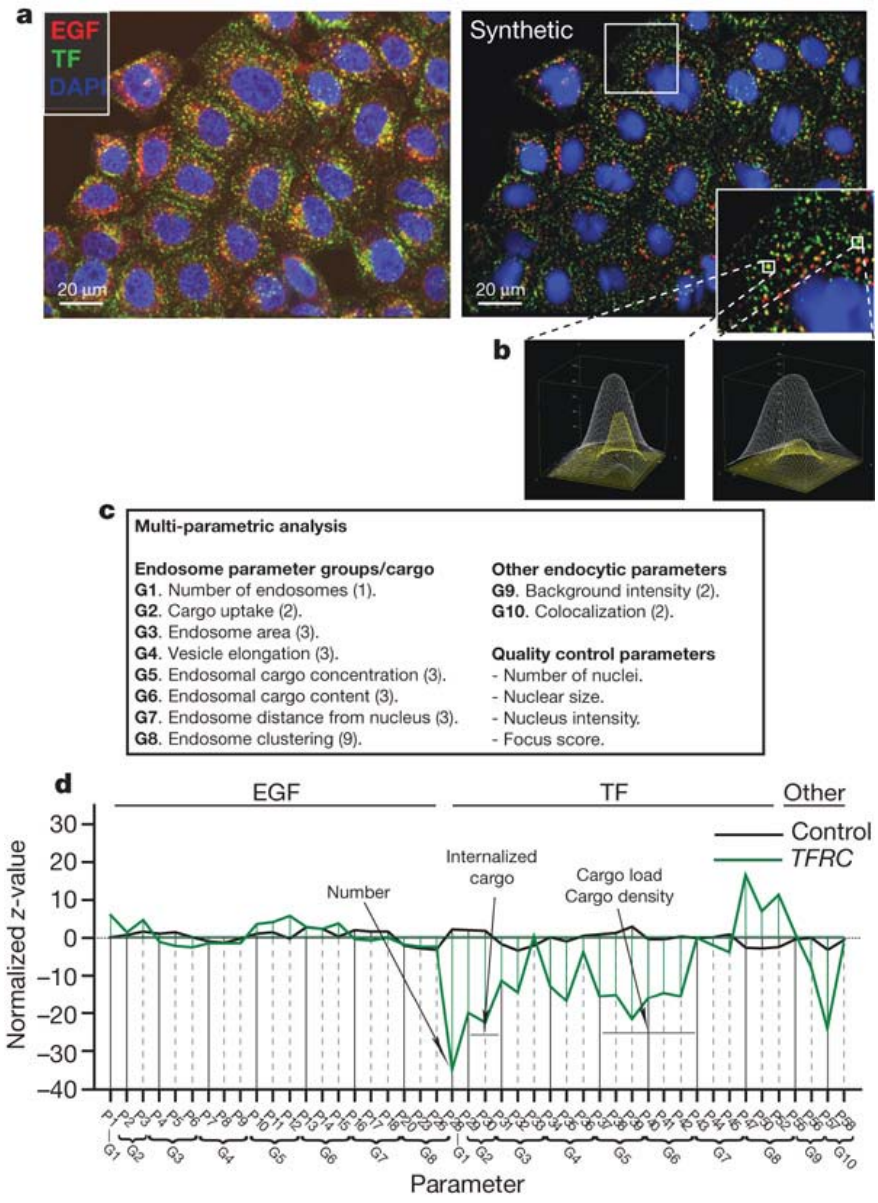

Figure 4. Multiparametric image analysis
a, Example of three-colour high-resolution images collected with the automated spinning disk confocal OPERA microscope (left). Nuclei and cytoplasm are pseudo-coloured in blue, Alexa-488-labelled EGF is pseudo-coloured in red and Alexa-647-labelled TF is pseudo-coloured in green. The synthetic image (right) was obtained after background intensity subtraction and modelling of endosomal structures. b, Close-ups show the model structure of individual endosomes. c, List of quantitative parameters divided into groups (G1–G10). Ten groups of parameters (number of parameters in each group indicated in brackets) were used to measure endosomal features and four parameters as image quality control. d, Example of QMPIA profile. The 46 most specific parameters are aligned on the x-axis and normalized z-values are plotted on the y-axis. Black continuous lines separate the parameter groups and grey dashed lines separate the parameters. The horizontal bars indicate the endocytic markers which the parameters refer to. Key parameters affected in the TFRC profile are indicated by arrows. Source: Collinet et al., Nature, 2010, Figure 1.

A new platform of the Cellenger image analysis program ('4D Cellenger') has been developed that allows fully automated tracking and tracing of cellular and subcellular structures in three dimensions over time. This software was adapted to the specific screening tasks (such as vesicle tracing and tracking) by developing the corresponding software ('rule sets').

High throughput enabling technology for discovery using electrochemiluminescence in cell-free, cell-based and animal systems
The MSD-based assays for EGF and PDGF have been optimized for using for subsets of libraries and on the hits acquired in the genome-wide screen and to test those using immunodetection of phosphoproteins by electrochemiluminescence.
MSD has played an instrumental role in establishing the followig assays in collaboration with the EndoTrack groups:

- specific ECL-based assays to measure internalization of biotinylated EGF, biotinylated PDGF, biotinylated ActivinA. For measuring biotinylated PDGF suitable antibodies and conditions were rapidly identified to set-up a sensitive assay. Translating this assay into meaningful measurements of biological samples proved to be challenging. One major obstacle was the inherent stickiness of PDGF to cells and plastic surfaces, which made it impossible to only measure the small fraction of biotin-PDGF that had actually entered the cells. By using cleavable linkers for biotinylation it was possible to remove the non-specific signal caused by PDGF sticking to cells and plastic.
- assay for SMAD2 phosphorylation to provide a read-out for ActivinA signaling. To this end a total of 120 antibody pairs were tested on cell lysates from different cell lines (the A341 cell line has been selected). The 8 most promising pairs were carried through assay optimization to yield a sensitive and robust assay. The assay is ready to be used as a read-out in ActivinA screens.

EGFR Autophosphorylation assay. Assays similar to those measuring the autophosphorylation of EGFR at specific tyrosine residues in a multiplex assay that distinguishes phosphorylated and nonphosphorylated forms of distinct EGFR epitopes have been developed to identify the critical Tyr autophosphorylation sites essential for recycling of the PDGFR or to identify binding partners of PDGFR, or the TGFb/ActivinA/BMP machinery.
Upon binding its ligand EGF, the EGFR forms hetero- or homo-dimers. Dimerization results in the activation of its intrinsic kinase activity and the phoshorylation of multiple tyrosines in the cytoplasmic domain, including Tyr992, 1068, 1086, 1148, and 1173. The MSD biomarker detection assays provide a rapid and convenient method for measuring the total and phosphorylated levels of protein targets within a small-volume sample.

Advanced Mass Spectrometry techniques for protein identification and post-translational modification EndoTrack has developed the ultra high resolution mass spectroscopy technical platform for characterizing protein and metabolite modifications, providing protein and metabolite identifications from complex mixtures and characterising protein/protein interactions.

These methods allow the identification of complex protein sample composition; the identification of protein/protein interactions and characterisation of post-translational modifications (PTMs) such as phosphorylation in both complex samples and purified proteins. The method development has focused on optimisation of electron- capture dissociation (ECD) and electron transfer dissociation (ETD) methodologies for analysing PTMs. The EndoTrack studies showed that ECD/ETD can in particular be used to resolve multisite phosphorylation events in proteins and identify PTMs that were not detected using current methods. The increased coverage of protein PTMs allowed by these methods significantly increased our understanding of the extent and complexity of phosphorylation in endocytosis and signalling. In parallel they have also developed new algorithms and software for analysing Mass Spectroscopy data and large scale proteomics datasets.

These methods have been applied to the analysis of proteins involved in propagation of signals via the FGF receptor. They generated large scale datasets of protein phosphorylation events downstream of FGF signalling and, using chemical genomics approaches, identified those events which are direct or indirect substrates for effector kinases of the Src family. It was shown that amplification of FGF receptors – a common occurrence in tumours – involved recruitment of novel protein partners into the signalling pathway.

In collaboration with other EndoTrack partners the group of John Heath has applied their techniques to specific problems in collaborations with EndoTrack consortium partners: SMAD signalling; P3 on Protein kinase C substrates; characterisation of the Wnt exosome complex, and ALK partners.

3. Progress beyond the state-of-the-art and impact

3.1. Progress beyond the state-of-the-art

Establishing a new interface between two different fields: trafficking and signaling

At the start of the EndoTrack project the available knowledge of the intracellular itinerary of GFR complexes on the one hand and signaling mechanisms on the other hand were fragmentary. There were many reasons for this. In part, the trafficking and signaling fields have been conceptually separated. In addition, knowledge of the molecular mechanisms underlying endocytic trafficking and signaling has been limited. By combining different types of expertise and experimental system, EndoTrack aimed at bridging this gap and, through the convergence of experimental efforts and the combination of an unprecedented set of tools directly relating to endocytosis or GF biology. EndoTrack has generated unique basic knowledge on the mechanisms whereby cells decode GF stimuli and translate them into a response, thereby exploiting the spatio-temporal regulation provided by the endocytic pathway. The integration of experimental strategies has led to information that is superior in value to the sum of its individual components. Knowledge on the trafficking routes was essential to track the endocytic itinerary of GFR complexes. In turn, analysis of the GF transport revealed information on the trafficking routes. Qualitative and quantitative changes of such trafficking itineraries helped to explain cell and tissue-specific differences in signaling output. The direct regulatory interaction of signaling and trafficking can be best exemplified using the TGFbeta pathway. TGFbeta receptors signal via the effector Smad2, with the assistance of SARA, in early endosomes, whereas the same receptors, when taken-up into caveolae (no Smad2 and SARA), will be degraded via the combinatory action of Smad7 and Smurf.

EndoTrack has thus addressed issues fundamental to cell biology in an integrated manner using a number of novel and/or strongly integrative approaches. This project has achieved a progress beyond the state-of-the-art. This partnership of groups at leading research institutions and selected biotech SMEs has also delivered new enabling technologies and proof of principle for modulation of trafficking as a mode of therapeutic intervention in disease. EndoTrack has developed and optimized several innovative laboratory assays (at the bench and at the high throughput scale) to dissect the pathways and regulatory mechanisms of endocytosis, and assess their impact on the regulation of signaling. EndoTrack has performed a pioneer work in functional genomics to benefit our ability to understand (and combat) disease. An multidisciplinary technology pipeline has been developed integrating RNAi based functional genomics, automated screening protocols, imaging and image analysis using a proprietary software with the help of high performance computing. The designed assay protocols are amenable to both RNAi and small molecule screening. Since the genes responsible for each RNAi or knockout phenotype are known, the genomic screening technologies can be used for the identification and interpretation of defects resulting from small molecule hits. The results of such screen provide a gene specific fingerprint of phenotypes that can be compared and combined in several screen set-ups. The models selected are amenable to medium-scale genetic manipulation and provide advantages for investigating the mechanisms in both physiological and pathological settings.

The EndoTrack quantitative approach to analyse endocytosis and trafficking sets a new quality standard, previously rarely used in Cell Biology. This was possible due to the development of the quantitative multiparametric image analysis software (QMPIA, MotionTrack software) in the Max Planck Institute (MPI-CBG), Dresden and its distribution to all relevant partners (Collinet et al., Nature, 2010).

Unique features of the multiparametric quantitative image analysis:
A pipeline that starting from the raw data leads to the hits identification and classification, allowing a precise description of the phenotype of each gene upon knockdown in the specific assay. One essential feature to have a high content rather than just high throughput screening is to process the raw images and extract as much information as possible, to obtain a quantitative multiparametric description of every single gene knock-down.
To overcome the technical challenges the methods developed range from low level algorithms for data correction, processing and feature extraction, to higher level analysis for hits identification (hits scoring) and subdivision in phenotypic classes (clustering).
On the image processing side we have developed several innovative solutions:

- image correction for chromatic shift and non-even illumination;
- image segmentation for vesicles, nuclei and cells;
- feature extraction for quantifying endosome morphometry and position, cell shape, cell context, subcellular vesicle accumulation, colocalization as well as some quality control parameters;

- normalization procedures for correcting the plate-by-plate variability and condensation procedures for increasing the signal-to-noise ratio and gaining statistical significance.

A powerful statistical description of phenotypes can be produced in a quantitative fashion. The newly developed methods for cell segmentation and cell shape feature extraction allow:

- performing cell population studies to assess phenotypic variability and penetrance;
- to do gating on cell subpopulations to consider endocytic phenotypes for this specific group of cells (e.g. cells expressing a certain level of a transfected protein, or having certain shape features);
- to perform a correlative analysis between two different levels of complexity (cellular and subcellular) to highlight design principles of cross-regulation between cell morphology and the endocytic pathway

To eliminate RNAi off-target artifacts a multiparametric mode seeking method was developed, which retains phenotypic traits shared by several different oligonucleotides and suppresses the signal relative to non-conserved effect. In this way only conserved feature patterns will be included in the final estimated gene profile.

The analysis of multivariate high-throughput biological data is a growing area of bioinformatics that becomes more important with the advent of high-content techniques. The basic tool used to explore this kind of data is clustering, with an aim to identify the classes sharing common properties, such as sets of genes producing similar phenotype upon knockdown. However, commonly used clustering methods such as k-means, hierarchical clustering and related algorithms are not well-suited for exploratory data analysis as they require more or less direct parameterization of the desired number of clusters and impose restrictions on cluster shape and relative size that are often unrealistic. EdnoTrack has explored innovative data mining approaches to resolve the complex dataset structure in an unbiased manner. The applied density-based approach has been shown to provide a VT-shift performance on synthetic and real-world multiparametric cell-based screening datasets that is substantially superior to that of mean-shift, hierarchical and k-means clustering.

The work within EndoTrack provided strong evidence that the processes of endocytosis and signal transduction are mechanistically linked at multiple levels. Identification of endocytic routes of growth factors, the components regulating them and the methods to specifically perturb endocytic transport now pave the way to employ endocytic components to selectively affect certain signaling processes. Initially, the results obtained within EndoTrack are of primary importance for fundamental research, as they open several avenues for further investigation. Furthermore, the future impact on biotechnology industry is very likely.

ENDOTRACK has undertaken several research approaches that have significant impact in the field of ActivinA/TGFb signaling: the trafficking route of ActivinA receptors, its modulation, and in a series of high throughput screens, yeast -2-hybrid, and proteomic approaches the molecular machinery involved in some aspects of both signalling and trafficking has been analysed.

The work provided insight into the mechanisms of Wnt signaling, one of the most important growth factor pathways involved in development and human disease.
The EndoTrack project made it possible to monitor the dynamics of Wnt11 mediated Frizzled7 endocytosis and intracellular trafficking in individual cells within gastrulating zebrafish embryos. While there is considerable expertise in analyzing intracellular trafficking in culture cells, monitoring these processes in the living organism has not yet been clearly demonstrated. They have provided a clear example by monitoring Wnt11 triggered intracellular trafficking of Frizzled 7 with high spatial and temporal resolution in vivo and to analyze the modulation of trafficking by different genes functioning in this process such Arrestin and Ryk.
The in vivo studies of Wingless trafficking have shown that Wingless secretion requires retromer-dependent retrograde transport of Wls/Evi, a multipass transmembrane protein that transports Wnts from the Golgi to the plasma membrane. Important contributions have been made to understanding the role of lipid modifications on Wnt and two new conserved components of the secretory pathway have been identified.
EndoTrack research has generated exciting results that were not anticipated in the original proposal. While assessing the role of Arrow and Frizzled2 in Wingless degradation, the unexpected observation was made that some aspects of wing development can occur in the absence of Wingless. This led to the identification of a novel feedback mechanism that ensures robust interpretation of the Wingless gradient.

A dual function of endocytosis in morphogen gradient formation and its interpretation has been shown regarding FGF8 spreading. Importantly, the data suggest that these two functions are independent of each other, thus adding a so far unanticipated regulatory layer necessary for tissue patterning.
The FGF8 studies were performed in vivo, however, the newly developed tools together with quantitative analysis methodology allowed to reach a level of data quality that is normally only achieved in tissue culture.
Two research papers were published in the course of the EndoTrack work emphasising the fact that in the early Xenopus embryo long-range signalling occurs through an extracellular route, and therefore that different embryos and different tissues use different mechanisms to establish positional information.
The main findings regarding the importance of clustering and endocytosis for Wnt signaling, which will guide our future work in this direction, to characterize better Wnt signalosomes, their composition, their endocytic routes, their dynamics, their distribution within the cell.

The EndoTrack work has elucidated mechanisms that control PDGF receptor internalization and sorting to degradation versus recycling. These mechanisms have been shown to be important for the magnitude of signaling from the receptors. Evidence has also been obtained that this is important, e.g. in cells transformed by mutation of Ras, which is very common in human tumors. These findings may therefore be of importance for the development of improved methods for the diagnosis, prognosis and treatment of human tumors.

The development and application of ultra high resolution mass spectroscopy methods for protein fragmentation has greatly enhanced appreciation of the extent and complexity of protein phosphorylation as a biologically significant post translational modification. In particular the development of techniques to resolve isobaric peptides (i.e. those which have the same mass but differ in the site of phosphorylation) has revealed for the first time that proteins can exist in multiple alternative phosphorylated forms, which may have considerable biological significance. Method development withtin EndoTrack has benefited instrument manufacturers and led to further collaboration.

The development of associated software and modelling packages has also improved the accuracy and power of methods for analysing large scale proteomics datasets, post-translational modifications as well as modelling cell signalling and trafficking.

Applying these techniques to specific case studies we have generated large scale datasets of the FGF and PKC pathways. These studies have substantially increased our understanding of these biomedically important pathways and led to collaboration with the pharmaceutical industry. EndoTrack (group of John Heath) was the first to study the impact of pharmacological inhibition of members of the Src kinase family on cell signalling pathways using a differential phosphoprotemics approach. This is being taken forward with industry collaborators to analyse inhibition of this pathway using novel agents that are in preclinical or phase 1 trials.

The discovery that amplification of FGFR signalling, which occurs in about 15% of breast tumours, involves the recruitment of novel pathway components (the Jak STAT pathway) suggests both novel biomarkers of tumour classification and novel drug therapies for FGFR amplified tumours. These are being taken forward by collaboration with clinical collaborators.

Contribution of SMEs to the EndoTrack project
MSD
Current commercially available assays to measure ligands, receptors, and their interactions rely on Western blotting and co-immunoprecipitation data. These assays are typically not sufficiently sensitive and quantitative. Within EndoTrack MSD has developed assays that very sensitively and reproducibly measure different GFR ligands. Key to the success of developing such assays is the identification of the best possible pair of capture and detection reagents, followed by assay optimization. For biotin-ActivinA, as an example, this has led to an assay that can accurately measure as little as 160 pg/ml biotin-ActivinA from a 25 µl sample. This is considerably more sensitive than current Western blot detection techniques, and will allow reducing sample size significantly. Together with a very easy to use assay, this is expected to make large-scale screens possible that should allow identifying modulators of ActivinA internalization. The same is true for other ligands MSD has been developing assays for.
MSD has developed a large set of assays that are based on immunodetection of phosphoproteins using electrochemiluminescence. These assays have been made available to the consortium to address the role of endosomal/trafficking proteins in signaling events. Optimized phosphoprotein multiplexes serve the purpose of screening for modulators of signaling and trafficking. Current methodology would likely

rely on semi-quantitative Western blot analysis of just a few analytes. The MSD phosphoprotein multiplexes provide a quantitative readout of key components of several signaling pathways in parallel. This is expected to provide considerably more information than would be possible with current methodology.

IMAXIO provided the academic laboratories with original tools useful for exploring and validating new potential therapeutic targets in oncology, using its aptamers approach. More globally, IMAXIO demonstrated the potential of this approach in drug discovery to find new hits focusing on protein-protein interaction.

3.2. Impact

Strategic impact

EndoTrack project aimed at a dual objective:

- First, to fill a gap in the basic knowledge, by providing extensive **new insights** into the question how cells transduce extracellular stimuli in the form of polypeptide GFs to changes in gene expression exploiting the enormous potential of the spatio-temporal regulation provided by the endocytic pathway through **different endocytic routes**.
- Second, to develop **new concepts** that can create *in the future* novel opportunities for therapeutic intervention in human disease. The results of EndoTrack are specifically relevant for cancer and neurodegenerative diseases.

The **societal impact** of EndoTrack lies in the opportunity to **change the current textbook model** of how cytoplasmic cascades transduce proliferation and differentiation signals from the plasma membrane to the nucleus of cells. The currently proposed models hardly recognize the importance of endocytic trafficking routes and, when addressing this possibility, dramatically ignore their complexity at the subcellular level. EndoTrack has gained novel **mechanistic insights** into the regulation of signaling pathways and how these are **integrated** in a multi-GF environment with the fundamental principles of **cellular organization** and its overall significance in embryogenesis. Besides the benefits in basic knowledge, advances along this line also have implications for *future* improvement of **health care** in severe diseases resulting from dysfunction of signal transduction and gene expression.

The **economic impact** is **not immediate** but will be of outmost value in the middle and long-term. EndoTrack itself did not carry out screenings of small molecule libraries to identify novel potential drug candidates in disease model systems. Nevertheless, the discovery of new mechanistic principles and the development of the technology pipeline, EndoTrack has provided the **groundwork** necessary to develop novel opportunities for intervention. Specifically, these opportunities are based on the **following developments**:
(1) The development of new cell-based multi-parameter assays that can be scaled up for high-throughput screenings (96-well and 384-well plate format) and amenable to the screenings of chemical libraries; (2) The identification of a large number of novel key signaling regulators that can serve as potential drug targets; (3) The generation of new cellular and animal model systems that recapitulate different aspects of human disease (cancer and neurodegenerative diseases). (4) Proof-of-principle for the value of intervention via trafficking regulators to modulate signaling functions required for normal development and which are altered in human diseases. Altogether, EndoTrack has delivered an entirely novel technology platform with the potential to provide higher efficiency of drug development, hence improved cost effectiveness of health care and increased competitiveness of European biotech companies.

Innovation

EndoTrack was in Europe the first large-scale and systematic effort to understand how the endocytic routes are regulated at the molecular level and how they contribute to the regulation of the signal transduction process. Innovation provided by EndoTrack has added value in two aspects:

- The domain of research and development, where mechanistic aspects related to basic cell functions such as endocytosis and signaling were largely incomplete and had not been studied in basic biological processes at the organism level.
- The development of technologies that enable a systematic dissection of the aforementioned processes under physiological and pathological conditions and, thus, foster the generation of new diagnostic and therapeutic tools to improve the efficiency of new drug developments.

The delivered main innovation aspects are summarised in the following table:

Weaknesses of knowledge and technology addressed in EndoTrack	EndoTrack innovation
Limited knowledge of transport routes undertaken by GF-GFR complexes	Systematic analysis of endocytic routes of selected GF-GFR complexes (EGF, PDGF, ActivinA, Fgf8, Wnt)
Identification of endocytic routes lacks molecular regulation	Discovery and functional characterization of endocytic transport regulators via systematic RNAi-based functional genomics screen
Signalling pathways are not completely understood	Discovery and functional characterization of GFR signalling effectors via systematic HT-functional genomics screens
Endocytic and signalling pathways are poorly integrated and cross-compared between different GF families	Cross-comparison between different GF systems and integration with respect to endocytic routes
Lack of cross-comparison between different cell systems	Comparative analysis of GFs between different cell systems
Lack of cross-comparison between different animal model systems	Comparative analysis of GFs between different animal model systems
Lack of integration between basic cellular mechanisms and disease model systems	Proof of principle that mechanisms deduced from cell and animal models are effective in established disease model systems (cancer, Huntington's disease)
Technology is inadequate for the analysis of both endocytic trafficking and signaling downstream GF-GFR complexes	New technological developments in assay development, target identification and validation in cell and animal models
Lack of quantitation in the analysis of signaling and trafficking	Enabling technology for quantitative multiparametric image analysis and electrochemiluminescence-based assays

Contributions to standards

The activities of EndoTrack defined new standards with respect to quantitative parameters, morphological resolution and mechanistic properties of the intracellular routes of GF-GFR complexes with respect to their signaling activity. Defining standards and quality controls has been critical for the following developments:

Quality control of reagents: in order to obtain maximum reproducibility special care has been taken to evaluate the quality of the reagents used and, more specifically, to ensure the purity and to standardize parameters. This was particularly important for the high-throughput screenings developed in cellular systems

Development of in vitro assay systems involved (i) the precise definition of endocytic routes opposite to the general term of endocytosis, (ii) assessment and optimization of signal to noise, reproducibility, and high-volume screening applications over time, (iii) standardization of growth and treatment conditions for cell lines, which was especially critical for the high-throughput screening technology.

Development of in vivo assay systems: The obtained EndoTrack knowledge regarding one or more endocytic routes and signalling of one GFR system was validated using other models or systems.

Development of disease model systems: standardized mouse models for cancer and neurodegenerative diseases have been developed, which can be used for validation of candidate therapeutic agents.

4. Future perspectives

MSD has introduced their platform to the EndoTrack consortium and several partners have made use of it. Presentations, discussions and practical courses have led to new ideas on how to tackle difficult questions related to modulation of signaling and trafficking. Making use of the multitude of assays already available and with additional new assays it should be possible to get a more complete picture of what happens to different signaling pathways upon perturbation of the system.

In order to identify new genes involved in the regulation of Wnt11 controlled Frizzled7 endocytosis/recycling, a highly robust and reliable assay system needs to be established. One possibility is to generate stable cell lines expressing different fluorescently tagged Wnt ligands and Frizzled receptors – ideally under the control of several promotors with different strengths - and use these lines to set up a robust and reliable screening assay for Wnt induced Frizzled endocytosis.

The other main finding is the input that the cell cycle has via LRP6 phosphorylation on Wnt signaling. The unexpected coupling of Wnt receptor activation with the cell cycle raises new questions that will be important to address in the future. One of these will be to determine the degree of cooperation between different LRP6 kinases. This is particularly relevant because of the cooperativity between the different PPPSPxS sites as well as the apparent difference in their Wnt dependent phosphorylation. Wnt signaling output is known to be context dependent, and the balance of LRP6 kinases in a cell is likely to be a crucial factor, either at the level of abundance, activity or localization. Perhaps parallel kinase inputs are required to fully prime LRP6 at all five PPPSP sites and allow full signaling in response to Wnt. Also, how is LRP6 phosphorylation regulated in non-dividing cells?
Another aspect that remains to be analyzed in more detail is whether, among the multiple phosphorylation sites present in LRP6, there are ones that function in processes distinct from Axin binding and Wnt pathway activation.

G2/M specific transcription of Wnt target genes also needs to be looked at in more detail. At which subphase of G2-M does Wnt target gene transcription take place and to what extent is expression also occurring at other phases? Are the transcriptional responses to Wnt signaling different during different cell cycle phases?

The first systematic characterization of PDGF trafficking achieved within the consortium will be continued and extended. The studies within EndoTrack employed only the PDGF-BB form of this growth factor, so it is planned to include in future work other ligand versions (AA, AB, CC or DD) which activate different forms of receptors (ββ, αα, αβ). Their routes of internalization and endocytic trafficking are unknown and will be compared by measuring the rates of endocytosis and the colocalization with various markers of endosomal compartments. Such analyses will be also performed in various cell lines, especially in cancer-derived cells whose oncogenic potential depends on PDGF-PDGFRs. Moreover, changes in PDGF-dependent gene expression will be assayed upon downregulation or overexpression of selected proteins modulating the endocytosis of PDGF-PDGFR complexes. Such project will employ the combination of experimental approaches based on quantitative confocal microscopy, protein biochemistry, molecular biology and cell-based physiological assays, using the tools and assays elaborated during the EndoTrack. Its results are expected to bring novel insights into the problem of how endocytic trafficking and endosomal compartments contribute to the intracellular signal transduction elicited by PDGF.

One major aim is to decipher the trafficking mechanisms that ensure the spread of the Wingless morphogen within developing epithelia. In this context, we will continue to investigate the regulation and role of endocytosis. Specifically, we will continue to characterize the role of Arrow, as it is this receptor that directs Wingless to degradation. However, much of our future research will also focus on the issue of Wnt secretion. This is because there is a growing realization that understanding trafficking through the secretory pathway may hold the key to understanding how Wingless is packaged for export and long range transport. We will specifically investigate the role of Wls/Evi trafficking. We will track the behaviour of this receptor after it has reached the cell surface. To this end we will take advantage of transgenic BACs that express tagged versions of Wls/Evi (devised during the period of EndoTrack). This will be used for surface labeling followed by fluorescence and electron microscopy. The behaviour of tagged Wingless (again from transgenic BACs devised during EndoTrack) will be followed in parallel. This will be used to delineate the precise in vivo trafficking route both during secretion and endocytosis of Wingless. Finally, our future work will also involve characterizing exosome formation, both genetically and biochemically, and investigating their relevance to Wingless gradient formation.

An important question at this stage is where active signaling complexes are located along the endocytic route. In our current work we assume that the ligand also to a large extend marks active signaling complexes, however, this needs to be proven. The development of in vivo sensors for receptor activation will be a task of high priority for the future.

The proteomics programme has uncovered a wealth of new components in the endocytic trafficking and signalling pathway which are now appropriate for further scrutiny and experimental investigation. In particular the discovery of complex multisite phosphorylation events in some “node’ proteins such as Dok1 and EPs8 is a particular challenge as it suggest these act as ‘flow control’ logic gates in trafficking rather than passive scaffolds as previously assumed. It will be challenging to develop appropriate tools to address these issues.

Further technology development in Mass Spectroscopy is being pursued especially with regard to resolution of isobabric peptides. Theoretical calculations suggest that these may in fact dominate the proteome but have not be previously observed due to limitations in methods.

Further proteomics software development is being pursued especially with regard to the development of integrated workflows and database searching using machine learning and genetic algorithms.

Further proteomic studies of the FGF pathway are being pursued in cell systems in which the FGF pathway has clear biological significance. These include human embryonic stem cells, and tumours with somatic mutations in the FGF pathway.

Further proteomic studies are being pursued on evaluating novel pharmacological inhibitors of the Src pathway using differential phosphoproteomics.

Collaborative work developed under EndoTrack is being pursued by studies of the PDGF/PKC and ALK /SMAD pathways.

Future work will expand on what has been discovered for Activin by moving on to other members of the transforming growth factor beta family, especially BMPs. Preliminary results suggest that BMPs will differ from Activin and nodals: an exciting prospect.

During the ENDOTRACK program a wealth of information was gathered regarding the interplay of trafficking and signalling using ActivinA as a model system. However, there are many unexplored findings, which will be investigated in the coming years. The main areas can be summarised as follows:

- The screens for interacting proteins of both ActivinA receptors and also SARA have identified several proteins which, when validated, will open horizons both in stem cell biology, neurobiology and Cancer.

- The results from the High-throughput siRNA screen have indicted several kinases not previously implicated in ActivinA signalling –their role will be investigated in the next years with respect to signalling and trafficking.

IMAXIO get highly specific aptamers against various proteins involved in tumorigenesis: these aptamers could be used, after bioactivity confirmation, in a new peptide-based therapeutic approach against cancer. Besides, IMAXIO discovered hits able to break some protein-protein target interaction in 2H assays, which could be furtherevaluated in *in vitro* specific assays and chemically improved to be developed as drug candidates.

The findings that perturbations of mechanisms controlling internalization and sorting of PDGF receptors can affect the magnitude of signaling via the receptors raise the question whether such perturbations occur in human tumors. Such studies should involve continued search for components of the sorting mechanisms and their function, as well as analyses of human tumor materials.

Another aspect that deserves to be explored is that not only the quantity of signaling via the receptors is affected by perturbation of sorting mechanisms, but also the quality of signaling. Thus, individual pathways might be enhanced or suppressed depending on where in the cell the activated receptor is located.

5. Lessons learned

The work within EndoTrack revealed and confirmed basic principles of mutual relationships between the processes of endocytosis and signal transduction, which occur for various signaling ligands and receptors at different intracellular levels. Endocytosis is important for the spatio-temporal regulation of signaling processes and several proteins involved in endocytic trafficking appear to modulate signal transduction at the same time. Therefore, they represent a potential target for the concomitant modulation of both processes, currently under experimental conditions with future potential for therapeutic approaches.

The main difficulty encountered concerned the generation of probes and tools enabling precise tracking of internalized growth factors. For certain growth factors, their biophysical properties (e.g. strong unspecific adhesion to glass or plastic, as in the case of PDGF, in purifying Wingless, generating functional tagged versions) make the development of such tools very difficult. For some others, the production of active recombinant molecules presents a challenge. In many cases, the available sensitivity of biochemistry- or microscopy-based detection methods imposes the use of higher than physiological concentrations of growth factors. The lack of suitable antibodies at the outset of EndoTrack was also a bottleneck. Nevertheless, even such imperfect experimental models (e.g. cultured cells treated with recombinant growth factors) allow describing the principles and identifying the pathway components for further tests in model organisms and disease models.

It has become clear that studies of the regulation of growth factors/receptors trafficking are dependent on the access to a number of different methods and technologies, such as RNAi screens, proteomic screens, ability to determine posttranslational modifications by mass spectrometry, techniques for transfection of genes and knockdown of genes in tissue culture, advanced microscopy techniques, and cell culture and animal models that can be used to monitor effects of perturbations of trafficking events, e.g. in cancer. Collaborations with laboratories with different skills are therefore a rate limiting.

The future EndoTrack work on the various signaling and trafficking pathways is strongly influenced by the lessons and techniques they have learned through EndoTrack and they expect long lasting collaborations. It was essential for achieving the EndoTrack aims to be able to redirect the research in order to take full advantage of actual developments and generated results.

6. Final plan for using and disseminating knowledge

All the dissemination and exploitation activities as well as a list of project exploitable results are described in the Appendix 1.

The dissemination of Endotrack knowledge took place at three levels:

a) Within the Endotrack Consortium.
The aim here was to inform the consortium of new findings of scientific interest, to discuss the progress of the project, the modifications needed and to plan future steps. The dissemination within the Endotrack consortium took place during internal meetings (Kick-off meeting in Dresden, 2005, 2. General assembly meetings in Athens, 2006, Midterm Review Meeting in Cracow, 2008, General Assembly meeting in Gent, 2009, and the EndoTrack Conference in Il Ciocco, 2009), training activities (Athens, Greece 2006, Leuven, Belgium 2007, Cambridge, UK 2007, Birningham, UK 2007, Dresden, Germany 2008, Leuven, Belgium 2010), and telephone conferences (June 2006, fall 2007, June 2009, October 2009, November 2009, March 2010, May 2010, June 2010, August 2010, September 2010 with the Steering Committee members). In addition, partners attended the same meetings.
The **EndoTrack Conference** ("On the Tracks of Signaling", November 2009, Il Ciocco, Italy) provided a great opportunity to meet the Endotrack consortium, to discuss further interaction and the generated data.

The **internal EndoTrack web page** was set up July 31, 2006 and continuously further developed as well as frequently updated. The aim of the page was and is to provide all the necessary and relevant information to the Endotrack partners concerning documents and guidelines, meetings, contact dates, reports and finances. It provides training material and information on the different training activities coming up within the project. It is up dated regularly by the project management and serves the partners also as a reference site for administrative issues.

The url for the internal web page is: www.Endotrack.org.

Furthermore, three different databases are established during the first two years of the project. The databases were described in more detail in the previous report.
A forum for students and postdocs within Endotrack has also been implemented in which they can discuss whatever topic among each other and ask questions. All these tools are meant to facilitate the communication between the single groups, to share the knowledge and results generated within Endotrack and to support and strengthen collaborations.

b) To the scientific Community
The dissemination to the scientific community aims at strengthening and reinforcing the European research activities by multiplication and initiation of networking and collaborations beyond the consortium. The consortium is publishing in international peer review journals (see publication list) and presenting Endotrack at national and international conferences (see also the attendance list of conferences in Appendix 1).
EndoTrack Conference, "On the Track of Signalling", November 9-11, 2009, Il Ciocco, Lucca-Tuscany, Italy, Organizers: Danny Huylebroeck and Marino Zerial. The scientific conference provided the opportunity to present the EndoTrack results to the broader scientific community and to establish further collaborations and interactions.

c) To the public
The Ednotrack consortium considers it as one of the major tasks to inform the potential stakeholders and the interested public of the scientific activities that are going on in the local and European research area. A press release has been sent in English and German to the "Generalverwaltung" of the Max Planck Society in the first funding period. The Max Planck Society is in charge of distributing the press releases of all the Max Planck institutes throughout Germany and Europe. The project's public web page was also set up and published within the first year. It aims at spreading awareness of the ongoing scientific activities in the local and European research area. It informs the public on the project's aims, summarizes the work that is to be done and provides information on administrative issues such as job openings and

contact dates. Also listed are meetings of the Endotrack consortium open for the public and published literature.
The url of the Endotrack web page is: www.endotrack.org which has also been mentioned in the press release.
The project management has made a **poster** that describes the goals of Endotrack, explains endocytosis and lists the different approaches Endotrack is taking to achieve its goal. This poster has been circulated to all partners and is presented at public events at the different partner institutes.

See Appendix 1 for more details.

The project manager is responsible for the implementation of the dissemination activities including the maintenance and updating of the existing web page for the public as well as the internal one for the Endotrack consortium.

Knowledge and technology transfer

Action has been taken to create a resource to translate the advances in basic science generated by EndoTrack into applications. This is the mission of the MPI-CBG Screening Facility called HT-Technology Development Studio (TDS) and has been established with the aim of preparing it for setting the ground of a future Biotech company.

The TDS played a key role in establishing and developing the technology pieline for high throughput and high content cell-based image analysis screening for the Endotrack consortium. The major goal of the central Screening Facility / TDS is to provide expertise in assay development towards high throughput applications and state-of-the-art technology for cell-based screening by automated microscopy. The Screening Facility / HT-Technology Development Studio (TDS) is an early-stage operation in Dresden hosted by the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG). The TDS is aiming to meet the necessary requirements to become shortly a “Center of Excellence” in the area of post-genomic screening and technology development for the economical benefit of the region. Innovative next-generation cell-based assays mainly at sub-cellular level are developed starting at the bench experiment scale of our academic partners (Max Planck Institutes, Technical University Dresden) towards validated high-content assays capable for high-throughput screening. The TDS is actively driving the technology development in high-content analysis by automated confocal microscopy, electronic pattern recognition and image analysis, electrochemiluminescence-based detection technology as well as in application of RNA interference in genome-wide scales together with our industrial partners Evotec Technologies, Definiens, Meso Scale Discovery, MSD and Cenix BioScience. The TDS is integrating such methods with chemical compound screening, gene expression analysis and generation of target-mutant model organisms by TILLING to achieve rapidly in-depth functional characterization of genes and identification of initial chemical compounds exhibiting pharmaceutical potential. The transfer of technology from academia to pharmaceutical industry will be accelerated as well as opportunities for assay development and high-content screening will be offered to future customers.

7. ENDOTRACK partners and contact

Contractors involved

- Marino Zerial, Max Planck Institute of Molecular Cell Biology and Genetics, MPI-CBG, Germany
- Carl-Phillipp Heisenberg, Max Planck Institute of Molecular Cell Biology and Genetics, MPI-CBG, Germany
- Danny Huylebroek, Laboratory of Molecular Biology (Celgen), Belgium
- Carl-Henrik Heldin, Ludwig Institute for Cancer Research, LICR, Sweden
- Christof Niehrs, Deutsches Krebsforschungszentrum, DKFZ, Germany
- Marta Miaczynska, International Institute of Molecular and Cell Biology, IIMCB, Polen
- Rüdiger Klein, Max Planck Institute of Neurobiology, MPIN, Germany
- Jean-Paul Vincent, National Institute for Medical Research, NIMR/MRC, Great Britain
- Michael Brand, Technische Universität Dresden, TUD, Germany
- John Heath, University of Birmingham, UBHAM, Great Britain
- Jim Smith, University of Cambridge UCAM/WCRUK, Great Britain
- Carol Murphy, Foundation for Research and Technology, Hellas Biomedical Research Institute, BRI, Greece
- Jérôme Denis, Imaxio SA, France
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Web page	www.EndoTrack.org	Project logo	Endotrack

Appendix 1

Plan for using and disseminating the knowledge

Section 1: Exploitable knowledge and its use

Dissemination of knowledge and technology to the research community

Scientific publications in peer-reviewed journals

2009-2010

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Presentations at Conferences and other institutions in 2009/2010

Conference name/location	Date	Your Institute	Title	Authors	Presentation (Talk/Poster/ Lecture)	Audience	Website address
FASEB Summer Research Conference on Glucose Transporter and Diabetes Lucca, Italy	6 - 11 September 2009	P1A MPI-CBG	"Systems analysis of endocytosis"	M. Zerial	Invited lecture	International scientists, students	http://src.faseb.org
EMBO Conference Series on Membrane Dynamics in Endocytosis: Macromolecular Complexes in Control of Cell Signalling and Tissue Morphogenesis Chania, Crete, Greece	3 - 8 October 2009	P1A MPI-CBG	"System analysis of endocytosis by functional genomics and quantitative multi-parametric image analysis"	M. Zerial		International scientists, students	http://www.embo.org/events/calendar/conference-series.html
Colloque inaugural du nouvel Institut Jacques Monod	20 October 2009	P1A MPI-CBG	"Systems analysis of endocytosis and signaling"	M. Zerial		International scientists	

Paris, France							
EC FP6 EndoTrack Conference "On the Tracks of Signalling" Lucca, Italy	9 - 11 November 2009	P1A MPI-CBG		Claudio Collinet, M. Zerial		International scientists	
hSR-MDC Joint PhD Student meeting Stresa, Italy	11 - 13 November 2009	P1A MPI-CBG	"System Analysis of Endocytosis"	M. Zerial		International scientists	
8th EMBO/Annaberg Workshop Goldegg, Austria	12 – 17 January 2010	P1A MPI-CBG	"System Analysis of Endocytosis by Functional Genomics and Quantitative Multi-Parametric Image Analysis"	M. Zerial		International scientists	
Biomedicum & Viikki Biocenter; Helsinki, Finnland	6 April 2009	P1a MPI-CBG	System Survey of Endocytosis by Functional Genomics and Quantitative Multi-Parametric Image Analysis	M. Zerial	Seminar	International scientists	

Conference on "Biochemical interactions: From molecules to function"; Velen, Germany	22 – 24 April 2009	P1a MPI-CBG	From systems biology to synthetic biology: Reconstitution of Rab- and SNARE-dependent membrane fusion by synthetic endosomes	M. Zerial	Invited oral presentation	International scientists	
Institute of Biotechnology; Prague, Czech Republic	29 May 2009	P1A MPI-CBG	System Survey of Endocytosis by Functional Genomics and Quantitative Multi-Parametric Image Analysis	M. Zerial	seminar	International scientists	
Alnylam Pharmaceuticals; Cambridge, MA, USA	9 June 2009	P1A MPI-CBG	From systems biology to synthetic biology: Reconstitution of Rab and SNARE-dependent membrane fusion by synthetic endosomes	M. Zerial	seminar	International scientists	
MIT; Cambridge; MA; USA	10 June 2009	P1A MPI-CBG	High Throughput Investigation of Cell Biology	M. Zerial	seminar	International scientists	
Interdisciplinary and Interinstitutional Neuroscience Seminar Series,	Nov.26, 2010	P2 VIB		Danny Huylebroeck	Seminar	International scientists	

Program in Neuroscience, Charité Medical University, Berlin, Germany (coords. C. Rosenmund and S. Sigrist)							
University of Birmingham (Dr. J.K. Heath)	Oct. 21, 2010	P2 VIB		Danny Huylebroeck	Seminar	International scientists	
IGBMC/ICS (Institute of Genetics and Molecular and Cellular Biology & Mouse Clinical Institute), Strasbourg-Illkirch, France (Dr. Y. Hérault)	May 5, 2010	P2 VIB		Danny Huylebroeck	Seminar	International scientists	
Gordon Research Conference on "Craniofacial Morphogenesis and Tissue Regeneration", Lucca/Il Ciocco, Italy	Apr.11-16, 2010	P2 VIB		Danny Huylebroeck	invited keynote lecture	International scientists	

(org. P. Trainor)							
1st International Workshop on "Modern Trends in BMP signaling", Berlin, Germany (P. Knaus, P. Seemann, D. Graf, orgs)	Sept.6-9, 2009	VIB		Danny Huylebroeck	(invited speaker; chair of session)	International scientists	
A*STAR Institute for Medical Biology, Singapore (Dr. M. Jones, Dr. A. Colman)	Mar. 23, 2009	P2 VIB		Danny Huylebroeck	Seminar	International scientists	
Developmental Biology program, Temasek Life Sciences Laboratory, Singapore (Dr. S. Cohen)	Mar. 20, 2009	P2 VIB		Danny Huylebroeck	Seminar	International scientists	
Dept Biological Sciences and Biotechnology, Tsinghua University, Beijing, China (Dr.	Mar.16-18, 2009	P2 VIB		Danny Huylebroeck	Seminar	International scientists	

Y-G. Chen)							
Meeting of the EC-FP6-TMR Network Epiplastcarcinoma (coord.: C. Hill), Leuven	Mar.12-13, 2009	P2 VIB	Insights into Sip1's cell autonomous and non-autonomous actions in ES cells and mouse embryos	Danny Huylebroeck	invited lecture	International scientists	http://www.epiplastcarcinoma.org/
de Duve Institute, Université Catholique de Louvain (UCL), Brussels (Dr. A. Goffinet)	Feb. 19, 2009	P2 VIB		Danny Huylebroeck	Seminar	International scientists	
171st Annual meeting of the Netherlands Anatomy Society, parallel symposium on "Signalling and stem cells in development and disease", Lunteren, The Netherlands, (Orgs.: M. de Ruiter, C. Mummery)	Jan. 9-10, 2009	P2 VIB		Danny Huylebroeck	invited lecture	International scientists	

5th International Meeting of the Stem Cell Network NRW	24-25 March 2010	German Cancer Research Center (DKFZ)	Role of Gadd45 in demethylation	C. Niehrs	Talk	International scientists	http://www.kongress.stammzellen.nrw.de/
ASIP 2009 Annual Meeting at Experimental Biology New Orleans, USA	18-22 April 2010	German Cancer Research Center (DKFZ)	Wnt signalling events at the plama membrane	C. Niehrs	Talk	International scientists	http://bscdb.ugent.be/index.php?id=next meeting
Seminar at the University of Virginia, USA	24 April 2010	German Cancer Research Center (DKFZ)	Wnt signalling events at the plama membrane	C. Niehrs	Talk	International scientists	http://www.asip.org/mtgs/eb09/
Embo Gold Medalist Workshop, Singapore	2-6 May 2010	German Cancer Research Center (DKFZ)	Wnt signalling events at the plama membrane	C. Niehrs	Talk	International scientists	http://www.virginia.edu/biology/
Seminar an der Tsinghua University, Department of Biological Sciences,	26 May 2010	German Cancer Research Center	Wnt signalling events at the plama membrane	C. Niehrs	Lecture	International scientists	

Beijing, China		(DKFZ)					
Santa Cruz Developmental Biology Meeting	30.6.2010	German Cancer Research Center (DKFZ)		C. Niehrs	Lecture	US, International scientists Devel. biologists	
Spetses Summer School	30-31 March 2010	German Cancer Research Center (DKFZ)		C. Niehrs	Lectur, tutorial	International PhD students, postdocs	
National Institute for Medical Research	23.09.2009	UCAM P10		Jim Smith	Lecture	International PhD students	
Institute of Cancer Research	26.11.2009	UCAM P10		Jim Smith	Lecture	International PhD students	
Cancer Research UK	27.11.2009	UCAM P10		Jim Smith	Lecture	International PhD students	
King's College London	16.11.2009	UCAM P10		Jim Smith	Lecture	International PhD students	
Hinxton Hall (MRC-LMCB Retreat).	01.02.2010	UCAM P10		Jim Smith	Lecture	International PhD students	

Institute of Medical Sciences, Aberdeen	03.02.2010	UCAM P10		Jim Smith	Lecture	International PhD students	
11th Chinese Conference on Cell Biology & Xi'an International Conference on Cell Biology 2009	July 5-7, 2009	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	International scientists	
German Society for Genetics Annual Meeting	September 16 to 19, 2009	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	International scientists	
EMBO Gold Medalist Workshop, Singapore	04/05/09	NIMR	Upstream and Downstream No-tail: Making Mesoderm in the Zebrafish Embryo	Prof. Jim Smith	Workshop	International scientists	
Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland on "Optical Techniques in Cell and Developmental Biology"/Dresden	29 November-1 December 2009	P5 IMCB	Tracking endocytosis of growth factors by quantitative confocal microscopy"	Marta Miaczynska	Invited oral presentation	International scientists	
EndoTrack International Conference "On the	9-11 November 2009	P5 IMCB	"Endosomal adaptors as regulators of signaling and transcription: the case of	Marta Miaczynska	Invited oral presentation	International scientists	

tracks of signalling"/Il Ciocco			APPL proteins"				
"Endocytic machineries in control of cell signalling and tissue morphogenesis"/Chania	3-8 October 2009	P5 IMCB	"Endosomal adaptors as regulators of signaling and transcription: the case of APPL proteins"	Marta Miaczynska	Invited oral presentation	International scientists	
36th Winter School of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University: Molecule interactions in health and disease / Zakopane	21-26 February 2009	P5 IMCB	"Endocytosis and the regulation of signal transduction – the case of APPL proteins"	Marta Miaczynska	Invited oral presentation	International scientists	
Gordon Research Conference on Lysosomes & Endocytosis / Andover, NH, USA	June 20-25, 2010	P5 IMCB	"Endocytic proteins in the regulation of transcription"	Marta Miaczynska	Invited oral presentation	International scientists	

Meeting of the Belgian Society of Cell and Developmental Biology. Leuven, Belgium	29-31 March 2009	NIMR (P7)	Control of positional information and growth by Wingless	Jean-Paul Vincent	Talk	International scientists	http://bscdb.ugent.be/index.php?id=next meeting
Endotrack meeting, Gent	25-27 June 2009	NIMR (P7)		Jean-Paul Vincent	Talk	International scientists	http://www.endotrack.org/
European "Wnt Meeting", Arolla, Switzerland	26-30 August 2009	NIMR (P7)		Jean-Paul Vincent	Talk	International scientists	http://wnt2009.epfl.ch/
European Drosophila meeting, Nice	18-21November 2009	NIMR (P7)		Jean-Paul Vincent	Talk	International scientists	
Meeting of the British Soc for Cell Biology Warwick, UK	12-15 April 2010	NIMR (P7)		Jean-Paul Vincent	Talk	International scientists	http://www.bscb.org/
Meeting of the Belgian Society of Cell and Developmental Biology/ Leuven	29-31 March 2009	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	

European "Wnt Meeting", Arolla	26-30 August 2009	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
European Drosophila meeting/ Nice	18-21 November 2009	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
UK Membrane trafficking symposium / London	14 December 2009	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
Meeting of the Israeli Society of Developmental Biology / Tel Aviv	23 Feb 2010	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
Meeting of the British Soc for Cell Biology Warwick	12-15 April 2010	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
Endocyte workshop /London	26-28 May 2010	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
Universite Libre de Bruxelles	15 oct 2009	NIMR (P7)		Jean-Paul Vincent	Scientific seminar	International scientists	
University of Porto	23 Oct 2009	NIMR (P7)		Jean-Paul Vincent	Scientific seminar	International scientists	

Pasteur-Curie Cell Biology course	3 Feb 2010 17 Jan 2011	NIMR (P7)		Jean-Paul Vincent	Lecture/discussion (Pasteur)	International Post-graduate students	
Symposium of the PhD program "Institute of Biomembranes" of the Graduate school of Life Sciences of Utrecht University	29-30 October 2009	NIMR (P7)		Jean-Paul Vincent	Lecture/discussion (Utrecht)	International Post-graduate students	
Endocyte workshop /London	26-28 May 2010	NIMR (P7)		Jean-Paul Vincent	Lecture	International scientists	
Macromelecular complexes / Frankfurt	June 5-6, 2009	LICR P15		C.-H. Heldin	Lecture	International scientists	
EMBO Gold Medalist workshop / Singapore	May 4-6, 2009	LICR P15		C.-H. Heldin	Lecture	International scientists	
From cell signaling to medical systems biology / Turku	June 11-12, 2009	LICR P15		C.-H. Heldin	Lecture	International scientists	
2009 EMBO Molecular medicine workshop /Torino	Sept 10-12, 2009	LICR P15		C.-H. Heldin	Lecture	International scientists	

Signal transduction in health and disease /Aachen	Sep 27-30, 2009	P15 LICR LICR P15		C.-H. Heldin	Lecture	International scientists	
Targeted cancer therapy/ Heidelberg	May 10-11, 2010	LICR P15		C.-H. Heldin	Lecture	International scientists	
EndoTrack International Conference "On the tracks of signalling", Il Ciocco	9-11 November 2009	IIMCB P5, P1a, P15	"Multifunctional adaptor protein APPL1 as an interacting partner of histone deacetylases and the nucleosome-remodeling complex NuRD" "Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) by microscopy"	Beata Pyrzynska, Magdalena Banach-Orlowska, Iwona Pilecka, Anna Torun, Marta Miaczynska Łukasz Sadowski, Patrick Keller, Yannis Kalaidzidis, Carina Hellberg, Carl-Henrik Heldin, Marta Miaczynska	Poster presentation	International scientists, students	
Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland on "Optical Techniques in Cell and Developmental Biology", Dresden	29 November-1 December 2009	IIMCB P5, P1a, P15	"Multifunctional adaptor protein APPL1 as an interacting partner of histone deacetylases and the nucleosome-remodeling complex NuRD"	Beata Pyrzynska, Magdalena Banach-Orlowska, Iwona Pilecka, Anna Torun, Marta Miaczynska Łukasz Sadowski, Patrick Keller, Yannis	Poster presentation	International scientists	

			"Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) by microscopy"	Kalaidzidis, Carina Hellberg, Carl-Henrik Heldin, Marta Miaczynska			
EMBO Conference "Endocytic machineries in control of cell signalling and tissue morphogenesis", Chania	3-8 October 2009	IIMCB MPI-CBG P1A, P5	"Features and Functions of APPL Endosomes"	Anna Hupalowska, Marta Olchowik, Yannis Kalaidzidis, Marino Zerial, Marta Miaczynska	Poster presentation	International scientists	
EMBO Conference "Endocytic machineries in control of cell signalling and tissue morphogenesis", Chania, Greece and Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland on "Optical	(3-8 October 2009) (29 November-1 December 2009)	IIMCB P5	"APPL proteins as components and regulators of histone deacetylase-containing complexes"	Anna Torun, Magdalena Banach-Orlowska, Sajid Rashid, Iwona Pilecka, Beata Pyrzynska, Marta Miaczynska	Poster presentation	International scientists	

Techniques in Cell and Developmental Biology", Dresden, Germany							
The EMBO Meeting 2009, Amsterdam, the Netherlands and Gordon Research Conference on Signal Transduction Within The Nucleus, Ventura, California (USA)	29 August-1 September 2009 29 March-3 April 2009	IIMCB	Endosomal adaptor proteins APPL1 and APPL2 are novel activators of β-catenin/TCF-mediated transcription"	Sajid Rashid, Iwona Pilecka, Anna Torun, Marta Olchowik, Beata Bielinska, Marta Miaczynska	Poster presentation	International scientists	
EMBO Conference on Cellular Signaling and Molecular Medicine, Cavtat (Dubrovnik), Croatia	21-26 May 2010	IIMCB P5, P1A, P15	"Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) by microscopy"	Łukasz Sadowski, Patrick Keller, Yannis Kalaidzidis, Carina Hellberg, Carl-Henrik Heldin, Marta Miaczynska	Poster presentation	International scientists	
2nd Vesicular Transport and Cancer Workshop, Turku, Finland	7-9 June 2010	IIMCB P5	"A role of endocytic proteins APPL1 and APPL2 in the regulation of glioblastoma cell growth"	Beata Pyrzynska, Marta Teperek, Magdalena Banach-Orlowska, Katarzyna Miekus, Grazyna	Poster presentation	International scientists	

				Drabik, Marcin Majka, Marta Miaczynska			

On the Track of Signaling, The EndoTrack International Conference, Il Ciocco Italy	11-13 Nov 2009	Endotrack consortium and external speakers	For details and title of presentations see Appendix 3	Endotrack consortium and external speakers	Talks and posters	International scientists	www.endotrack.org

Presentations at Conferences and other institutions in 2007/2008

Conference name/location	Date	Your Institute	Title	Authors	Presentation (Talk/Poster/ Lecture)	Audience	Website address
International Symposium in Developmental Neurobiology on TGFβs: Signaling and Roles in Neural Development, Maintenance and Disease, Heidelberg University (org. Dr. K. Unsicker)	Sept. 20-23, 2007	VIB	"Functional analysis of TGFbeta signaling in the central and peripheral nervous systems	Danny Huylebroeck (invited speaker)	Talk	Internat.scientists	http://www.izn.uni-heidelberg.de/
ESF Meeting on Stem Cell Niches, Barcelona	Jan. 10-12, 2008	VIB	Smad-interacting proteins as versatile regulators of embryonic cell fate, differentiation and function	D. Huylebroeck	Invited speaker	Stem cell scientists	http://stemcells.idibell.org/?p=program
7th International Conference on Bone Morphogenetic Proteins, Lake Tahoe, CA, USA	Jul. 9-13, 2008	VIB	Smads and partners: from stem cell function and differentiation to disease	D. Huylebroeck	Invited speaker	Developmental biologists and regenerative medicine	http://www.bmpconferences.org/

171st Annual meeting of the Netherlands Anatomy Society, parallel symposium on "Signalling and stem cells in development and disease", Lunteren, The Netherlands	Jan. 9-10, 2009	VIB	Smads and partner proteins in the regulation of mouse development	D. Huylebroeck	Invited speaker	Developmental biologists, medical docters	http://www.anatomen.nl (click Meeting 2009 for the program)
Cold Spring Harbor meeting "The Ubiquitin family", Cold Spring Harbor, NY	April 25-29 2007	LICR-UPP	ALIX INVOLVEMENT IN C-CBL-PDGFRβ COMPLEX REGULATES RECEPTOR DOWNREGULATION"	Piotr Wardega	Talk or poster	International Scientists	http://meetings.cshl.edu/meetings/ubiq07.shtml
The Norwegian Cancer Symposia: Intracellular Transport and Signal Transduction in Cancer Biomedicine, Stalheim, Norway	May 19-23 2007	LICR-UPP	Loss of T-cell protein tyrosine phosphatase induces recycling of the PDGF beta-receptor but not the PDGF alpha-receptor	Carina Hellberg	Talk	international scientists	http://www.radium.no/stalheim2007/
2007 winter meeting of the Norwegian Biochemical Society	Feb 1-4 2007	LICR-UPP	Signal transduction via receptors for PDGF and TGFβ- possible targets in	C-H Heldin invited speaker	Talk	international scientists	

Lillehammer, Norway			tumor therapy				
5th International Aachen Symposium on cytokine signalling, Aachen, Germany	March 29-31 2007	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets for tumor therapy	C-H Heldin invited speaker	Talk	international scientists	http://www.uk aachen.de/go/ changelangua ge?ID=49495 33&DV=3&C OMP=page& NAVID=4949 533&NAVD V=3
Annual meeting of the American Association for Cancer Research, Los Angeles, USA	April 14-18, 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	http://www.aa cr.org/home/s cientists/meeti ngs-- workshops/pr evious- annual- meetings/annu al-meeting- 2007.aspx
Annual meeting of the American Society for Biochemistry and Molecular Biology,	April 28-30 2007	LICR-UPP	Signal transduction via PDGF receptors	C-H Heldin invited speaker	Talk	international scientists	

Washington, USA							
Federation of American Societies for Experimental Biology meeting on Growth factor Receptor Tyrosine kinases in Mitogenesis, Morphogenesis and Tumorigenesis, Tucson, USA	Aug 11-16 2007	LICR-UPP	Targeting PDGF receptors in cancer	C-H Heldin invited speaker	Talk	international scientists	
International meeting on the tumor-vessel interface, Kloster Seeon, Germany	Sept 22-25 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	http://www.angiogenese.de/Index_english.html
VIII Sao Paulo Research Conference, Sao Paulo, Brazil	November 12-14, 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	
4th International conference on Ubiquitin, Ubiquitin-like proteins and	Feb 7-9, 2008	LICR-UPP		Carina Hellberg	Poster	international scientists	http://www.mdanderson.org/featured_sites/sentrin/

cancer, Houston, USA							
4th International Ubiquitin, Ubiquitin-like Proteins and Cancer Conference, Houston, USA	7-9 Feb. 2008	LICR-UPP	Activation of PKC induces recycling of the PDGF β-receptor	C. Hellberg, S. Karlsson, C. Schmees and Carl-Henrik Heldin.	poster presentation		
ELSO Meeting on Frontiers of cellular, developmental and molecular biology, Nice, France	August 20th – September 2nd 2008	LICR-UPP	At the crossroads – Differential trafficking of PDGF receptor isoforms	Schmees C, Hellberg C, Karlsson S, Heldin CH.	poster presentation		
EMBO Meeting on Cellular Signalling and Molecular Medicine Cavtat/Dubrovnik, Croatia	May 29th – June 4th 2008	LICR-UPP	At the crossroads – Differential trafficking of PDGF receptor isoforms	Schmees C, Karlsson S, Hellberg C, Heldin CH.	poster presentation		
Eight ESH Conference on Angiogenesis, Paris, France	May 9-11, 2008	LICR-UPP	Effects of PDGF antagonists on tumor angiogenesis and stroma	C-H Heldin	invited speaker		
Symosium at Center of	May 15 2008	LICR-UPP	Molecular mechanisms for signaling via receptors for	C-H Heldin	invited speaker		

Biotechnology, Oslo, Norway			PDGF and TGF-β				
Protein phosphorylation Meeting, La Jolla, USA	August 15-18, 2008	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets in tumor treatment	C-H Heldin	invited speaker		
Molecular Mechanisms and Mouse Models in Cancer, Amsterdam, Holland	November 27-28, 2008	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets in tumor therapy	C-H Heldin	invited speaker		
DKFZ-ZMBH Alliance Inauguration Meeting, Heidelberg, Germany	December 5, 2008	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets in tumor therapy	C-H Heldin	invited speaker		
Seminar, University of Bergen, Norway	January 24, 2008	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets in tumor therapy	C-H Heldin	invited speaker	Norwegian scientists	
Seminar, University of Tromsö, Norway	August 27, 2008	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets in tumor therapy	C-H Heldin	invited speaker	International PhD course	

8th International Conference of Anticancer Research, Kos, Greece	17-22 October 2008		*HD-PTP IS IMPORTANT REGULATOR OF PDGFRβ DEGRADATION*	Piotr Wardega, Johan Lennartsson	poster		
Freie Universität Berlin, Germany	10 October, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	German scientists	http://www.fu-berlin.de/en/index.html
Stowers Institute for Medical Research Kansas City, Missouri USA	5 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	US scientists	http://www.stowers-institute.org/
UK London Cancer Research Institute London UK	7 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	UK scientists	http://www.london-research-institute.org.uk/
Epigenome Networks in Mammalian Development, Medical Research Institute, Tokyo Japan	11-15 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.tmd.ac.jp/mri/English/mri_top-e.html

Workshop „From developmental biology to tissue regeneration“ Santiago Chile	8-11 January, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.usach.cl/
IBMS Davos Workshop: Bone Biology & Therapeutics, Davos Switzerland	9-14 March, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.ibmsonline.org/Meetings/IBMSDavos/tabid/74/Default.aspx
Meeting „Molecular Cell Dynamics: from signaling to organ formation“ Münster Germany	15-18 May, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.ibmsonline.org/Meetings/IBMSDavos/tabid/74/Default.aspx
Russian-European workshop on Epigenetic Mechanisms and DNA Repair St. Petersburg Russia	end of June, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	

German society for research on DNA repair: major meeting, Berlin, Germany	2-5 September, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.dna-rep-net.de/html/conferences.html
12th International Xenopus Conference, Leiwen/Trier, Germany	8-12 September, 2008	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.xenbase.org/community/static/12th_Xenopus_conf.jsp

Cancer Research UK - London Research Institute Room 626 44 Lincoln's Inn Fields London, UK	11 May 2007	NIMR	Trafficking of the Wingless signal in developing epithelia	Jean-Paul Vincent	Talk	UK scientists	http://www.london-research-institute.co.uk/
Department of human anatomy Univ of Oxford	25 May 2007	NIMR	Trafficking of the Wingless signal in developing epithelia	Jean-Paul Vincent	Talk	UK scientists	
Institut de Biologie du Développement de Marseille-Luminy	15 June2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	French scientists	http://www.ibdml.univ-mrs.fr/

FRANCE							
Centre for Developmental Genetics Sheffield England	6 July 2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	UK scientists	http://cdbg.shef.ac.uk/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function	12-14 Sept 2007	NIMR	Secretion of Wingless requires the retromer complex	Jean-Paul Vincent	Talk (selected speaker)	International scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
Journées Annuelles des Drosophilistes Francophones Nice	10-13 Oct2007	NIMR	Secretion of Wingless requires the retromer complex	Jean-Paul Vincent	Talk (selected speaker)	International scientists	http://www-cbd.ups-tlse.fr/congres/droso2007/index.php
EMBO Members Meeting in Barcelona	26-29 Oct2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	International scientists	<http://www.embo.org/members_meeting07/.>http://www.embo.org/members_meeting07/

Department of Biology University of York United Kingdom	3 Dec 2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	UK scientists	http://www.york.ac.uk/depts/biol/
Endocytosis-exocytosis' meeting Batz sur Mer France	31 May-3June 2008	NIMR	Trafficking of Wingless in epithelia	Jean-Paul Vincent	Talk (invited speaker)	International scientists	
Institut für Neurobiologie Badestr. 9 D-48149 Münster Germany	12 June 2008	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	German scientists	<http://www.uni-muenster.de/Biologie.NeuroVer/Neuro/welcome.html>http://www.uni-muenster.de/Biologie.NeuroVer/Neuro/welcome.html
EMBO Workshop on the MOLECULAR AND DEVELOPMENTAL BIOLOGY OF DROSOPHILA Crete	22-28 June 2008	NIMR	Interpretation of the Wingless gradient	Jean-Paul Vincent	Talk	International scientists	<http://flybase.org/data/news/announcements/meetings/crete/>http://flybase.org/data/news/announcements/me

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Institut für Neurobiologie Münster, Germany	13 June 2008	NIMR (P7)	Formation and interpretation of the Wingless gradient in developing epithelia	Jean-Paul Vincent	Lecture	Students and scientists	http://www.uni-muenster.de/Biologie.NeuroVer/
The Sixteenth International Workshop on the Molecular and Developmental Biology of Drosophila, Crete	22-28 June 2008	NIMR (P7)	Interpretation of the Wingless gradient	Jean-Paul Vincent	Talk	Principal investigators	http://flybase.org/data/news/announcements/meetings/crete/
Gradients and Signalling: from chemotaxis to development. Okinawa, Japan	November 17-21, 2008	NIMR (P7)	Control of positional information and growth by Wingless	Jean-Paul Vincent	Talk	Students and scientists	http://www.irp.oist.jp/signalling08/
Weatherall Institute, Oxford	8 Dec 2008	NIMR (P7)	Control of positional information and growth by Wingless	Jean-Paul Vincent	Lecture	Students and scientists	http://www.imm.ox.ac.uk/
Curie Institute, Paris	15 Jan 2009	NIMR (P7)	Control of positional information and growth by Wingless	Jean-Paul Vincent	Lecture	Students and scientists	http://www.curie.fr/recherche/themes/detail_unites.cfm/lang/_fr/id/54.

							htm
Cells into organs' EU Grant (Symposium oganisation by 2 participants of Claudio Stern and Antonio Jacinto), Lisbon, Portugal	31 August 2007	UCAM/WCRUK	Identifying T-targets	Jim Smith (invited speaker)	Talk	International scientists	www.**cellsintoorgans**.net
UMR, CNRS, Paris, France	18 September 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.**parisgeo**.**cnrs**.fr
Erasmus MC (Symposium on Stem Cells), Royal Tropical Institute, Amsterdam	1 October 2007	UCAM/WCRUK	Making mesoderm: intercellular signals and transcriptional responses	Jim Smith (invited speaker)	Talk	International scientists	www.**erasmusmc**.n
SARS, International Centre for Marine Molecular Biology, Bergen, Norway	15 October 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.**sars**.no/about
EMBL, Heidelberg, Germany	14 November 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.**embl**-**heidelberg**.de/
Endotrack Internal Workshop, Cambridge, UK	5-7 December 2007	UCAM/WCRUK	Introduction to training workshop	Jim Smith (co-organiser)	Introduction talk	International scientists of Endotrack	www.**endotrack**.org

						grant	
Institute of Molecular and Cell Biology, Singapore	13 February 2007	UCAM/WCRUK	T-box genes: genetic regulatory networks	Jim Smith (invited speaker)	Talk	International scientists	www.imcb.a-star.edu.sg/
BSDB Meeting, Warwick, UK	31/3/08 – 3/4/08	UCAM/WCRUK	TBA		Invited speaker	International scientists	http://www.bms.ed.ac.uk/services/websp ace/bsdb/WELCOME.HTM
EU Grant "Cells into Organs" Plenary Meeting, Amsterdam	8-10 April 2008	UCAM/WCRUK	Attendance at meeting only			International scientists participating in EU grant	www.cellsintoorgans.net
Endotrack Review Meeting, Poland	16-18 April 2008	UCAM/WCRUK	Attendance at meeting only			International scientists participating in EU grant	www.endotrack.org
12th International Xenopus Conference, Germany	8-12 September 2008	UCAM/WCRUK	TBA		Invited speaker	International scientists	www.uni-ulm.de/12thxenopus
Institute for Stem Cell Research, University of Edinburgh	23/05/08	NIMR	Mesoderm induction revisited	Prof. Jim Smith	Seminar	Edinburgh	

MRC Centre for Developmental Neurobiology King's College London	04/07/08	NIMR	Zebrafish gradients revisited	Prof. Jim Smith	Seminar	London	
University of Oxford	01/12/08	NIMR	Mesoderm induction revisited	Prof. Jim Smith	Seminar	Oxford	
European Zebrafish conference, Amsterdam, Netherlands	12.-15. July, 2007	TUD Biotec	Cellular Mechanisms controlling spreading of Fgf protein in the early CNS primordium	M. Nowak, M. Kolandzyk, Marta Luz, Michael Brand	poster	Internat.scientists	http://www.zebrafish2007.org/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function, Villars-sur-Ollon, Switzerland	Sept. 18 – 23, 2007	MPI-CBG	Role of endocytosis for mesoderm cell migration during zebrafish gastrulation	Carl-Philipp Heisenberg (invited speaker)	Talk	Internat. scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html

EMBO/FEBS/ISF Workshop on Systems Dynamics Of Intracellular Communication - Overcoming Distance In Signalling Networks, Maale HaChamisha, Jerusalem Hills, Israel	March18 – 22, 2007	MPI-CBG	Functional genomics studies of endocytosis and signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.bionorth.org.il/BioNorth/Templates/showpage.asp?TMID=100&FID=120&PID=4450&IID=5018
2007 ASBMB Annual Meeting Washington DC	April 28 - May 2, 2007,	MPI-CBG	Unraveling the design principles of endocytosis and signaling using multi-parametric image analysis	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.asbmb.org/ASBMB/site.nsf/web/A822CEF502DE9513852571B1005CE7B6
EMBO/FEBS/ISF Workshop on Systems Dynamics Of Intracellular Communication - Overcoming Distance In Signalling Networks, Maale HaChamisha,	March18 – 22, 2007	MPI-CBG	Genome-wide Quantitative Image Analysis of Endocytosis and Signaling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.weizmann.ac.il/conferences/spatial2006/index.html

Jerusalem Hills, Israel							
The Norwegian Cancer Symposia: Intracellular Transport and Signal Transduction in Cancer Biomedicine, Stalheim, Norway	May 19 – 23, 2007	MPI-CBG	Regulation of endocytic membrane trafficking by Rab GTPases	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.radium.no/stalheim2007/
32nd FEBS Congress, Vienna, Austria	July 7 – 12, 2007	MPI-CBG	Genome-wide Quantitative Image Analysis of Endocytosis and Signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.febs2007.org/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function, Villars-sur-Ollon, Switzerland	Sept. 18 – 23, 2007	MPI-CBG	Molecular Design Principles of Endosome Transport and Signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
2008 Keystone Symposium on Molecular Basis for Biological Membrane Organization, Big Sky, Montana, USA	Jan. 12 – 17, 2008	MPI-CBG	Genome-Wide Survey of Endocytosis by Quantitative Multi-Parametric Image Analysis	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.keystonesymposia.org/Meetings/ViewMeetings.cfm?MeetingID=925

2008 Hunter Cell Biology Meeting, Pokolbin NSW, Australia	April 1 – 4, 2008	MPI-CBG	to be decided	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://hcbm.mtci.com.au/
EMBO Meeting on Cellular Signaling & Molecular Medicine, Dubrovnik, Croatia	May 29 - June 4, 2008	MPI-CBG	to be decided	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.dubrovnik-conference.org
FASEB SRC 2008 on Small GTPases, Saxtons River, VT, USA	July 13 – 18, 2008	MPI-CBG	Rab5 and the Endocytic Pathway	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://src.faseb.org/
German Conference on Bioinformatics 2008, Dresden, Germany	Sept. 9 – 12, 2008	MPI-CBG	to be decided	Marino Zerial (keynote speaker)	Talk	Internat. scientists	http://www.biotec.tu-dresden.de/gcb2008
Joint Meeting of the British and Spanish Developmental Biology Societies, Seville/ Spain	September 24-27	MPI-CBG	Tissue mechanics during zebrafish gastrulation	CP Heisenberg (invited speaker)	Talk	Developmental and Cell Biologists	
Barcelona BioMed Conference Morphogenesis and Cell Behavior, Barcelona/ Spain	October 6-8	MPI-CBG	Tissue mechanics during zebrafish gastrulation	CP Heisenberg (invited speaker)	Talk	Developmental and Cell Biologists	

Santa Cruz Developmental Biology Meeting, Santa Cruz/ USA	June 26-29	MPI-CBG	Tissue mechanics during zebrafish gastrulation	CP Heisenberg (invited speaker)	Talk	Developmental and Cell Biologists	
9th International Neuroscience Winter Conference, Sölden, Austria	March 24-29, 2007	MPIN	Bi-directional Eph/ephrin signaling in axon guidance and synaptic plasticity	Rüdiger Klein	Keynote Lecture	Internat. scientists	http://inwc.sambax.com/?site=home
Gordon Research Conference on neurotrophic factors, Newport, RI, USA	June 17-22, 2007	MPIN	Eph and Ret receptor signaling during axonal guidance and growth	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	http://www.grc.org/programs.aspx?year=2007&program=neurtrop
FASEB Summer Research Conference on Growth factor receptor tyrosine kinases in mitogenesis, morphogenesis and tumorigenesis, Tucson, Arizona, USA	August 11 – 16, 2007	MPIN	Bi-directional eph/ephrin signaling in axon guidance"	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	https://secure.faseb.org/faseb/meetings/Summrconf/Programs/11589.pdf
EMBO WORKSHOP Common Regulatory Mechanisms in Haemopoiesis and Neurogenesis,	October 3-5, 2007	MPIN	Ephrin/Eph signaling during remodeling of neuronal and vascular networks	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	www.embo.org

Heidelberg. Germany							
Wings for Life-meeting, Salzburg,	April 28 - May 4, 2008	MPIN	Axon guidance events regulated by the bi-directional Eph/ephrinB signaling system	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	
Brain Development Symposium 2008 Regents College, London	12-13 May 2008	MPIN	Repulsive cell-cell communication regulating the wiring of the nervous system	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	
Gordon Research Conference on Growth Factors and Signaling, Magdalen College, Oxford, United Kingdom	August 3-7, 2008	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	http://www.grc.org/programs.aspx?year=2008&program=growth
Keystone Symposium, Axonal Connections: Molecular Cues for Development and Regeneration, Keystone, Colorado, USA	Feb 17 - 22, 2009	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	www.keystonesymposia.org.
EMBL PhD Symposium 'Decision making in Biology', EMBL Heidelberg,	October 23-25, 2008	MPIN	Molecular mechanisms of repulsive axon guidance	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	

Germany							
A Century of Axon Navigation Research An international symposium, Uppsala University, Uppsala, Sweden	December 5, 2008	MPIN	Eph-ephrin signalling during axon guidance	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	
11th Chinese Conference on Cell Biology & Xi'an International Conference on Cell Biology 2009	July 5-7, 2009	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	
German Society for Genetics Annual Meeting	September 16 to 19, 2009	MPIN	Eph receptor signaling in neuronal development and plasticity	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	
First collaboration day of Lyonbiopole (Lyon, France)	Octobre 12, 2007	Imaxio	New anticancer drugs	Jerome Pansanel	Poster	French scientists	
Second collaboration day of Lyonbiopole (Grenoble, France)	Octobre 24, 2008	Imaxio	New anticancer drugs	Jerome Pansanel	Poster	French scientists	
ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	APPL-positive endosomes: a biochemical and microscopical characterization	Marta Miaczynska (invited speaker)	talk	International scientists	http://www.elso.org/index.php?id=elso2007

ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	Transport of endocytic cargo through an APPL compartment.	Anna Hupalowska, Marta Brewinska, Jochen Rink, Yannis Kalaidzidis, Anna Zarebska, Patrick Keller, Marino Zerial, Marta Miaczynska	poster	International scientists	http://www.elso.org/index.php?id=elso2007
ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	An electrochemiluminescence (ECL) assay for detection of internalized biotinylated PDGF-BB.	Łukasz Sadowski, Patrick Keller, Carina Hellberg, Marino Zerial, Marta Miaczynska	poster	International scientists	http://www.elso.org/index.php?id=elso2007
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function; Villars-sur-Ollon (Switzerland)	September 18-23, 2007	IIMCB	Endocytic Cargo Transport via an APPL-positive Compartment.	Marta Brewinska, Anna Hupalowska, Jochen Rink, Yannis Kalaidzidis, Anna Zarebska, Patrick Keller, Marino Zerial, Marta Miaczynska	poster	International scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
Meeting of Polish Research Network on Cell Motility, Warsaw, Poland	October 25-26, 2007	IIMCB	The role of endosomes in signal transduction: a link through APPL proteins	Marta Miaczynska (invited speaker)	talk	Polish scientists	

Dept. of Biochemistry, The Norwegian Radium Hospital, Oslo (Norway)	30 October 2007	IIMCB	Endocytosis and signaling: a link through APPL proteins	Marta Miaczynska (invited speaker)	talk	Department members	
MPI-CBG/IMG Trilateral Workshop for Young Scientists from Germany, Czech Republic and Poland, Prague, Czech Republic	November 15-18 2007	IIMCB	Signaling from Endosomes to the Nucleus: the Role of APPL Proteins	Marta Miaczynska (invited speaker)	talk	Scientists from Germany, Czech Republic and Poland	
EMBO Meeting on Cellular Signaling and Molecular Medicine Dubrovnik, Croatia	29 May - 4 June 2008	IIMCB	Endocytic cargo transport via an APPL-positive compartment	Marta Miaczynska (selected speaker)	Talk	International scientists	
Meeting of HHMI International Scholars; Lisbon, Portugal	19-22 June 2008	IIMCB	The dual role of APPL proteins in the regulation of endocytic transport and signal transduction	Marta Miaczynska	Talk	HHMI International Scholars	
The Congress of Biochemistry and Cell Biology, Olsztyn, Poland	7-11 September 2008	IIMCB	Endocytosis – mechanisms and a role in signal transduction	Marta Miaczynska (invited speaker)	Talk	Polish scientists	

EMBO Meeting: At the interface of cell biology and cellular microbiology Villars-sur-Ollon, Switzerland	20-25 September 2008	IIMCB	The dual role of APPL proteins in the regulation of endocytic transport and signal transduction	Marta Miaczynska (invited speaker)	Talk	International scientists	
Gordon Research Conference on Growth Factors and Signaling, Magdalen College, Oxford, UK	3-8 August 2008	IIMCB	Tracking the endocytic pathways of internalized platelet-derived growth factor (PDGF) by microscopy and electrochemiluminescence assays	L Sadowski, P Keller, Y Kalaidzidis, C Hellberg, C-H Heldin, M Miaczynska	Poster	International scientists	
The Golgi Meeting 2008, Pavia, Italy	4-9 September 2008	IIMCB	Transport of endocytic cargo through an APPL-positive compartment	M Olchowik, A Hupalowska, J Rink, Y Kalaidzidis, A Urbanska, P Keller, M Zerial, M Miaczynska	Poster	International scientists	
The Golgi Meeting 2008, Pavia, Italy	4-9 September 2008	IIMCB	APPL-positive endosomes, a biochemical characterization	A Urbanska, M Miaczynska	Poster	International scientists	
University of Liverpool Physiological Society	14.2.08	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Physiologists and cell biologists	
CRUK Annual Researchers	20.9.07	UBHAM	FGF signaling in Cancer	John K. Heath (invited speaker(	Talk	Physiologists and cell	

conference. Birmingham						biologists	
Norwegian Radiumhospital Cancer Centre Oslo	17.6.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Cancer biologists and clinicians	
Paterson Institute Manchester	30.1.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Cancer scientists	
University of York	15.1.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk		
Microsoft Converging Sciences"University of Trento	9.1.07	UBHAM	Process calculus for modeling signal transduction	John K. Heath (invited speaker(	Talk	Computer scientists	
17th International Mass Spectrometry Conference, Prague	August 2006,	UBHAM	On-line Data-dependent Electron Capture Dissociation of Phosphotyrosine Peptide	Sweet, Cunningham, Creese, Heath, Cooper	Poster presentation		
4th Uppsala International Conference on Electron Capture and Transfer Dissociation Mass Spectrometry- Fundamentals and	December 2006	UBHAM	Neutral Loss Triggered ECD for the Identification of Sites of Phosphorylation in Proteins	" Sweet, Creese, Cooper	Oral presentation		

Applications Hong Kong							
4th Proteomics Methods Forum Meeting Liverpool	,June 2007	UBHAM	The Role of ETD/ECD in Phosphorylation Analysis	Sweet, Cunningham, Creese, Cooper	Oral presentation		
BSPR-EBI Meeting "Integrative Proteomics: from molecules to systems, Cambridge	July 2007,	UBHAM	Identification of phosphorylation-dependent protein interactions	Sweet, Ryan, Fares, Payne, Heath	Poster presentation		
BMSS 29th Annual Meeting, Edinburgh	September 2007	UBHAM	Electron capture dissociation in phosphoproteomics	Sweet, Cunningham, Cooper	Poster presentation		
Research potential support program, University of Ioannina Medical School, Ioannina, Greece	5 - 6th October, 2007	BRI	ERBIN is a novel SARA-interacting protein on early endosomes that negatively regulates TGF-β/Activin signaling	George Sflomos	Talk	Greek Scientists	
59th National Conference of Biochemistry and Molecular Biology, Athens, Greece	December 7-9th, 2007	BRI	ERBIN is a novel SARA-interacting protein on early endosomes that negatively regulates TGF-β/Activin signaling	George Sflomos, Eleftherios Kostaras, Ekaterina Panopoulou, Nikos Pappas, Theodore Fotsis and Carol Murphy	Talk	Greek Scientists	http://www.eebmb.gr/el/index.html

ESF Meeting on Stem Cell Niches Barcelona	Jan. 10-12, 2008	VIB		Danny Huylebroeck	invited speaker		

Presentations at Conferences and other institutions in 2007

Conference name/location	Date	Your Institute	Title	Authors	Presentation (Talk/Poster/Lecture)	Audience	Website address
International Symposium in Developmental Neurobiology on TGFβs: Signaling and Roles in Neural Development, Maintenance and Disease, Heidelberg University (org. Dr. K. Unsicker)	Sept. 20-23, 2007	VIB	"Functional analysis of TGFbeta signaling in the central and peripheral nervous systems	Danny Huylebroeck (invited speaker)	Talk	Internat.scientists	http://www.izn.uni-heidelberg.de/
Cold Spring Harbor meeting "The Ubiquitin family", Cold Spring Harbor, NY	April 25-29 2007	LICR-UPP	ALIX INVOLVEMENT IN C-CBL-PDGFRβ COMPLEX REGULATES RECEPTOR DOWNREGULATION"	Piotr Wardega	Talk or poster	International Scientists	http://meetings.cshl.edu/meetings/ubiq07.shtml

Conference name/location	Date	Your Institute	Title	Authors	Presentation (Talk/Poster/Lecture)	Audience	Website address
The Norwegian Cancer Symposia: Intracellular Transport and Signal Transduction in Cancer Biomedicine, Stalheim, Norway	May 19-23 2007	LICR-UPP	Loss of T-cell protein tyrosine phosphatase induces recycling of the PDGF beta-receptor but not the PDGF alpha-receptor	Carina Hellberg	Talk or poster	international scientists	http://www.radium.no/stalheim2007/
2007 winter meeting of the Norwegian Biochemical Society Lillehammer, Norway	Feb 1-4 2007	LICR-UPP	Signal transduction via receptors for PDGF and TGFβ- possible targets in tumor therapy	C-H Heldin invited speaker	Talk	international scientists	
5th International Aachen Symposium on cytokine signalling, Aachen, Germany	March 29-31 2007	LICR-UPP	Signaling via receptors for PDGF and TGFβ- possible targets for tumor therapy	C-H Heldin invited speaker	Talk	international scientists	http://www.ukaachen.de/go/changelanguage?ID=4949533&DV=3&COMP=page&NAVID=4949533&NAVDV=3
Annual meeting of the American Association for Cancer Research, Los Angeles, USA	April 14-18, 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	http://www.aacr.org/home/scientists/meetings--workshops/pre

							vious-annual-meetings/annual-meeting-2007.aspx
Annual meeting of the American Society for Biochemistry and Molecular Biology, Washington, USA	April 28-30 2007	LICR-UPP	Signal transduction via PDGF receptors	C-H Heldin invited speaker	Talk	international scientists	
Federation of American Societies for Experimental Biology meeting on Growth factor Receptor Tyrosine kinases in Mitogenesis, Morphogenesis and Tumorigenesis, Tucson, USA	Aug 11-16 2007	LICR-UPP	Targeting PDGF receptors in cancer	C-H Heldin invited speaker	Talk	international scientists	
International meeting on the tumor-vessel interface, Kloster Seeon, Germany	Sept 22-25 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	http://www.angiogenese.de/Index_english.html
VIII Sao Paulo Research Conference, Sao Paulo, Brazil	November 12-14, 2007	LICR-UPP	Singaling via receptors for PDGF and TGFβ-possible targets in tumor treatment	C-H Heldin invited speaker	Talk	international scientists	

Freie Universität Berlin, Germany	10 October, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	German scientists	http://www.fu-berlin.de/en/index.html
Stowers Institute for Medical Research Kansas City, Missouri USA	5 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	US scientists	http://www.stowers-institute.org/
Conference name/location	Date	Your Institute	Title	Authors	Presentation (Talk/Poster/Lecture)	Audience	Website address
UK London Cancer Research Institute London UK	7 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	UK scientists	http://www.london-research-institute.org.uk/
Epigenome Networks in Mammalian Development, Medical Research Institute, Tokyo Japan	11-15 December, 2007	DKFZ	Mechanisms of Wnt signaling	Christof Niehrs	Talk	International scientists	http://www.tmd.ac.jp/mri/English/mri_top-e.html
Cancer Research UK - London Research Institute Room 626 44 Lincoln's Inn Fields	11 May 2007	NIMR	Trafficking of the Wingless signal in developing epithelia	Jean-Paul Vincent	Talk	UK scientists	http://www.london-research-institute.co.uk/

London, UK							
Department of human anatomy Univ of Oxford	25 May 2007	NIMR	Trafficking of the Wingless signal in developing epithelia	Jean-Paul Vincent	Talk	UK scientists	
Institut de Biologie du Développement de Marseille-Luminy FRANCE	15 June2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	French scientists	http://www.ibdml.univ-mrs.fr/
Centre for Developmental Genetics Sheffield England	6 July 2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	UK scientists	http://cdbg.shef.ac.uk/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function	12-14 Sept 2007	NIMR	Secretion of Wingless requires the retromer complex	Jean-Paul Vincent	Talk (selected speaker)	International scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
Journées Annuelles des Drosophilistes Francophones Nice	10-13 Oct2007	NIMR	Secretion of Wingless requires the retromer complex	Jean-Paul Vincent	Talk (selected speaker)	International scientists	http://www-cbd.ups-tlse.fr/congres/droso2007/index.php

EMBO Members Meeting in Barcelona	26-29 Oct2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	International scientists	<http://www.embo.org/members_meeting07/.>http://www.embo.org/members_meeting07/
Department of Biology University of York United Kingdom	3 Dec 2007	NIMR	Formation and interpretation of the Wingless gradient	Jean-Paul Vincent	Talk (invited speaker)	UK scientists	http://www.york.ac.uk/depts/biol/
Cells into organs' EU Grant (Symposium oganisation by 2 participants of Claudio Stern and Antonio Jacinto), Lisbon, Portugal	31 August 2007	UCAM/WCRUK	Identifying T-targets	Jim Smith (invited speaker)	Talk	International scientists	www.cellsintoorgans.net
UMR, CNRS, Paris, France	18 September 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.**paris**geo.**cnrs**.fr
Erasmus MC (Symposium on Stem Cells), Royal Tropical Institute, Amsterdam	1 October 2007	UCAM/WCRUK	Making mesoderm: intercellular signals and transcriptional responses	Jim Smith (invited speaker)	Talk	International scientists	www.erasmusmc.n
SARS, International Centre for Marine	15 October 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.**sars**.no/about

Molecular Biology, Bergen, Norway							
EMBL, Heidelberg, Germany	14 November 2007	UCAM/WCRUK	Making mesoderm: signals and responses	Jim Smith (invited speaker)	Talk	International scientists	www.embl-heidelberg.de/
Endotrack Internal Workshop, Cambridge, UK	5-7 December 2007	UCAM/WCRUK	Introduction to training workshop	Jim Smith (co-organiser)	Introduction talk	International scientists of Endotrack grant	www.endotrack.org
Institute of Molecular and Cell Biology, Singapore	13 February 2007	UCAM/WCRUK	T-box genes: genetic regulatory networks	Jim Smith (invited speaker)	Talk	International scientists	www.imcb.a-star.edu.sg/
European Zebrafish conference, Amsterdam, Netherlands	12.-15. July, 2007	TUD Biotec	Cellular Mechanisms controlling spreading of Fgf protein in the early CNS primordium	M. Nowak, M. Kolandzyk, Marta Luz, Michael Brand	poster	Internat.scientists	http://www.zebrafish2007.org/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function, Villars-sur-Ollon, Switzerland	Sept. 18 – 23, 2007	MPI-CBG	Role of endocytosis for mesoderm cell migration during zebrafish gastrulation	Carl-Philipp Heisenberg (invited speaker)	Talk	Internat. scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html

EMBO/FEBS/ISF Workshop on Systems Dynamics Of Intracellular Communication - Overcoming Distance In Signalling Networks, Maale HaChamisha, Jerusalem Hills, Israel	March18 – 22, 2007	MPI-CBG	Functional genomics studies of endocytosis and signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.bionorth.org.il/BioNorth/Templates/showpage.asp?TMID=100&FID=120&PID=4450&IID=5018
2007 ASBMB Annual Meeting Washington DC	April 28 - May 2, 2007,	MPI-CBG	Unraveling the design principles of endocytosis and signaling using multi-parametric image analysis	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.asbmb.org/ASBMB/site.nsf/web/A822CEF502DE9513852571B1005CE7B6
EMBO/FEBS/ISF Workshop on Systems Dynamics Of Intracellular Communication - Overcoming Distance In Signalling Networks, Maale HaChamisha, Jerusalem Hills, Israel	March18 – 22, 2007	MPI-CBG	Genome-wide Quantitative Image Analysis of Endocytosis and Signaling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.weizmann.ac.il/conferences/spatial2006/index.html

The Norwegian Cancer Symposia: Intracellular Transport and Signal Transduction in Cancer Biomedicine, Stalheim, Norway	May 19 – 23, 2007	MPI-CBG	Regulation of endocytic membrane trafficking by Rab GTPases	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.radium.no/stalheim2007/
32nd FEBS Congress, Vienna, Austria	July 7 – 12, 2007	MPI-CBG	Genome-wide Quantitative Image Analysis of Endocytosis and Signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.febs2007.org/
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function, Villars-sur-Ollon, Switzerland	Sept. 18 – 23, 2007	MPI-CBG	Molecular Design Principles of Endosome Transport and Signalling	Marino Zerial (invited speaker)	Talk	Internat. scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
9th International Neuroscience Winter Conference, Sölden, Austria	March 24-29, 2007	MPIN	Bi-directional Eph/ephrin signaling in axon guidance and synaptic plasticity	Rüdiger Klein	Keynote Lecture	Internat. scientists	http://inwc.sambax.com/?site=home
Gordon Research Conference on neurotrophic factors, Newport, RI, USA	June 17-22, 2007	MPIN	Eph and Ret receptor signaling during axonal guidance and growth	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	http://www.grc.org/programs.aspx?year=2007&program=neurtrop

FASEB Summer Research Conference on Growth factor receptor tyrosine kinases in mitogenesis, morphogenesis and tumorigenesis, Tucson, Arizona, USA	August 11 – 16, 2007	MPIN	Bi-directional eph/ephrin signaling in axon guidance"	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	https://secure.faseb.org/faseb/meetings/Summrconf/Programs/11589.pdf
EMBO WORKSHOP Common Regulatory Mechanisms in Haemopoiesis and Neurogenesis, Heidelberg. Germany	October 3-5, 2007	MPIN	Ephrin/Eph signaling during remodeling of neuronal and vascular networks	Rüdiger Klein (invited speaker)	Talk	Internat. scientists	www.embo.org
First collaboration day of Lyonbiopole (Lyon, France)	Octobre 12, 2007	Imaxio	New anticancer drugs	Jerome Pansanel	Poster	French scientists	
ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	APPL-positive endosomes: a biochemical and microscopical characterization	Marta Miaczynska (invited speaker)	talk	International scientists	http://www.elso.org/index.php?id=elso2007
ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	Transport of endocytic cargo through an APPL compartment.	Anna Hupalowska, Marta Brewinska, Jochen Rink, Yannis Kalaidzidis, Anna Zarebska, Patrick	poster	International scientists	http://www.elso.org/index.php?id=elso2007

				Keller, Marino Zerial, Marta Miaczynska			
ELSO Meeting 2007 Dresden, Germany	September 1-4, 2007	IIMCB	An electrochemiluminescence (ECL) assay for detection of internalized biotinylated PDGF-BB.	Łukasz Sadowski, Patrick Keller, Carina Hellberg, Marino Zerial, Marta Miaczynska	poster	International scientists	http://www.elso.org/index.php?id=elso2007
EMBO/FEBS Workshop on Endocytic Systems: Mechanism and Function; Villars-sur-Ollon (Switzerland)	September 18-23, 2007	IIMCB	Endocytic Cargo Transport via an APPL-positive Compartment.	Marta Brewinska, Anna Hupalowska, Jochen Rink, Yannis Kalaidzidis, Anna Zarebska, Patrick Keller, Marino Zerial, Marta Miaczynska	poster	International scientists	http://www.unige.ch/sciences/biochimie/MeetingsAndWorkshops/WorkshopOnEndocyticSystems.html
Meeting of Polish Research Network on Cell Motility, Warsaw, Poland	October 25-26, 2007	IIMCB	The role of endosomes in signal transduction: a link through APPL proteins	Marta Miaczynska (invited speaker)	talk	Polish scientists	
Dept. of Biochemistry, The Norwegian Radium Hospital, Oslo (Norway)	30 October 2007	IIMCB	Endocytosis and signaling: a link through APPL proteins	Marta Miaczynska (invited speaker)	talk	Department members	
MPI-CBG/IMG Trilateral Workshop for Young Scientists	November 15-18 2007	IIMCB	Signaling from Endosomes to the Nucleus: the Role of APPL Proteins	Marta Miaczynska (invited speaker)	talk	Scientists from Germany,	

from Germany, Czech Republic and Poland, Prague, Czech Republic						Czech Republic and Poland	
University of Liverpool Physiological Society	14.2.08	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Physiologists and cell biologists	
CRUK Annual Researchers conference. Birmingham	20.9.07	UBHAM	FGF signaling in Cancer	John K. Heath (invited speaker(	Talk	Physiologists and cell biologists	
Norwegian Radiumhospital Cancer Centre Oslo	17.6.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Cancer biologists and clinicians	
Paterson Institute Manchester	30.1.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk	Cancer scientists	
University of York	15.1.07	UBHAM	Systems Biology of FGF signaling	John K. Heath (invited speaker(	Talk		
Microsoft Converging Sciences"University of Trento	9.1.07	UBHAM	Process calculus for modeling signal transduction	John K. Heath (invited speaker(	Talk	Computer scientists	
17th International Mass Spectrometry Conference, Prague	August 2006,	UBHAM	On-line Data-dependent Electron Capture Dissociation of Phosphotyrosine Peptide	Sweet, Cunningham, Creese, Heath, Cooper	Poster presentation		

4th Uppsala International Conference on Electron Capture and Transfer Dissociation Mass Spectrometry-Fundamentals and Applications Hong Kong	December 2006	UBHAM	Neutral Loss Triggered ECD for the Identification of Sites of Phosphorylation in Proteins	” Sweet, Creese, Cooper	Oral presentation		
4th Proteomics Methods Forum Meeting Liverpool	,June 2007	UBHAM	The Role of ETD/ECD in Phosphorylation Analysis	Sweet, Cunningham, Creese, Cooper	Oral presentation		
BSPR-EBI Meeting “Integrative Proteomics: from molecules to systems, Cambridge	July 2007,	UBHAM	Identification of phosphorylation-dependent protein interactions	Sweet, Ryan, Fares, Payne, Heath	Poster presentation		
BMSS 29th Annual Meeting, Edinburgh	September 2007	UBHAM	Electron capture dissociation in phosphoproteomics	Sweet, Cunningham, Cooper	Poster presentation		
Research potential support program, University of Ioannina Medical School, Ioannina, Greece	5 - 6th October, 2007	BRI	ERBIN is a novel SARA-interacting protein on early endosomes that negatively regulates TGF-β/Activin signaling	George Sflomos	Talk	Greek Scientists	
59th National Conference of	December 7-9th, 2007	BRI	ERBIN is a novel SARA-interacting protein on early	George Sflomos, Eleftherios Kostaras,	Talk	Greek Scientists	http://www.eebmb.gr/el/inde

Biochemistry and Molecular Biology, Athens, Greece			endosomes that negatively regulates TGF-β/Activin signaling	Ekaterina Panopoulou, Nikos Pappas, Theodore Fotsis and Carol Murphy			x.html

Presentations at Conferences and other institutions 2006

Conference name/location	Actual Date	Your Institute	Title	Authors	Presentation (Talk/Poster/Lecture)	Audience	Website address
6th International Conference on Bone Morphogenetic Proteins, Dubrovnik, Croatia (org. Dr. S. Vukecivic)	Oct.11-15, 2006	VIB	Smads and Smad-interacting proteins in BMP and Nodal signaling in vertebrate embryos and human disease	Danny Huylebroeck (invited speaker, co-chair of session)	Talk	International scientists	
Dept of Cell Biology & Molecular Medicine, Cardiovascular Research Institute, UMDNJ	Mar. 30, 2006	VIB	Connecting TGFβ family actions in vertebrate embryogenesis and human disease	Danny Huylebroeck	Talk	International scientists	

- New Jersey Medical School, Newark, NJ, USA (host: Dr. V. Gaussin)							
The Trilateral Workshop "Cell Biology of Intracellular Transport Processes" at the International Institute of Molecular and Cell Biology (IIMCB) Warsaw	Nov. 16-17 2006	IIMCB	Compartmentalization in the endocytic pathway – the role of APPL proteins and their interacting partners	Marta Miaczynska	Talk	Young Scientists from Germany, Czech Republic and Poland	http://www.iimcb.gov.pl/the_trilateral
43d Winter Meeting of the Norwegian Biochemical Society, Lillehammer, Norway	Feb 1-3 2007	LICR-UPP	Signaling via receptors for PDGF and TGFbeta-possible targets for tumor therapy	Carl-Henrik Heldin	Talk	International Scientists	
Targeting the kinome, Basel, Switzerland	Dec 4-6 2006	LICR-UPP	Signaling via receptors for PDGF and TGFbeta-possible	Carl-Henrik Heldin	Talk	International Scientists	

			targets for tumor therapy				
Keystone meeting Snowbird Resort Snowbird, Utah	April 7 - 12, 2006	DKFZ	Wnt and beta-Catenin Signaling in Development and Disease	Christof Niehrs	Talk	International scientists	
Arolla, Switzerland	August 21.-27.2006	DKFZ	Frontiers in Genetics	Christof Niehrs	Talk	International scientists	
Departement de Biologie Cellulaire Universite de Geneve Switzerland (Host Didier Picard)	7 Feb 2006	NIMR	'Trafficking of the Wingless signal in developing epithelia'	Jean-Paul Vincent	Talk	Students, post-docs and PIs	http://www.cellbio.unige.ch
The Fifteenth International Workshop on the Molecular and developmental biology of Drosophila Kolymbari, Crete	June 18-23, 2006	NIMR	Oriented cell divisions contribute to germ band elongation in Drosophila embryos	Jean-Paul Vincent	Talk	International Scientists	http://flybase.net/
Congres annuel francais sur la drosophile	11-14 Oct 2006	NIMR	Trafficking of the Wingless signal in developing	Jean-Paul Vincent	Talk	International Scientists, incl Students and	http://congres.igh.cnrs.fr/DrosoImbours/2006/index.p

St Jean Cap-Ferrat France			epithelia			Postdocs	hp
Division of Cell & Developmental Biology, University of Dundee	16 Novb 2006	NIMR	Trafficking of the Wingless signal in developing epithelia	Jean-Paul Vincent	Talk	Students, post-docs and PIs	http://www.maths.dundee.ac.uk/~simbios/cell.htm
IGBMC Illkirch France	1 Dec 2006	NIMR	Cell biological parameters that modulate the range of Wingless in Drosophila	Jean-Paul Vincent	Talk	Students, post-docs and PIs	http://www-igbmc.u-strasbg.fr/
UK Membrane Trafficking 2006	18 Dec 2006	NIMR	Wingless modifications: how sugar and lipids affect the range of wingless	Franz Wendler	Talk	UK scientists	http://www.ucl.ac.uk/lmcb/
Cerebellar Development – from Bench to Bedside, International Conference, Washington , DC	Nov 9-12 2006	TUD Biotec	Development of the zebrafish cerebellum	Michael Brand (invited speaker)	Talk	Internat.scientists	

International Zebrafish conference, Madison, USA	Jun 14-18, 2006	TUD Biotec	Maternal control of dorso-ventral axis formation and epiboly by the POU domain protein spg/pou2/Oct4	G. Reim, Michael Brand	poster	Internat.scientists	http://www.zebrafish2006.org/
International Zebrafish conference, Madison, USA	Jun 14-18, 2006	TUD Biotec	Efficient Generation of knock-outs in Zebrafish	Michael Brand et al.	poster	Internat.scientists	http://www.zebrafish2006.org/
International Zebrafish conference, Madison, USA	Jun 14-18, 2006	TUD Biotec	Transport of Wnt8 in the Neural Plate: Spiel mit Grenzen	Marta Luz, Muriel Rhinn, Michael Brand	poster	Internat.scientists	http://www.zebrafish2006.org/
International Zebrafish conference, Madison, USA	Jun 14-18, 2006	TUD Biotec	Parallel and synergistic patterning of the retina through Fgf Signals	A. Picker, Michael Brand	poster	Internat.scientists	http://www.zebrafish2006.org/
Max Planck Society-BMS mission to India,	March 6-12 2006	MPI-CBG	"Functional genomics studies of endocytosis and signalling"	Marino Zerial, Invited speaker	Talk	Max Planck Society and Indian scientists	
Gordon Research Conference on Lysosomes &	June 25-30, 2006	MPI-CBG	"Quantitative analysis of endosome	Marino Zerial, Invited speaker	Talk	International scientists	http://www.grc.uri.edu/06sched.htm

Endocytosis, Andover, NH, USA,			dynamics"				
Conference on Interface of Cell Biology and Cellular Microbiology, Sant Feliu de Guixols, Spain,	September 23-28, 2006	MPI-CBG	"Rab GTPases and their effectors in endocytosis and phagocytosis"	Marino Zerial, Invited speaker	Talk	International scientists	www.esf.org
46th ASCB Annual Meeting, San Diego, CA, USA,	December 9-13, 2006	MPI-CBG	"Mechanisms of Endocytosis and Signalling Revealed through Functional Genomics and Quantitative Image Analysis"	Marino Zerial, Invited speaker	Talk	International scientists	www.ascb.org/meetings/am2006/
British Society for Developmental Biology Dundee	November 2006	UoB	Systems Biology of FGF signaling	J Heath	Talk	International Scientists	
International conference on Converging Sciences Trento	October 2006	UoB	Systems Biology of cell signaling pathways	J Heath	Talk	International Scientists	

Intrenational conference on ECD	December 2006	UoB	ECD methods for postranslational protein modifications	S Sweet	Talk	International Scientists	

PR activities – National science fairs

Participation in work shops, open door events, science and society events - 2007

Type of activity	Actual/Planned Date	Your Institute	Title	Who	Description	Type of Audience
Talk to Biology teachers in the Cambridge area	10 January 2007	UCAM/WCRUK	How do cells know where they are?	Jim Smith & Jordan Raff	Providing information to school teachers about science undertaken at the Institute	Science School Teachers
Talk to Primary School Children – part of Institute Outreach activities	2 March 2007	UCAM/WCRUK	Heads and tails, how our bodies build themselves	Jim Smith	Part of the Institute's outreach activities – informing school children about science	School children aged between 8 & 10 years old
Talk to Sixth Form Children – part of National Science Festival	16 March 2007	UCAM/WCRUK	How an egg becomes a human being	Jim Smith	Part of the University's Science Festival	School children aged between 16 & 18 years
Art and Science: a dialogue Barber Institute Birmingham	14.12.07	UBHAM	Art and Science: a dialogue	John K. Heath		General Public

Science fort he public	04.07.2007	MPI-CBG	Lange Nacht der Wissenschaften	Marino Zerial	Poster	General Public
Science for the public	08.11.2008	MPIN	Open day at the Max-Planck Institutes in Munich-Martinsried	Rüdiger Klein	Part of the Institute's outreach activities	General Public – 4500 visitors
Formal Lecture	22/05/08	NIMR	Emmanual College, Cambridge - "University of the Third Age"	Prof. Jim Smith	How the egg becomes a human being: lessons learned from fish and frogs	Students
Seminar	23/05/08	NIMR	Imperial College Campus of Hammersmith – "Fabrics of Life Workshop II"	Prof. Jim Smith	Frogs as model organisms	Students
Informal Talk	03/12/08	NIMR	Abbey College, Cambridge – "Cells into embryos"	Prof. Jim Smith	Cells into embryos	Students

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Outreach activities					about science	
Talk to Sixth Form Children – part of National Science Festival	16 March 2007	UCAM/WCRUK	How an egg becomes a human being	Jim Smith	Part of the University's Science Festival	School children aged between 16 & 18 years

Section 2: Dissemination of knowledge

Date	Type	Audience	Countries addressed	Size of audience	Partner responsible
July 31, 2006	Dissemination to the public: Public web page	General public	All		MPI-CBG
July 14, 2006	Dissemination to the public: Press release	General public	All		MPI-CBG
During the entire Endotrack project	Dissemination to the scientific community: Publications and Conferences	Scientific community	All		MPI-CBG
During the second year	Poster	General public	Partner countries		MPI-CBG
November 9-11, 2009	On the Track of Signaling, the International Endotrack Conference	Scientific community	All		MPI-CBG, VIB

Dissemination to the public: **Public web page**

The project's public web page was set up and published July 31, 2006. It aims at spreading awareness of the ongoing scientific activities in the local and European research area. It informs the public on the project's aims, summarizes the work that is to be done and provides information on administrative issues such as job openings and contact dates. Also listed are meetings of the Endotrack consortium open for the public and published literature. The url of the Endotrack web page is: www.endotrack.org which has also been mentioned in the press release.

Dissemination to the public: **Press release**

The press release has been sent in English and German to the "Generalverwaltung" of the Max Planck Society July 14, 2006. The Max Planck Society is in charge of distributing the press releases of all the Max Planck institutes throughout Germany and Europe.

The press release was forwarded to

- all German science journalists
- to www.alphagalileo.com the world's leading independent resource for European research news, providing a fast and effective way to reach approved journalists around the world

to www.eurekalert.org an online, global news service operated by AAAS, the science society. EurekAlert! provides a central place through which universities, medical centers, journals, government agencies, corporations and other organizations engaged in research can bring their news to the media.

Dissemination to the public: **Poster**

The poster summarizes the goals of Endotrack and describes in a very general way the work that is being done by the partners. It is presented at public events such as open door events.

Dissemination to the public and to the scientific community:

Partner 7 has produced two **podcast** that were uploaded to the journal websites:

Vincent J-V and VanHook AM (2009) Science Signalling Podcast: October 6, 2009, Science Signaling 2

Vincent J-V and Karniol K (2009) Cell Podcast (paper clip): January 6, 2009, Cell 136

Dissemination to the public: **Open door at the Endotrack partner institutes**

MPI-CBG, Dresden

Lange Nacht der Wissenschaften (Dresden Science Night):

Once a year, research institutions open their doors for the interested Dresden people and present their work with information about the various funding organizations and projects.

In 2009, the Long Night of Science was a great success for the MPI-CBG: 1400 people visited the institute between 6pm and 1am, altogether in Dresden 35,000 visitors used this night to get a vivid idea on science.

A specific kids programme is also organized.

In 2010, a VIP tour with Prof. Sabine von Schorlemer, **Saxon Minister of Science and the Fine Arts**, Dirk Hilbert, Deputy Mayor for Economic Affairs of the Dresden City Administration, and other prominent science-related people started at the MPI-CBG: Debojyoti Chakraborty played the sitar, a band with Indian, Polish, and German scientists played a song blending Indian and Western music - this was meant to show that science is an international endeavour: 60% of the MPI-CBG staff comes from abroad, 45 nations work under one roof in the institute. The VIP guests had tried some pipetting in a competition vs. pipetting robots in the MPI-CBG HT-TDS.

Juniordoktor Programme: Kids visit a number of Dresden research institutions in the natural sciences, medicine, or art field and collect stamps for all stations in their junior PhD pass. After that, they have to give correct answers to a number of questions on the research they looked deeper into. If they meet all these requirements, they will get their Junior PhD, together with a certificate and even a doctoral cap.

The target group is students attending grade 3-12 in a Dresden primary school.

In 2009, 408 kids took part, of which 150 got their Juniordoktor.

The MPI-CBG is one of the financing partners of this programme and offers various talks and tours. In 2009, the MPI-CBG could offer 10 events for almost 300 kids. One of the stations was the TDS Screening Facility.

MPI-IN

Open day at the institute: 4500 visitors during open day at the two MPIs in Martinsried
Institute broschure has information on the work performed in EndoTrack in German and English.

Section 3: Publishable results

Overview of exploitable results, their future use and practical applications

A number of publications has been published or submitted for publication during the running period of the project in peer-reviewed journals describing the work that has been done within Endotrack. Endotrack partners have also taken part in the dissemination of knowledge by giving talks at international conferences or by poster presentations (See section 1 and 2).
Given in this section is an overview of the exploitable results the project achieved within the funding period of the project and of how these results are going to be used in the future research.

Exploitable result	Practical/Future use	Anticipated exploitation
Work package 1		
Tools to monitor endocytic trafficking and signaling in cellular systems	These tools allow the analysis of the trafficking of growth factors and growth factor receptors along endocytic routes, the localization of these factors and they allow the measurement of the kinetics. The generation of tools has been completed by all partners and will continue throughout the project if specific tools are need during the course of the project. The tools will be described and published in peer-reviewed journals and thereby will be made accessible to the scientific community.	At this stage this remains basic research.
Work package 2		
Genome-wide screens (genomics and proteomics) to study and identify regulators of endocytosis using forefront technologies	The analysis of the screen is ongoing and will identify further new components of the molecular machinery responsible for the intracellular trafficking in relation to signalling of growth factor complexes. The genome-wide screen has been conducted at MPI-CBG. Candidate genes will be confirmed using secondary assays. The results obtained will be made available to the scientific community via publications. One paper on the GWS is under revision	At this stage this remains basic research. After the data have been obtained and the hypothesis tested in animal model systems we will be in the position to consider filing Intellectual property-
With their optimized	Such a compound could inform the design of a	MRC is further

signaling assay, Partner 7 identified a new class of compounds that affect Wnt signaling (in collaboration with A. Russell; U. of Oxford)	inhibitor that could be used as part of anti cancer therapy	testing and optimizing this compound.
Work package 3		
Animal model systems for characterization of the signaling and trafficking machinery	These animal model systems will be characterized and later be available for the scientific community for further research questions. These model systems can be used in the future by Biotech- and Pharma companies for target and compound validation.	At this stage this remains basic research.
Work package 4		
Generation of disease model systems	P1b, P2 and P3 are about to generate mouse model systems to study tumorigenesis, neurodegenerative and congenital heart diseases. The findings of these studies will be published in peer-review journals and will be accessible to the scientific community.	At this stage this remains basic research.
	MPIN has generated a novel genetic model for Parkinson's disease (DAT-cre;Retlx/lx;DJ-1 mice). This model shows progressive and adult-onset reduction (50%) of nigral dopaminergic neurons.	
Work package 5		
Development of image analysis software	The quantitative multiparametric image analysis software (MotionTracking) developed for the analysis of intracellular compartments/structures showing very different fluorescent intensities and sizes. The software provides a very flexible solution that can be adjusted to the specific needs of the particular screening assay.	Potentially can be used directly in cell-based image analysis screening campaigns for functional genomics or chemical compound screens in the Pharmaceutical and Biotech industries
Development of new technologies enabling quantitative image-based genome	The integrative technology pipeline can be used for basic research and is also useful for biotech and pharma companies. The approach is based on robotics for pipetting and automatic imaging and image	

wide screens	analysis using either functional genomics based on siRNA or small molecule screening of a wide range of primary cells (even human samples) or cell lines	
Production of specific aptamers	Specific aptamers against 2 proteins involved in different signaling pathways (proteins SARA and APPL1). These tools will support the characterization of the function of these 2 proteins	At this stage this remains basic research.
Establishment of new biochemical assays	Basic research	A date for exploitation is not yet foreseen.
New cell culture models	Basic research	We are still at an early stage to foresee any date for exploitation.

Work package 2

The transforming growth factor β (TGFβ) family of ligands consists of evolutionary conserved pleiotropic secreted cytokines, which include TGFβ1, Activins and bone morphogenetic proteins (BMPs). Individual members of this family play crucial roles in multiple processes throughout development and in the maintenance of tissue homeostasis in adult life. As a consequence, deranged signalling by TGFβ family members has been implicated in many human diseases, including cancer, fibrosis, autoimmune and vascular diseases. TGFβ ligands trigger heteromeric complex formation between specific transmembrane type I and type II Ser/Thr kinase receptors, in which the type II receptor transphosphorylates and activates the type I receptor. R-SMADs are phosphorylated by type I receptor, and in turn can form heteromeric complexes with SMAD4. SARA, a protein localised on early endosomes, recruits non-phosphorylated SMAD2/3 to the activated receptors for phosphorylation by controlling their localisation. Receptor-induced phosphorylation of SMADs causes their dissociation from SARA and formation of SMAD2/3-SMAD4 complexes, which subsequently translocate to the nucleus, where they directly or indirectly bind to specific promoter region on target genes together with transcription factor and/or co-activators/repressors. As TGFβ receptor trafficking influences the signalling output, the detailed investigation of the interconnection between TGFβ family receptor signalling and trafficking is expected to provide molecular targets for the development of therapeutic interventions in several important human diseases.

One important filed of exploitation is the area of vasculogenesis/angiogenesis, in which TGFβ family signalling plays a significant role. Indeed, increased expression of TGF-β1 in cancer cells enhances tumor angiogenesis. The regulatory role of TGF-β family members on angiogenesis is further supported by targeted inactivation of the *TGF-β1* or *TβRII* gene, which results in embryonic lethality owing to defective vasculogenesis and angiogenesis. Angiogenesis-defective phenotypes are also seen in mice with null mutations of the genes encoding *TβRI (ALK-5)*, *ALK-1*, *Smad5*, and *endoglin*. On the contrary, inactivation of activin A gene has not been reported to cause any defects on angiogenesis. With respect to exploitation to applications in the vascular system, two main lines can be envisaged.

i) Inhibition of angiogenesis in tumors and ocular diseases

Treatment of diseases by pharmacological intervention at the level of the ongoing angiogenesis process is a very attractive and intense point of drug development in the industry worldwide. In cancer, by blocking the development of new blood vessels, it is hoped to cut off the tumor's supply of oxygen and nutrients, and therefore its continued growth and spread to other parts of the body. Anti-angiogenic drugs are not as likely to cause bone marrow suppression, gastrointestinal symptoms, or hair loss - symptoms characteristic of standard chemotherapy treatments. Moreover, since anti-angiogenic drugs target normal endothelial cells, which are genetically stable, drug resistance may not develop. So far, resistance has not been a major problem in long-term animal studies or in preliminary clinical trials. For these reasons, the pharmaceutical industry has exhibited an intense interest in developing anti-angiogenic drugs for cancer treatment. Another area of intense interest is the development of anti-angiogenic interventions for neovascular eye diseases such as diabetic retinopathy and age-related macular degeneration (AMD). These are common chronic diseases in the Western societies with a large market for the industry.

ii) Regeneration of vessels

Another domain with huge market for the industry is the treatment of cardiovascular diseases. Cardiovascular disease (CVD) accounts for approximately 30% of all deaths in the United States. Thus, therapeutic angiogenesis/vasculogenesis holds great promise for a potential cure. The logic behind angiogenesis/vasculogenesis is to improve the spontaneous healing process by supplementation of vascular progenitor cells (VPCs) either as cell transplants or in the form of tissue-engineered vascular grafts. Human embryonic stem (hES) cells have generated much interest because of their capacity for self-renewal and pluripotency. In practical terms, hES cells can be cultured indefinitely ex vivo, and can differentiate into virtually any cell type in the adult body. Recently, reprogramming of terminally differentiated cells to iPS cells opened a new gate for cell transplantation-based regenerative medicine by overcoming the ethical controversy over ES cells. Activin A and BMP-4, both members of the TGFβ subfamily, are important in determining pluripotency or initiation of differentiation of hES/iPS cells towards mesendoderm, including hemangioblasts, respectively.

Hemangioblasts are bipotential progenitors that have the capacity to differentiate into both hematopoietic and endothelial cells. The ability to generate an unlimited supply of these cells from hES cells could have important clinical ramifications. It has been shown that hES-derived hemangioblasts homed to the site of injury and showed robust reparative function of damaged vasculature, when injected into animals with diabetes or ischemia/reperfusion injury of retina. The cells showed also a similar regenerative capacity in animal models of both myocardial infarction (50% reduction in mortality rate) and hind-limb ischemia, with restoration of blood flow to near normal levels within a month. In fact, hemangioblasts are tripotential generating also smooth muscle cells, thereby forming multilayered blood vessels in animal models.

As TGF-β family members can both enhance or inhibit vasculogenesis and angiogenesis, investigation of the signalling cascades emanating from the TGF-β family receptors is expected to lead to the discovery of novel targets for pharmacological intervention for either positive or negative regulation of angiogenesis. Exploitation of these targets for angiogenesis-related drug development by the pharmaceutical industry in Europe will definitely improve the competitiveness and the market opportunities of the latter.

WP3 Mouse Models:

The **Ttrap knockout mouse model** established at VIB_P2 is now studied further in the direction of DNA repair (with Keith Caldecott, Sussex) as it has been identified as the second tyrosyl DNA phosphodiesterase (Tdp) that repairs topoisomerase-mediated DNA damage, but the only one displaying 5' Tdp activity. This mouse model is now crossed with Tdp1 knockout mice (which are also viable, Tdp1 being the gene causing progressive neurodegenerative disease in humans; see Cortes Ledesma et al. Nature 2009;461:674). This work also opens the possibility to investigate Tdp1/Tdp2 co-operation and Ttrap function in senescence-associated inflammatory cytokine signalling in conditions of persistent DNA damage signaling. In addition, the Ttrap knockout mouse will enter the German Mouse Clinic (Neuherberg, Munich) for phenotyping in their dual pipeline system and older Trrap knockout mice are also being screened in additional tests assessing neurodegenerative disease. Preliminary data show a significant increase in these latter mice of the formation of Lewy bodies in their brains, pointing at a function of Ttrap/Tdp2 deficiency in Parkinson's disease (PD). This is in line with previously published data obtained in tissue culture by other groups showing that mutant PARK7/DJ-1, which causes PD in humans, binds more tightly to Ttrap inhibiting the neuroprotective action of Ttrap in neuorblastoma cells (Zucchelli et al. Cell Death Diff 2009;16:428). These data also open perspectives to cross the Ttrap knockout mouse model with the conditional DJ-1 knockout mouse models used by R. Klein in EndoTrack either using CamKII-iCre based approaches or – in the midbrain – DAT-Cre based approaches. The Ttrap knockout mouse model can also be crossed in the acknowledged Eu-Myc transgenic mouse that develops lymphoma, and where the p53 pathways, including those of DNA repair, have been studied intensively. Moreover, Ttrap has been found to be a novel p53-interacting protein. Finally, the group of M. Tafit (Lausanne) has identified a missense mutation (in the TNFR/CD40, TRAF and Smad-interacting N-terminal segment) in Ttrap as a candidate mutation causing narcolepsy. Biochemical analysis of this mutant Ttrap is now ongoing at VIB_P2, including assessment of its rescue of its phenotype in ttrap zebrafish morphants, as developed and published by P2 through EndoTrack) and analysis of the sleep pattern of Ttrap knockout mice.

The **Sip1 conditional knockout mice**, in particular those removing the gene in the ventral forebrain (see PAR_37-54), are also giving valuable insight into the function of this transcriptional repressor in directional migration of GABAergic interneurons in the embryonic and early post-natal forebrain. Some of these mouse models develop myoclonic seizures 3 weeks after birth, explaining in part why many Mowat-Wilson syndrome patients develop seizures and epilepsy. In addition, the developed Sip1 knockout mouse models in EndoTrack, including the one targeting premigratory neural crest (Wnt1-Cre) and the Sip1+/- heterozygous mice reveal a crucial function of Sip1 in the formation of the transient boundary cap stem cell compartment needed in the embryo to generate sensory neurons of dorsal root ganglia. As a result of the deficient establishment of this stem cell compartment, the adult Sip1+/- mice develop a hypoalgesia phenotype, which is further investigated with pain researchers at the Univ. Bonn.

Overview of exploitable results, their future use and practical applications

Results generated	Owners	Sector of application	Exploitation/use potential measures	Timetable for use or exploitation
WP1				
Tools to monitor endocytic trafficking and signaling in cellular systems (reagents, cells)	Partners participating in generation of tools	Basic research aimed at understanding signaling and trafficking	The tools will be made available to the scientific community (subject to MTA)	We are still at an early stage to foresee any date for exploitation.
WP2				
Genome-wide screens (genomics and proteomics) to study and identify regulators of endocytosis using forefront technologies	Partners participating in the screens are MPI-CBG, BRI, MRC	Identification of regulators of trafficking and signaling, basic research	The assays and the hits obtained will be made available to the international scientific community via publications	One screen has been conducted already by the MPI-CBG, but the expected time point for use or exploiting cannot be defined at this point.
WP3				
Animal model systems for characterization of the signaling and trafficking machinery	Partners MPI-CBG, MPI-IN, MRC, DKFZ, LMB are generating animal model systems and analyse signaling and trafficking	Basic research aimed at understanding the molecular trafficking and signaling machinery in aimal model systems	The research results will be made available to the international scientific community via publications	The generation and characterization of animal model systems is ongoing. A time point for exploitation is not yet foreseen. These model system can later be used for target and compound validation
WP4				
Generation of disease model	MPI-CBG, MPI-IN,	Basic research, Biotech and	The findings of these studies will be	The generation of disease model

systems	LICR, LMB	pharma companies	published in peer-review journals and will be accessible to the scientific community	systems is ongoing.
WP5				
Development of new technologies enabling quantitative image-based genome wide screens	MPI-CBG, MRC, BRI	Basic research, biotech and pharma companies	Publication in peer reviewed journals	The new techniques developed within the Endotrack project can be used to test and develop new drugs
New technology for data analysis and network reconstruction	MPI-CBG, BRI, MRC, IIMCB	Basic research, biotech and pharma companies	Publication in peer reviewed journals	The new techniques developed within the Endotrack project can be used to test and develop new drugs
Establishment of new biochemical assays	MPI-CBG, BRI, IIMCB, LICR	Basic research	Publication in peer reviewed journals	
Small molecules active in two-hybrid assays	Imaxio	Cancer therapy	They have to check the activity of the hits in cellular assays and in disease animal models. Further chemical developments are also planned.	Compounds have been developed and tested in vitro and in vivo. A date for exploitation is not yet foreseen as we have to await the results.
New cell culture models	All	Basic research	Publication in peer reviewed journals	

Innovation related activities

The Endotrack project has developed new forefront technologies and set very high quality standards in functional genomics applied to signalling and endocytosis. However, there has not been any commercial exploitation during the project such as patenting, licensing or founding of spin-off companies.

Some of the technologies are still being further developed and refined and the results and the data obtained so far are not yet suitable for commercial exploitation. At this point it is impossible to foresee an exploitation plan of the data obtained by then.