Project no.
LSHM-CT-2004-503228

Project acronym
SIGNALLING & TRAFFIC

Project full name
Signalling and membrane trafficking in transformation and differentiation

Instrument type: **Specific targeted research project**

Priority name: **Priority 1 Life science and health**
- Life sciences, genomics and biotechnology for health

Signalling & Traffic Publishable Final Activity Report

2007-12

Period covered: from **May 1st 2004 to November 30th 2007**
Date of preparation: December 2007

Start date of project: **May 1st 2004** Duration: **43 months**

Project coordinator name: **Thierry Galli**
Project coordinator organisation name: Institut national de la santé et de la recherche medicale

Publishable executive summary

❖ Project objectives

Cancer and brain-related diseases are two of the principal causes of morbidity and mortality in Europe and diseases with major socio-economic impact. Our research is ultimately finalized to develop targets for therapeutic procedures and new diagnostic tools to better the health conditions and ultimately the standard of living. From the public health point of view, the social impact of our research is only too obvious. In addition, from the economical point of view, sensible gains are to be projected for the various national health systems, once more potent management tools will be available, by reducing, for example, the need for radical intervention and therefore save on the ensuing rehabilitation costs. In the case of cancer, the ultimate goal indeed is to shift the therapeutic emphasis, for some tumours, from surgical to pharmacological intervention, thus limiting the hospitalisation costs and the financial, psychological and physical burden on the patients and their families. In the case of neurodegenerative diseases such as Alzheimer's, Hungtington's disease and neuropathies, the lack of an understanding of the complex physiopathological links between gene defects and symptoms and the absence of efficient therapeutics demonstrate the need to search for new strategies. Several of the gene products that have been studied in this project (rab proteins and their partners, SNAREs and their partners, growth factors, cell adhesion molecules and metalloproteases) have already been linked to cancer and brain-related diseases.

❖ Educational goals

We intended to foster education, training and exposure of young scientists to cultural diversity, both from the scientific and social point of view. ‘Signalling and Traffic’ offered training opportunities for technicians, PhD students and post doctoral fellows to perform their own professional scientific development in a highly skilled and competitive consortium. The participating laboratories offered, in our admittedly "biased" assessment, the highest standards of scientific rigor and international competitiveness and therefore provided an excellent opportunity for younger scientist to develop to full maturity and start their own scientific enterprises. We gave particular attention to maintaining the gender balance at all levels and to involving the most promising fellows in the inter-group meetings, to offer them the opportunity of travelling, coming into contact with new scientific and national realities and participating in the management of science. Standard scientific dissemination through congresses, workshops and publications, and in classes in the universities of the participants serve as important vehicles for information transfer and training. Our web site http://www.signallingtraffic.com/ was the core of the communication of the network with students. Through this platform, we have tried to foster education on the molecular and cellular basis of Signalling and Traffic in normal and pathological situations. We particularly emphasized the molecular and cellular mechanisms of cancer, brain degenerative diseases and neuropathies that have a Signalling and Traffic component. Additionally public awareness actions designed to inform the general public of 'research' and the 'benefits of research to society' were performed by the partners and through our final public meeting.

❖ State-of-the-art – General Background

Intracellular communication in eukaryotes is mainly achieved by the trafficking of membranes carrying membrane-bound and/or intra-vesicular signalling molecules. Inside cells, vesicles circulate from one intracellular compartment to another, thus facilitating vectorial transport of signals. Between cells, membrane anchored and secreted molecules mediate extracellular signalling. Exocytosis and endocytosis of receptors control the response of sensitive cells to extra cellular signals. The routes of membrane trafficking are controlled by cell signalling pathways; however, the underlying molecular machinery is scarcely characterized. Moreover, membrane trafficking has been studied mainly in differentiated cells and rarely in cells changing phenotype during differentiation or de-differentiation. Such drastic phenotypic changes occur during development when cells mature to differentiated cells, such as complex as neurons, and during malignant transformation.

❖ Working hypothesis

Our working hypothesis was that inter- and intracellular signalling and membrane trafficking are interdependent and co-coordinately regulated during the course of differentiation and malignant transformation.

❖ General Aim

Our goal was to establish the connections between signalling pathways and membrane trafficking in the context of highly dynamic migrating, dividing and adhering mammalian cells. Through the study of membrane traffic in the course of cell differentiation, de-differentiation, and during mitosis, we aimed to unravel how important signalling pathways remodel the intracellular trafficking routes and conversely, how membrane traffic can influence signalling cascades. Using modern cell biological approaches, we studied several cellular models including neurons differentiating in culture and establishing contacts and cancer cells dividing and migrating. We investigated proteins that play central roles in membrane trafficking and secretion such as rabs, SNAREs and their partners. We focused on the trafficking of signalling molecules including cell-cell and cell-substrate adhesion molecules, growth factors and their receptors. Through these approaches we have started to define the connections between Signalling and Traffic in normal and pathological conditions.

Specific Aims

Within this general framework, we pursued the following specific objectives:

1. Identify the secretory mechanism of signalling molecules involved in malignant transformation: **Work Package 1**
2. Study the function of the endosomal system in signaling: **Work Packages 2 & 3**
3. Assess the importance of membrane trafficking in the migration of mammalian cells: **Work Package 5**
4. Reveal the role of membrane trafficking in the mitosis of mammalian cells: **Work Package 4**
5. Demonstrate the importance of membrane trafficking in the establishment of contacts between mammalian cells: **Work Package 6**

❖ Contractors involved

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project**	Date exit project**
CO	1	Institut National de la Santé et de la Recherche Médicale	INSERM	France	Month 1	Month 43
CR	2	German Cancer Research Center	DKFZ	Germany	Month 1	Month 43
CR	3	Institut de Recerca de l'Hospital Universitari Vall d'Hebron	IR-HUVH	Spain	Month 1	Month 43
CR	4	Instituto de Tecnologia Quimica e Biológica	ITQB	Portugal	Month 1	Month 43
CR	5	IFOM fondazione Istituto FIRC di oncologia molecolare	IFOM	Italy	Month 1	Month 43
CR	6	Institut Curie – Division de Recherche	IC-SR	France	Month 1	Month 43
CR	7	Università di Torino di Scienze Oncologiche	UNITO	Italy	Month 1	Month 43
CR	8	Johannes Gutenberg-Universität Mainz	University of Mainz	Germany	Month 1	Month 43
CR	9	INSERM Transfert SA	Inserm Transfert	France	Month 1	Month 43

❖ Coordinator contact details

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❖ Work performed

The STREP Signalling and Traffic was launched on May 1 2004 and ended November 30 2007. The work performed has consisted in **organizing the network** particularly through four meetings - *kick off* meeting in Paris on October 2004, mid-term meeting in Lisbon October 2005, Milan meeting October 2006, Heidelberg final public meeting October 2007, the web site http://www.signallingtraffic.com/, and **carrying a large series of experiments** that were the base to achieve most of the milestones and complete most of the deliverables. Part of the work has already been published or is about to be. Several publications associate authors from different partners.

❖ Results achieved so far

WP1 has found that L1 and ADAM10 have to cooperate to achieve enhanced motility and invasiveness in tumors and that exosomes represent an important novel platform of ADAM10-mediated cleavage (P2). Other studies have shown that the recycling of pro-TGF-alpha is essential for the activity of the EGFR (P3). Progress has also been made in the knowledge of cell surface glycosylation in ovarian carcinomas (P4) and the role of ubiquitination, which is necessary and sufficient for internalisation of the EGFR through a non-clathrin endocytic (NCE) pathway, has been shown to preferentially commit the receptor to degradation, rather than recycling.
WP2 has led to the characterization of the role of Eps15, Eps15R and Epsin proteins in EGFR trafficking, by showing that they participate in the clathrin endocytic pathway, although they are not essential, and that they are indispensable for NCE. KD of expression of these three genes abolishes NCE and skews EGFR fate towards recycling through the CME pathway (P5). Other studies have shown an indirect role for TI-VAMP in the regulation of EGFR endocytosis (P1). Work is underway to study the *in vivo* dynamics of Rab proteins and endosomal compartments, using Rab35 an endosomal Rab (P6).
In **WP3**, novel components of the molecular circuitry linking trafficking and signalling within cells have been identified. RN-tre, a regulator of endocytosis, has been found to interact with two centrosomal proteins controlling at distinct steps the mitotic phase of the cell cycle: the dual phosphatase Cdc14A, and the kinesin motor protein KIF3A. The biological relevance of both this interactions and their molecular circuitry have been depicted (P7). Rab5 has been shown to induce the activation of Rac, most likely on early endosomes. Subsequently, activated Rac is recycled to the plasma membrane, presumably in order to direct actin remodeling. This pathway ensures localized signaling leading to the formation of spatially-restricted actin cytoskeleton remodeling (P5).
WP4 has demonstrated the existence of pathways, involving trafficking proteins, which act with a progressive mechanism to ensure proper cell division (P6 and P7). In particular, a novel pathway required for the separation of the duplicated centrosome at the onset of mitosis and for proper spindle assembly involving RN-tre, Rab5, Rabex-5 and KIF3A has been identified and characterized (P7). The function of Rab6A' in the control of the metaphase to anaphase transition and in the activation of the mitotic checkpoint has been demonstrated and it provides a relevant a link between trafficking and cell cycle regulation (P6).
WP5 has lead to the demonstration that the exocytic pathways mediated by cellubrevin and TI-VAMP play a role in cell migration, the first by regulating cell adhesion, the second by regulating lysosomal secretion (P1). WP5 also showed that L1 and ADAM10 are released in exosomes and promote cell invsion and migration (P2).
WP6 has demonstrated that in neurite outgrowth, at L1-mediated contacts vesicles containing the t-SNARE syntaxin 1 and the exocyst subunit Sec6 accumulate suggesting these are sites of exocytosis (P1). Time-lapse video microscopy showed that L1 is recruited from endosomal pools for the formation of new contacts (P1). Endocytic trafficking routes of three oligodendroglial/myelin proteins have been defined (P8). The effect of ligating oligodendroglial F3 with the candidate neuronal ligand L1 has been analysed at the level of fyn kinase activation, and tyrosine phosphorylation of the protein HnRNPA2 which plays a role in mRNA trafficking has been shown (P8). SNARES expressed by oligodendroglial cells playing a role in trafficking of the main myelin protein PLP have been defined (P8). Glial exosomes have been characterised (P8).

❖ Expected end results

Our wish was to be able to unravel the links between membrane trafficking and cell signaling leading to cancer and brain diseases. We have already made very significant progress towards this goal. In order to achieve all of their goals, the participants have intensively exchanged data, reagents, and students and have decided to even increase the exchange of scientists during the second term of the project. While most if not all deliverables and milestones have been achieved, the collaborations initiated in this consortium will continue in the next few years.

❖ Intentions for use and impact

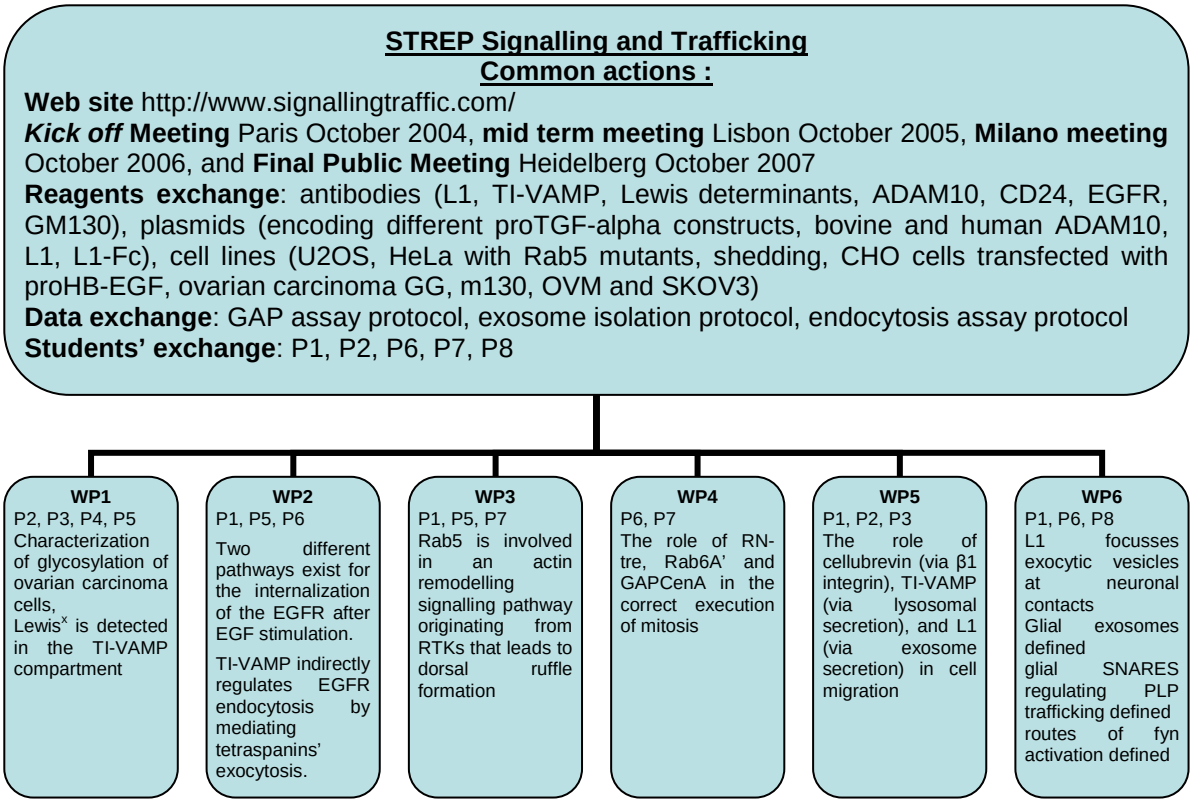
Each member of our consortium is already involved in the dissemination of the knowledge generated in this STREP in his/her home country. As a common action, we decided to organize a European meeting, free, open to the public and advertised. This meeting took place in Heidellberg in October 2007 at the end of the STREP. We presented our own work and invited two other scientists working in the same field: Jean Gruenberg and Ivan Dikic as speakers. We also extended the invitation to biotechnology companies interested by the link between signalling and trafficking.

❖ The main elements of the publishable results

Thierry Galli (P1) has demonstrated that TI-VAMP regulates epithelial cell motility and indirectly regulates EGFR endocytosis and signaling through a novel mechanism. Indeed, TI-VAMP was shown to regulate the cell surface expression of tetraspanins including CD82 which in turn interacts with and regulates EGFR at the cell surface. Furthermore, we have found an important functional link between TI-VAMP and the GTPase Rab21. These results have been published in PNAS 2005, Biology of the Cell 2007, or are submitted.
Peter Altevogt (P2) has found that L1 expression augments cell motility, invasiveness and tumor growth in vivo. Cleavage of L1 by the metalloproteinase ADAM10 was localized in exosomes that can be released from cells by a variety of stimuli. These results have been published in the International Journal of Cancer 2005 and the Biochemical Journal 2005. L1 is also a substrate for presenilins/g-secretase and this is important for the nuclear translocation and singalling of L1 (Oncogene 2007 and submitted).
Joaquín Arribas (P3) has shown that the recycling of proTransforming Growth Factor-alpha (proTGF-alpha) and the trafficking of the metalloprotease ADAM17, regulate the activation of the EGFR, opening the possibility that defects in the trafficking the growth factor and the metalloprotese may contribute to the development of tumours. These results have been published in the Journal of Biological Chemistry in 2005 and 2007 and in EMBO Journal in 2006. In addition, as published in Mol Cell Biol in 2006, several novel substrates of ADAM17 and ADAM10 have been found.
Júlia Costa (P4) has characterized the glycosylation of ovarian carcinoma cell lines (Int. J. Oncol. 2006). *N*-glycans from ADAM-10 play functional roles in the enzyme processing, in vivo and in vitro activities (under revision for publication in Biochimica Biophysica Acta). The biosynthesis and functional role of the fucosylated Lewis^{x} determinant have been elucidated in human NT2N neurons (J. Neurosci. Res. 2007).
Pier Paolo Di Fiore (P5) has demonstrated that, following EGF stimulation, two different internalization pathways for the EGFR direct the receptor towards different fates. The ubiquitin-independent, clathrin-dependent pathway allows EGFRs, by-and-large, to be recycled to the cell surface, while a second, non-clathrin-dependent internalization pathway in which the receptor becomes ubiquitinated, preferentially commits the EGFR to degradation. These results have been submitted to Nature for publication and the manuscript is currently under revision. P5 has also elucidated how endocytic trafficking/recycling of Rac is required for the spatial restriction of Rac-dependent actin reorganization, an observation that may be extremely relevant to understanding the molecular mechanisms of cell motility. The results of this work have been submitted to Nature, and are currently under review. In parallel work P5 characterized a possible candidate regulator of actin remodelling via Rab5, Rabex5.The crystal structure of the two ubiquitin binding domains (UBDs) of Rabex5 was resolved in complex with Ub. The two UBDs bind Ub on distinct surfaces, allowing Ub to interact simultaneously with different UBDs, thus opening new perspectives in Ub-mediated signalling. This work was published in Cell in 2006. Finally, a portion of the work of P5 depended on the use of RN-tre, which had previously been demonstrated by P5 and P7 to be a Rab5-GAP. However, this finding had been questioned by others, and P5 and P7 felt it was of paramount importance to address these concerns. P5 and P7 were able to confirm all their previous conclusions on RN-tre and its biochemical and biological functions. These findings were submitted to Nature Cell Biology for publication.
Bruno Goud (P6) has shown that the Golgi-associated Rab6A' GTPAse is required for the metaphase/anaphase transition. Rab6A', in association with p150^{Glued} and GAPCenA, regulate the dynamics of the dynein/dynactin complexe at kinetochores and consequently the inactivation of the Mad2-spindle checkpoint. This work has been accepted for publication in EMBO Journal.

Letizia Lanzetti (P7) and **Pier Paolo Di Fiore (P5)** have demonstrated cross-talk between the actin-remodelling signalling pathway activated by PDGF stimulation of cells and the endocytic pathway. Rab5, a well-known endocytic regulator, was shown to participate in RTK-induced actin remodeling, probably through its effector RN-tre. This work was published in Nature in 2004. P7 has also published the findings concerning the novel RN-tre-binding protein Cdc14A. RN-tre is phosphorylated by Cdk/cyclin complexes at mitosis and dephosporylated by Cdc14A. Such post-translational modification finely tune the RN-tre GAP activity towards Rab5 (J Biol Chem 2007). Furthermore the study on the involvement of RN-tre, Rab5 and KIF3A in centrosome cohesion and spindle assembly has beeen recently submitted.
Jacqueline Trotter (P8) has demonstrated that oligodendrocytes exhibit different transport routes for the three myelin proteins MAG, MOG and PLP (J Cell Sci. in press) and shown that PLP is transported from intracellular sites to the plasma membrane using VAMP 3 and TI-VAMP (manuscript in preparation). She has defined the components of glial exosomes which also contain PLP (Proteomics Clin. Appl.). She has shown that F3 cross-linking using antibodies as well as F3 ligation using L1-FC stimulates tyrosine phophorylation of HnRNPA2 in oligodendrocytes which is involved in trafficking of MBPmRNA in glia. This work has been submitted for publication and the manuscript is being revised.

- **Diagrams or photos illustrating the work**



- **Project logo and public web site link**

Signalling & Traffic

http://www.signallingtraffic.com/