LSHG-CT2005-518181

AMPKIN

Systems Biology of the AMP-activated protein kinase pathway

Specific targeted research project STREP

Life Sciences, Genomics and Biotechnology for Health

AMPKIN Final report

Period covered: from 1 January 2006 to 30 June 2009
Draft 1
Date of preparation of this draft: 30 September 2009

Start date of project: 1 January 2006 Duration: 42 months

Project coordinator name: Stefan HOHMANN
Project coordinator organisation name: University of Gothenburg UGOT

Publishable final activity report

1. Project execution

Summary description of project objectives

AMPKIN aims at a better understanding of how cells and organisms monitor changes in energy homeostasis and properly respond to such changes. A better understanding of such signal transduction processes and their dynamics requires multi-disciplinary research (biology, physics, mathematics, computer sciences) to collect quantitative data and to reconstruct processes in the computer. AMPKIN aims at providing generic tools for research and novel knowledge on signalling processes, which eventually can be used to diagnose and treat human diseases.

The overall objective of this project is to generate mathematical models of the AMPK pathway that can then be used in drug target identification and drug screening. In addition, the project is expected to deliver substantial new knowledge on the function, operation and physiological roles of AMPK in yeast and mammalian cells, new tools for experimental research such as in single cell analysis and improved software tools for systems biology.

This overall project objective leads to the following specific scientific, technical and innovation objectives:

1. To establish and critically compare the network structures of the AMPK pathway from activation to response in yeast and mammalian cells using existing data and knowledge from literature, databases and own research. Expected results: Detailed models based on knowledge and data from literature, databases and research of consortium members of the network structure of the AMPK pathways in yeast and mammalian cells, including pathway activation, signal transmission, crucial intersecting regulatory pathways (e.g. Snf3/Rgt2, TOR, PKA) as well as transcriptional and post-translational targets of the pathway.
2. To generate, optimise and verify assay systems for as many different steps as possible in the AMPK pathway of yeast and mammalian cells in order to generate quantitative data and maximise the use of real data in modelling. Expected results: Tools for quantitative data generation, such as epitope-tagged and GFP (and GFP variant)-tagged proteins and genes for use in flow cytometry, microscopy and bioimaging as well as *in vitro* and *in vivo* kinase assays, antibodies and other reagents to monitor specifically modified proteins. In addition, an optical manipulation platform will be further developed for quantitative data collection.
3. To generate reference quantitative dynamic datasets following activation and deactivation of the AMPK pathway in yeast and mammalian cells. This reference data set will be used for generating dynamic models of the pathways and to optimise parameters that can not be determined experimentally. Expected results: A reference set of "dynamic" (time course) experimental data following activation and deactivation of the pathway, including for instance rates of change of relevant phosphoproteins, protein interactions, protein localisation as well as level of RNAs, proteins and metabolites.
4. To generate and critically compare dynamic models for the yeast and mammalian AMPK pathway. To use information from the yeast model to complement gaps in the mathematical description of the mammalian model. Expected results: Kinetic models of activation and deactivation of the AMPK pathway in yeast and mammalian cells. Since data density will be higher in the yeast system it will be tried to use yeast data and parameters as much as possible to fill gaps in the model of the mammalian system.
5. Generation of tools for system perturbation, which will be used to generate data for model testing and iterative model improvement and potentially for development of drug screening

approaches. Expected results: A large set of tools and experimental approaches (mutants, growth conditions, compounds etc) for system perturbation are already available. This objective aims at generating constructs for expression in yeast of mammalian AMPK subunits and in particular yeast-human chimera of such components. Such tools are expected to be highly useful also in drug screening programmes. In addition, novel and existing compounds affecting AMPK activity will be tested here.

6. To provide "dynamic" datasets from experiments employing a range of defined system perturbations in both yeast and mammalian cells with the aim to test and iteratively improve the models and to optimise the underlying parameters. Expected results: The process of iterative model improvement will be repeated several times. The outcome of this objective/work package will be datasets from experiments in which activation and deactivation of the system will be perturbed in different ways.

7. To generate iteratively improved mathematical models in order to determine system properties and to provide an assessment of similarities and dissimilarities of the models in yeast and mammalian cells and hence of the significance and the limitations of the approach of comparative modelling from experimental and theoretical perspectives. Expected results: Several rounds of iterative model improvement will be performed. The outcome will be models that reproduce with increasing accuracy experimental scenarios of the dynamic operation of the pathways and with increasing capability to correctly predict the outcome of experiments. Model optimisation should lead to an assessment of the similarity or dissimilarity of different modules between yeast and mammalian cells.

8. To use the mathematical models to predict the result of pharmacological system perturbations and where possible assess those experimentally, thereby implementing the models in drug screening programmes. Expected results: Models of increasing accuracy will be implemented in drug screening programmes leading to definition of potential targets and predictions of drug effects.

Contractors involved

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	University of Gothenburg	UGOT	SE	Month 1	Month 42
CR	2	University of Rostock	SBIR	DE	Month 1	Month 42
CR	3	Imperial College	MRC	UK	Month 1	Month 42
CR	4	Technical University of Denmark	DTU	DK	Month 1	Month 24
CR	5	Arexis AB	AREXIS	SE	Month 1	Month 18
CR	6	Chalmers University of Technology	CHALMERS	SE	Month 25	Month 42

*CO = Coordinator, CR = Contractor

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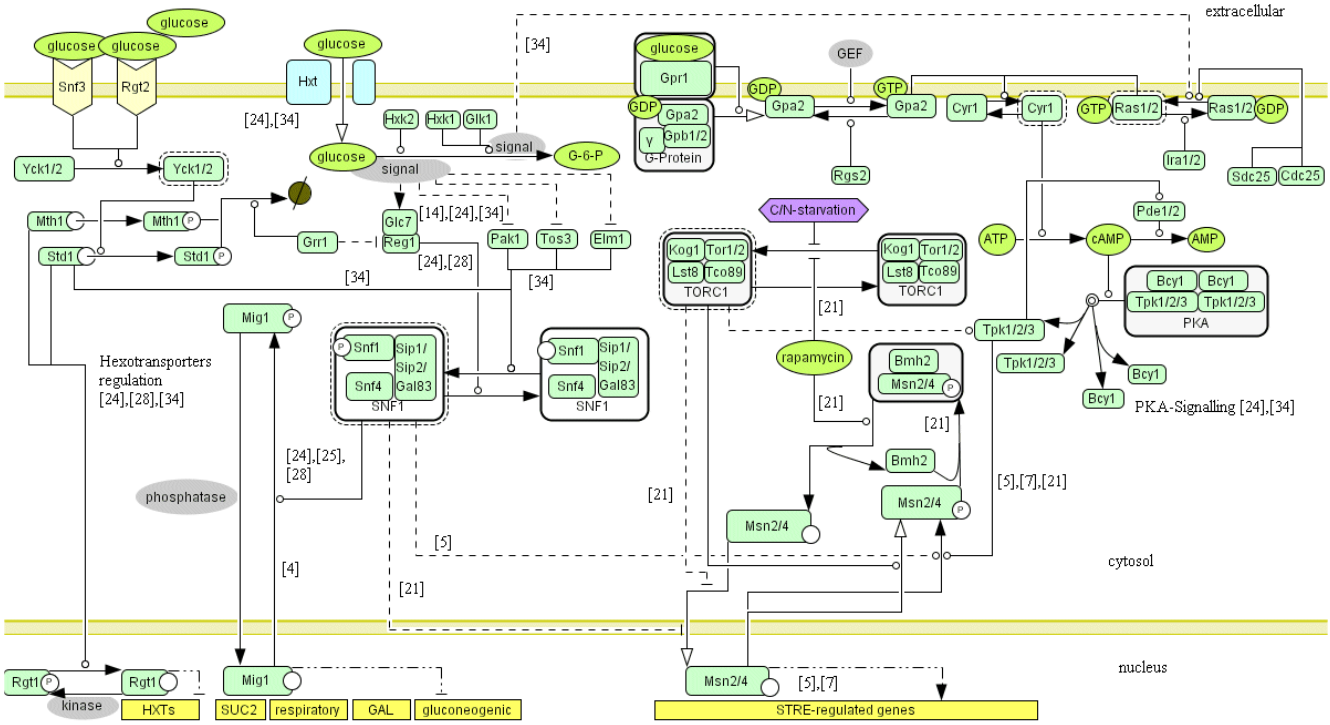
Achievements with respect to objectives and the state-of-the-art

The consortium regards AMPKIN as a major success – significant advances have been made in our understanding of AMPK signalling and the development of quantitative experimental tools and principles of mathematical modelling. The consortium believes that it has shown great flexibility with adjusting the initial project plan to the development of new technologies and abandon approaches that turned out to be too ambitious or out of date. Some work also had to be re-scheduled due to the fact that partner 4 decided to leave the consortium during the reporting period.

The project was arranged around eight research objectives, which directly translated into eight work packages. The following is a summary of the results achieved.

1. **To establish and critically compare** the network structures of the AMPK pathway from yeast and from mammalian cells using existing data and knowledge from literature, databases and own research.

The network structures of the yeast Snf1 and the mammalian AMPK signalling systems have been generated. These network structures do not constitute computational models to be used, for instance, in simulations of experiments, but rather constitute a formalised representation of present knowledge. The Snf1 pathway is shown as example below. The network structure is based on large-scale data (protein interaction, genetic interaction, gene expression data) and literature information.



This graphic representation will be complemented with a critical assessment of available information on the signalling networks in the form of a review article. The network structure will continuously be updated during the course of the project.

The main goal of AMPKIN is to build dynamic models of the operation of the signalling pathways in yeast and mammalian cells. To generate dynamic computational models requires time course data that capture the changes over time of concentrations of biomolecules in the system. The consortium concluded that such data are not available in the literature or in data resources and that they have to be generated exclusively within the consortium.

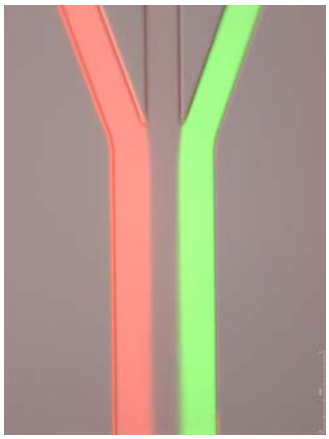
Self assessment: The objective has been fully achieved already in the first reporting period. In parallel partner 6 generated a reconstruction of the cellular Snf1 network in yeast, which showed that yeast and mammalian AMPK have similar targets and physiological roles.

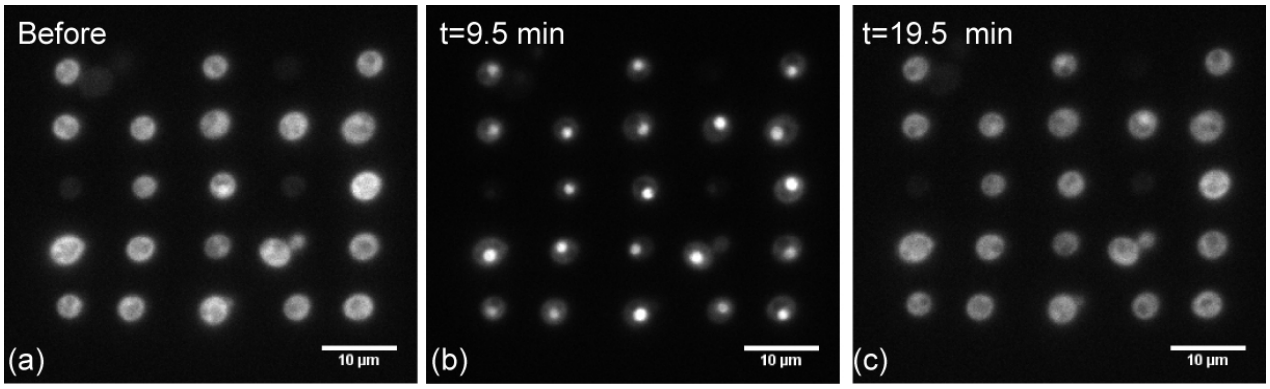
2. **To generate, optimise and verify assay systems** for as many different steps as possible in the AMPK pathway of yeast and mammalian cells in order to generate quantitative data and maximise the use of real data in modelling.

AMPKIN has generated numerous assay systems that allow monitoring the dynamic operation of the AMPK pathway in yeast and mammalian cells. Those in turn allowed generating datasets for dynamic modelling and addressing crucial questions concerning the direction of information flow (via kinases or phosphatases), contributing parallel routes, noise and threshold control and feedback mechanisms.

Important tools developed and/or established include among others (1) antibodies that allow monitoring over time the phosphorylation state – and thereby activity – of the AMP-activated protein kinase as well as novel approaches to monitor altered phosphorylation pattern in proteins of interest; (2) approaches to monitor the absolute concentration and their change over time of all components of the core yeast Snf1 signalling pathway; (3) promoter-XFP fusions that allow monitoring at single cell level by FACS analysis Snf1 pathway activation and hence to study population distribution profiles, noise and thresholds, which are critical to interpret results obtained with cell extracts; (4) an optical manipulation platform that together with suitable XFP-tagged proteins allows monitoring directly and in real time signalling through the yeast Snf1 pathway with nuclear shuttling of a signalling molecule as read-out.

The optical manipulation platform is one highlight of AMPKIN. The picture below illustrates the microfluidic flow chamber as well as cell arrays on the chamber surface where nuclear import and export of Mig1 is followed in response to changing glucose concentrations.

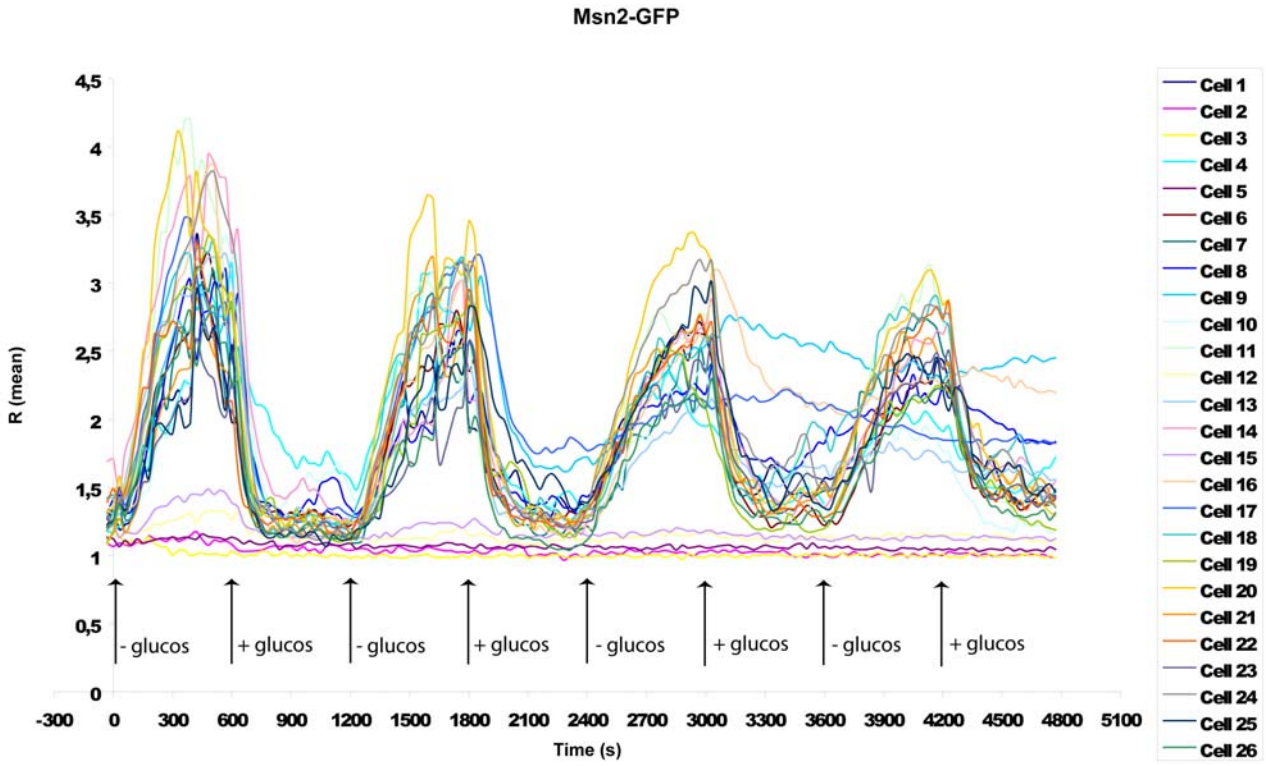




Self assessment: The objective has been achieved for mammalian cells and more than fully achieved for yeast cells, where an impressive repertoire of tools, many suitable beyond the organism, have been generated.

3. **To generate reference quantitative dynamic datasets** following activation and deactivation of the AMPK pathway in yeast and mammalian cells. This reference data set will be used for generating dynamic models of the pathways and to optimise parameters that can not be determined experimentally.

The AMPKIN consortium has generated dynamic datasets for the operation of the yeast Snf1 pathway in yeast and also the AMPK pathway in mammalian cells. This dataset includes time course data over different time scales including metabolic fluxes, concentration of signalling components, changes in the phosphorylation state of critical pathway components (Snf1 kinase, Mig1 regulator) as well as pathway output (gene expression, enzyme activities). These data obtained from cell extracts and hence cell populations have been complemented with data from single cells using flow cytometry (population profiles) as well as optical manipulation and microfluidics for monitoring signalling directly over time. Such analyses indicate that activation (shift from glucose to absence of glucose) of the pathway occurs in minute time scales while pathway deactivation (glucose addition) operates in a sub-minute time scale. This alone is novel information.



The illustration shows single cells scans of Msn2 nuclear entry and exit following altered glucose availability, obtained using the optical manipulation platform developed in work package 2.

Self assessment: The datasets generated allowed building mathematical models of the yeast Snf1 pathway. The available data, however, limited to some extent modelling of the mammalian AMPK. The objective was fully achieved with respect to yeast.

4. **To generate and critically compare dynamic models** for the yeast and mammalian AMPK pathway. To use information from the yeast model to complement gaps in the mathematical description of the mammalian model.

A kinetic model of the Snf1 pathway based on ordinary differential equations has been implemented by partner 2 using MATLAB. The basic model encompasses (1) glucose available to the cells; (2) its conversion to glucose-6-phosphate; (3) the phosphatases and kinases that are activated or inactivated by a glucose-induced signal and control the Snf1 phosphorylation state; (4) Snf1 itself; (5) active and inactive Mig1 as Snf1 target (cf. Snf1 pathway map from WP 1, D 4).

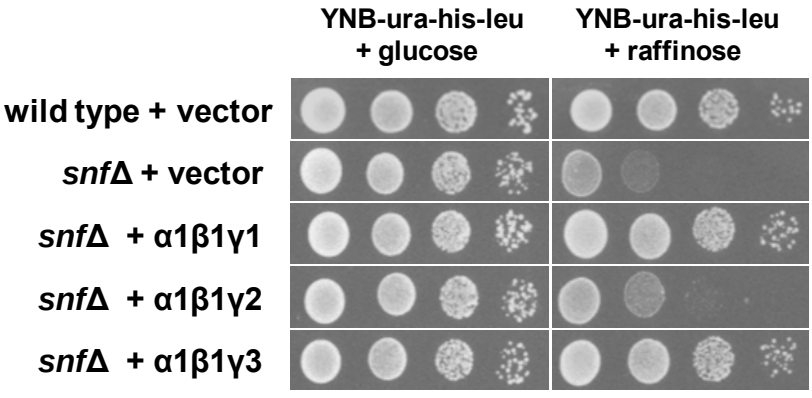
Establishing a dynamic model of the mammalian AMPK activation pathway in analogy to the yeast Snf1 pathway became a less central issue during the course of the project. While it was clear that AMPK's main upstream kinases were LKB1 and CaMKKβ, with the latter having its main role in the calcium stress response system, the mechanism of regulation was not clear, quantitative data were not available and those from the yeast system could not be matched directly to the mammalian system as there were three seemingly redundant upstream kinases of Snf1.

Self assessment: The objective was only fully achieved for the yeast Snf1 pathway.

5. **Generation of tools for system perturbation**, which will be used to generate data for model testing and iterative model improvement and potentially for development of drug screening approaches.

Chimeric constructs between yeast and mammalian gamma subunits were constructed. The yeast Bateman 2 domain (aa 176-320) was replaced by the rat-γ1 Bateman 2 domain (187-338) to construct the Snf4-γ1 chimeric protein. Growth complementation was confirmed on ethanol and raffinose plates. While it appears that phosphorylation of Snf1 is necessary for this complementation, Snf1 phosphorylation at T210 was not upregulated at low glucose in this strain. Hence, the chimeric constructs were not optimal for the purpose of the project.

Instead it was attempted to express the entire mammalian complex in a yeast strain lacking all complex components. All possible combinations of the AMPK subunits were transformed into the *snf*Δ mutant in which all the genes encoding the yeast Snf1 complex (*SNF1*, *SNF4*, *SIP1*, *SIP2*, *GAL83*) have been deleted. The AMPK α1β1γ1 and α1β1γ3 complexes are able to rescue the growth defect of the *snf*Δ mutant on raffinose plates, indicating that the mammalian AMPK complex functions and recognises Snf1 targets in yeast in vivo. Further analyses showed that the heterologous is functional, glucose-regulated and able to perform glucose-regulated Mig1 phosphorylation. The data described above demonstrates that mammalian AMPK complexes can be functionally expressed in yeast. The picture below illustrates that the mammalian AMPK is able to complement the growth defect on raffinose of the yeast mutant lacking the Snf1 complex.



Preliminary studies indicate available compounds known to active AMPK in mammalian cells do not do so in yeast cells. Probably this is due to lack of uptake or high-rate export of the compounds. This is analysed in subsequent studies.

Self assessment: The success of expressing an entire heterologous complex in yeast is a major breakthrough. At this point it is not known why the mammalian complex expressed in yeast does not respond to compounds that are effective in mammalian cells. There are different hypotheses that are tested by subsequent work.

6. **To provide "dynamic" datasets** from experiments employing a range of defined system perturbations in both yeast and mammalian cells with the aim to test and iteratively improve the models and to optimise the underlying parameters.

A significant number of different dynamic datasets was generated in the second project period. Those datasets were in most cases not only useful to generate data for modelling, but also allowed addressing and solving significant biological questions. The experiments included:

- Analysis of Snf1 phosphorylation in cells expressing single activating kinases
- Over-expression of the Snf1 activating kinase *SAK1* to solve the questions why Snf1 can be activated without phosphorylating Mig1
- The effect on Snf1 deactivation after glucose addition caused by deletion of hexokinases
- The effect on Snf1 deactivation caused by deletion of *PGI1* and *ZWF1*
- Deletion of *HXK2* and *REG1* as well as *REG2* affects on the phosphorylation of Snf1
- The effect of the phosphatase Glc7/Reg1/Reg2 complex towards Mig1 in high and low glucose
- The effect of Snf1 phosphorylation under salt stress
- Growth characteristics of the deletion mutants that affect Snf1 activity
- Transcriptional dynamics in the mutants during the transition
- Metabolite dynamics during the transition experiments
- Analysis of Snf1 phosphorylation during the transition
- Dose response and time course for phosphorylation and activation of AMPK following treatment with thrombin
- Incubation of HUVEC with the calcium ionophore ionomycin to activate AMPK and treatment of the cells with a calcium chelator to block the effect of thrombin on AMPK phosphorylation
- AMPK phosphorylation and activity in response to thrombin in the presence of the CaMKK inhibitor STO-609

Self assessment: A wealth of quantitative and dynamic data has been generated both for the yeast and the mammalian AMPK pathways. This objective has been fully fulfilled.

7. **To generate iteratively improved mathematical models** in order to determine system properties and to provide an assessment of similarities and dissimilarities of the models in yeast and mammalian cells and hence of the significance and the limitations of the approach of comparative modelling from experimental and theoretical perspectives.

For a better understanding of the overall processes governing growth of yeast cells, we adapted and expanded an existing cybernetic growth model for yeast in fermentors and batch cultures. The cybernetic model encompasses both variables for the relevant nutrients, cell mass and oxygen as well as abstractions of internal enzyme levels, using one "key enzyme concentration" variable per

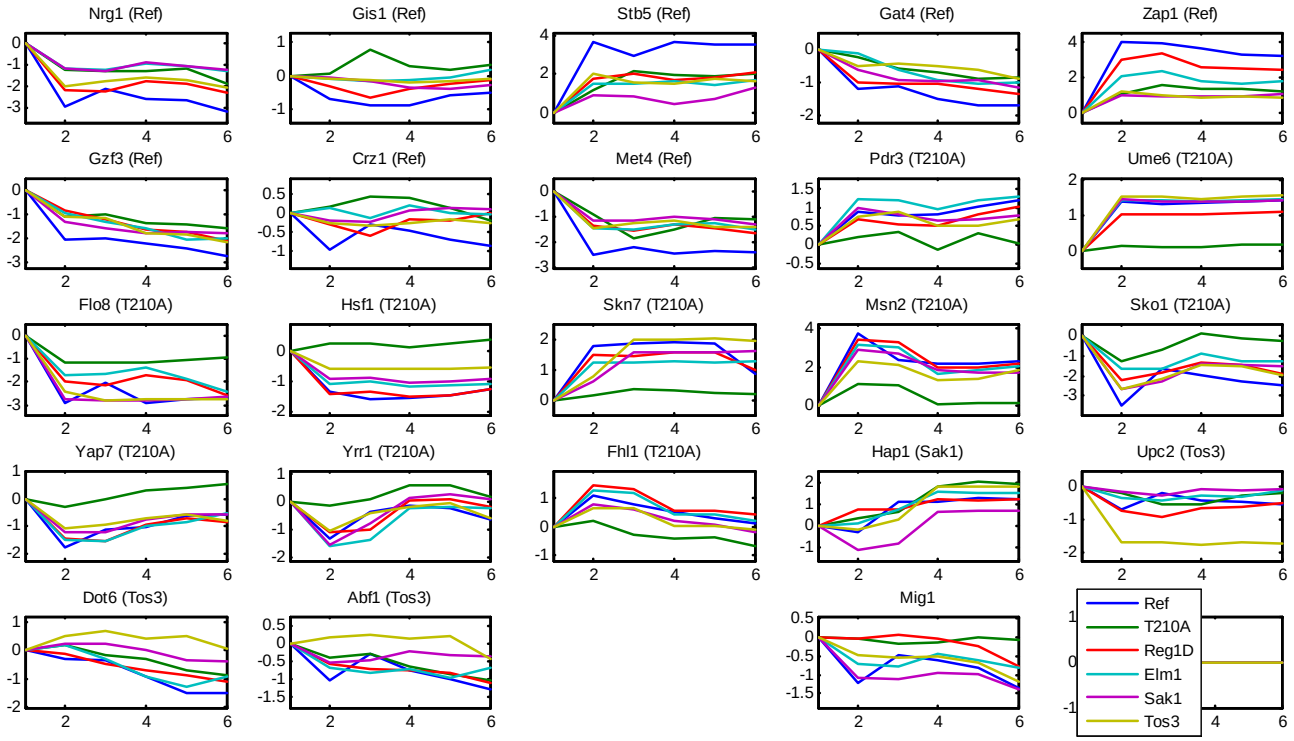
fundamental growth process (glucose fermentation, glucose oxidation, ethanol oxidation). The model is able to reproduce the observed behaviour both under aerobic and anaerobic conditions

For each of the six strains from the upstream kinase knockout experiment, six time points of the expression profiles of all known yeast genes were available. We used Network Component Analysis in order to infer information about transcription factor activity levels from these data. To our knowledge, NCA has not been applied on a full genome scale so far. We explored different ways of optimising the NCA algorithm, its sensitivity to its initialisation (an approximation has to be perfomed) as well as different ways of applying NCA. The resulting >100 TF profiles cover a wide range of the profile space and do not show obvious patterns at first. What can be seen from the profiles is that if a TF profile differs in one strain compared to the other five, it is mostly in the T210A strain. This means that the mutation in this strain is more significant on the TF level than any of the others, which is consistent with the observation that the strain shows the most altered growth pattern.

The Snf1-pathway model was developed further. Experimental data for extracellular glucose, cell density, Snf1 and Mig1 intensities provided the basis for the development of the mathematical model. Details of the signal that is generated during the first step of glycolysis and accounts for regulation of Snf1 are still not known. It is also still unknown whether this signal regulates Snf1 via the upstream kinases (UKs), the phosphatase (PP1) or both. We therefore established nine different models, differing in the upstream regulator (UK, PP1 or both) as well as including an X-factor that binds to Snf1P and defends it from becoming dephosphorylated. For all nine models, the differential equations for OD and external glucose (and the transporters Hxt1 and Hxt2) are the same. All models could reproduce the experimental data - but to very different levels of quality. The overexpression experiment of UK could only be reproduced with the UKPP1_X2 model (regulation of both, UK and PP1, and Snf1PX and Snf1P are kinases for Mig1). The simulation showed that Snf1P forms a complex with X, so that no free X remains but all X ends up in the Snf1PX complex. Thus, the Snf1PX complex is robust for Snf1P increase (e.g. through UK overexpression). For this observation, X_{tot} must be smaller than $Snf1_{tot}$. The simulation shows, that Mig1 regulation can be achieved by PP1 regulation alone, Snf1PX constantly phosphorylating Mig1.

The picture below shows reconstructed transcription factor profiles based on a model.

Self assessment: The objective was well achieved for the yeast system. Modelling combined with experimentation allowed addressing different relevant research questions, such as the role of feedback and feed-forward loops. In this way, modelling was closely integrated with experimentation to address and solve research problems.



8. **To use the mathematical models** to predict the result of pharmacological and genetic system perturbations and where possible assess those experimentally and thereby test the predictive value of the models.

Mammalian AMPK subunits (α1, α2, β1, β2, γ1, γ2 and γ3) were sub-cloned into yeast expression vectors in order to allow co-expression of the heterotrimeric AMPK complex in yeast (lacking expression of SNF1). Complexes containing a1 were expressed and rescued growth of yeast on raffinose. Moreover, expression of AMPK resulted in increased phosphorylation of Mig1, a downstream target for SNF1. These findings confirm that complexes (containing α1) can be expressed as functionally active AMPK in yeast. In order to determine whether the system could be used to screen for AMPK activators, 3 compounds (5-amino-4-imidazolecarboxamide riboside (AICAR), metformin and phenformin) that have previously been shown to activate AMPK in mammalian cells were used. However, incubation of yeast with varying concentrations of all of these compounds did not result in activation of AMPK. Our findings indicate that compounds that activate AMPK indirectly in mammalian cells (requiring some form of metabolism for their actions) may not activate AMPK in yeast. It will be important to determine whether direct activators of AMPK activate in the yeast system. To date, only one direct activator of AMPK has been described (A769662). We have been unable to obtain sufficient quantities of this compound to test in the yeast system.

Self assessment. This objective was party achieved. A problem in this context was that yeast Snf1 does not respond to compounds that are effective on mammalian AMPK. Remarkably, also AMPK expressed in yeast does not respond to those compounds. This may be due to limited uptake and rapid export of the compounds from yeast, but could also indicate that the effects of those compounds is indirect and requires proteins that are not part of the AMPK complex. Those alternatives are presently explored.

Project impact

The AMPKIN consortium did not produce results of potential commercial value. Instead, the main goal of AMPKIN was to increase our understanding of the dynamics of Snf1 and AMPK signalling and to advance quantitative, "systems-level" analyses of signalling pathways. For this, AMPKIN chose to bring together groups that perform experiments and collect data with a group that generates mathematical models of entire signalling systems are parts thereof. In addition, the project also planned to employ modelling in the context of drug development. Despite the fact that the industrial partner Arexis/Biovitrum first changed business model and then left the project, this goal was at least partly achieved

The integrated approach of AMPKIN has been extremely successful. Also the group in the project that did not previously collaborate with modellers, did see the value of quantitative analyses. Also the idea of basing experimental studies on computational simulations was followed to a significant, although perhaps not to the maximally possible extend.

AMPKIN ensured its impact in the very first place by publication in the scientific literature. Although the project has published a number of individual and joint papers already during its lifetime, numerous of the results of AMPKIN will only be published within about 12-18 months after the end of the project.

AMPKIN has further ensured its impact via organising two conferences/workshops. The first one was held on Rostock in September 2006 and confined to 40 participants. It brought together three related EC-funded projects QUASI, AMPKIN and COSBICS. This workshop has resulted in valuable interaction between those projects. In addition, AMPKIN participated in organisng the 2008 International Conference on Systems Biology in Göteborg, where a project poster was presented with the conference "Arena". This setting allowed interaction with other EC_funded projects and further initiatives.

The tools that AMPKIN generated for quantitative analyses, such as for instance for single cell analysis, inhibition of protein kinases and mathematical modelling are generally applicable, i.e. not confined to the use of yeast as experimental organism. Those tools are already being used in other projects both by groups involved in AMPKIN and other groups. Tools and reagents will be used further in the UNICELLSYS project, where kinetic modelling will be pushed significantly ahead within a consortium of leading experimentalists and modellers, including the AMPKIN partners 1 and 6.

AMPKIN has generated new knowledge on Snf1/AMPK signalling, including knowledge on different feedback mechanisms or via direct, phosphorylation-mediated feedback on signalling components, the link between signalling and metabolism and comparative analysis in two different organism. Obtained with yeast as a model system this knowledge has general applicability given the conservation of the basic design of Snf1/AMPK pathway and their importance for human health. AMPKIN has and will publish its results in high impact journals thereby ensuring dissemination of its results.

2. Dissemination and use

Section 1 - Exploitable knowledge and its Use

AMPKIN has not produced any results of commercially exploitable nature but foresees that the following results could have such value:

1. Yeast strains expression mammalian AMPK components or yeast-mammalian chimera of such components could be suitable for drug screening.
2. Computational models of the mammalian AMPK pathway could have potential value in drug target discovery and drug development.

Overview table

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Time table for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved
Yeast strains expression mammalian AMPK components or yeast-mammalian chimera	Yeast strains expression mammalian AMPK components or yeast-mammalian chimera	Drug screening	Unknown	Possible	Partners 1 and 3
Computational models of the mammalian AMPK pathway	Computational models of the mammalian AMPK pathway	Drug target identification and drug development	Unknown	Possible	Partners 2 and 3

Section 2 – Dissemination of knowledge

Overview table

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Spring 2006	Project web-site	Research/ public	Global	Hundreds	Partner 1 and 2
Fall 2006	Workshop	Research	Europe	40	Partner 2/All
Constantly	Publications	Research	Global	Hundreds	All
Spring 2006	Posters	Research/public	Determined by EC	Thousands	Partner 1
Spring 2006	Flyers	Research/public	Determined by EC	Thousands	Partner 1
Fall 2008	Project poster	Research, policy makers	Global	1,050	Partner 1

1. Press release

None

2. Conference

A joint workshop has been held together with QUASI and COSBICS. This is deliverable 38.

AMPKIN has been actively involved in the International Conference on Systems Biology, organised by the coordinator. An "Arena" (extended poster presentation) was presented at the conference featuring the goals and main results of the project.

3. Publications

1. Optical systems for single cell analyses K. Sott, E. Eriksson, E. Petelenz, and M. Goksör Expert Opinion on Drug Discovery, 3 (11), 1323-1344, (2008).
2. Optical manipulation and microfluidics for studies of single cell dynamics E. Eriksson, J. Scrimgeour, A. Graneli, K. Ramser, R. Wellander, J. Enger, D. Hanstrop, and M. Goksör Journal of Optics A Pure and Applied Optics 9 (8), S113-S121, (2007).
3. A microfluidic system in combination with optical tweezers for analyzing rapid and reversible cytological alterations in single cells upon environmental changes E. Eriksson, J. Enger, M. Goksör, K. Ramser, D. Hanstorp, N. Erjavec, T. Nyström and S. Hohmann Lab on a Chip, 7, 71-76, (2007).
4. Acquisition of Single Cell Data in an Optical Microscope Kristin Sott, Emma Eriksson and Mattias Goksör
5. Lab on Chip Technology (Vol 2): Biomolecular Separation and Analysis (2009) Herold K.E. and Rasooly A. (Eds) Caister Academic Press, UK

6. A micro-fluidic system for studies of stress response in single cells using optical tweezers Optical Trapping and Optical Micromanipulation. A. Granéli, E. Eriksson, J. Enger, K. Ramser, M. Goksör, S. Hohmann and D. Hanstorp. Proceeding of SPIE, 6326, (2006).

7. Holographic optical tweezers combined with a microfluidic device for exposing cells to fast environmental changes. Emma Eriksson, Jan Scrimgeour, Jonas Enger and Mattias Goksör Bioengineered and Bioinspired Systems III, Proceeding of SPIE, 6592, 0P1-3, (2007).

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9. Denison, F.C., Hiscock, N.J., Carling, D. and Woods, A. (2009). Characterisation of an alternative splice variant of LKB1. J. Biol. Chem. 284, 67-76.

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12. Carling, D. (2007). The role of the AMP-activated protein kinase in the regulation of energy homeostasis. Novartis Found. Symp. 286, 72-81.

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14. The pathway by which the yeast protein kinase Snf1p controls acquisition of sodium tolerance is different from that mediating glucose regulation. Microbiology. 2008 Sep;154 (Pt 9):2814-26. (Tian Ye, Karin Elbing and Stefan Hohmann)

15. Genetic basis of arsenite and cadmium tolerance in Saccharomyces cerevisiae. BMC Genomics. 2009 Mar 12;10:105. (Thorsen M, Perrone GG, Kristiansson E, Traini M, Ye T, Dawes IW, Nerman O, Tamás MJ.)

16. S.Frey, Th.Millat, S.Hohmann, O.Wolkenhauer: How quantitative measures unravel design principles in multi-stage phosphorylation cascades. Journal of Theoretical Biology, 254, 27-36, May 2008

17. S.Frey, O.Wolkenhauer, T.Millat: Quantifying properties of cell signaling cascades. In 'Control-Theoretic Approaches in Systems Biology', P.A.Iglesias and B.Ingalls (editors), MIT press, to appear November 2009

18. R. Usaite; J. Nielsen; L. Olsson (2008) Physiological characterization of glucose repression in the strains with SNF1 and SNF4 genes deleted. J. Biotechnol. 133:73-81

19. R. Usaite; J. Wohlschlegel; J. D. Venable; S. K. Park; J. Nielsen; L. Olsson; John R. Yates III (2008) Characterization of global yeast quantitative proteome data generated from the wild type and glucose repression Saccharomyces cerevisiae strains: the comparison of two quantitative algorithms. J. Proteom Res. 7:266-275

20. R. Usaite; M. C. Jewett; A. P. Oliveira; J. R. Yates III; L. Olsson; J. Nielsen (2009) Reconstruction of the yeast Snf1 kinase regulatory network reveals its role as a global energy regulator. Mol. Sys. Biol., in press

21. Farzadfard, F. M.L. Nielsen, J. Nielsen, G.N. Vemuri. Metabolic and transcriptional dynamics during the transition from carbon limitation to nitrogen limitation in Saccharomyces cerevisiae (in preparation)

22. G.N. Vemuri, F. Farzadfard, A. Bittig, O. Wolkenhauer, J. Nielsen. 2009. Dissecting the role of kinases of Snf1 in Saccharomyces cerevisiae (in preparation).

4. Contributions to conferences

A. Bittig, J.Hofmeyr. O.Wolkenhauer: An abstract cell model. Winter Simulation Conference (WSC), December 2006

2. S.Frey, T.Millat, K.Rateitschak, O.Wolkenhauer: Quantitative measures to characterize properties of signal transduction pathways. Winter Simulation Conference (WSC), December 2006

A. Bittig: SysBioMed Winter School, Tenerife, February 2007

3. S.Frey: SysBioMed Winter School, Tenerife, February 2007

4. J. Zhang: Glucose Regulation and Snf1 Protein Kinase in Saccharomyces cerevisiae. 2nd Danish Conference on Molecular Biology and Biotechnology May 24 - 25, 2007, Vejle, Denmark

5. J. Zhang: Glucose Regulation and Snf1 Protein Kinase in Saccharomyces cerevisiae. ICSB 2007 : The Eighth International Conference on Systems Biology October 1 - 6, 2007, Long Beach, CA, US

. Wolkenhauer: "Omics: Assembling Systems Biology“ conference, June 2007, Ascona.

. Wolkenhauer: Keynote speech at the 1st MathWorks Computational Biology Conference, Basel, Mai 2007

6. D. Carling: “The AMPK pathway in health and disease.” 7th Symposium on Type 2 diabetes and obesity, Stockholm, Sweden (April, 2006)

7. D. Carling: “The AMPK pathway in health and disease.” 4th AMPK meeting, Snowmass, USA (August 2006)

8. D. Carling: “The AMPK pathway in health and disease.” 42nd EASD Annual Meeting, Copenhagen, Denmark (September 2006)

9. D. Carling: “The AMPK pathway in health and disease.” Novartis Foundation Symposium, Beijing, China (October 2006)

10. D. Carling: “The AMPK pathway in health and disease.” AMPK in Obesity, Quebec, Canada (October 2006)

11. D. Carling: “The AMPK pathway in health and disease.” Steiner Foundation, Metabolism and Cancer, Brunnen, Switzerland (November 2006)

12. D. Carling: “The AMPK pathway in health and disease.” ASBMB Meeting, Washington USA (May 2007)

13. J. Nielsen (2006) Model driven data integration in yeast systems biology, Genome to Systems. Manchester, UK

14. J. Nielsen (2006) Reporter features: A tool for mapping global control in metabolism through model driven data analysis. ISSY25, Helsinki, Finland

15. J. Nielsen (2007) Integrated analysis of yeast metabolism. ASM Annual Meeting, Toronto, Canada

16. 42nd EASD Annual Meeting, Copenhagen, Denmark (2006).

17. Novartis Symposium on Fatty Acids and Lipotoxicity in Obesity and Diabetes, Beijing, China (2006).
18. Steiner Foundation, Metabolism and Cancer, Brunnen, Switzerland (2006).
19. AMPK in obesity, Quebec, Canada (2006).
20. ASBMB Meeting, Washington, USA (2007).
21. 5th AMPK meeting, Copenhagen, Denmark (2008).
22. Gordon Research Conference (Growth Factors and Signaling), Oxford, UK (2008).
23. 69th ADA Scientific Sessions, New Orleans, USA (2009)
24. G. Beltran, D. Bosch, R. García-Salcedo, K. Elbing and S. Hohmann: Systems Biology of the yeast AMP-activated Protein Kinase Pathway. Yeast 2007. XXIII International Conference on Yeast Genetics and Molecular Biology. Melbourne, Australia, July 2007
25. G. Beltran, D. Bosch, R. García-Salcedo, K. Elbing and S. Hohmann: Systems Biology of the yeast AMP-activated Protein Kinase Pathway. Yeast Genetics Meeting Cold Spring Harbor. New York, August 2007.
26. R. Garcia-Salcedo, K. Elbing, S. Manchala and S. Hohmann: Quantitative and functional análisis of Snf1-pathway components. 9th International Conference on Systems Biology (ICSB). Gothenburg, Sweden, August 2008
27. R. Garcia-Salcedo, K. Elbing, S. Manchala and S. Hohmann: Quantitative and functional análisis of Snf1-pathway components. Annual meeting of the Swedish Society for Biochemistry and Molecular Biology (SFBM). Gothenburg, Sweden, September 2008
28. Tian Ye, Karin Elbing and Stefan Hohmann The functional relationship between Hxk2 and the Snf1 pathway in yeast carbon catabolite repression. Annual meeting of the Swedish Society for Biochemistry and Molecular Biology (SFBM). Gothenburg, Sweden, September 2008
29. Tian Ye, Karin Elbing and Stefan Hohmann The functional relationship between Hxk2 and the Snf1 pathway in yeast carbon catabolite repression. Annual meeting of the Swedish Society for Biochemistry and Molecular Biology (SFBM). Gothenburg, Sweden, September 2008
30. S.Frey, T.Millat, S.Hohmann, O.Wolkenhauer: How Quantitative Measures unravel design Principles in multi-stage Phosphorylation Cascades. 9th International Conference on Systems Biology (ICSB), Gothenburg, Sweden, August 2008
31. S.Frey, H.Schmidt, K.Rateitschak, O.Wolkenhauer, G.Beltran, R.Garcia-Salcedo, K.Elbing, D.Bosch, T.Ye, S.Hohmann: Modelling Snf1 regulation in Saccharomyces Cerevisiae. 9th International Conference on Systems Biology (ICSB). Gothenburg, Sweden, August 2008
32. Arne T. Bittig, David Carling & Olaf Wolkenhauer: A Mathematical Model of the Regulation of AMPK Activation, Poster the ICSB 2008, Göteborg, Sweden, August 2008, also at the Systems Biology for Medical Applications Summer School, Tenerife, September 2008
33. Arne T. Bittig, Henning Schmidt: Format Overflow? Handling of Modeling Projects in Systems Biology, Poster for the ICSB 2008, Gothenburg, August
34. G.N. Vemuri, J. Nielsen, 2008. Rational vs combinatorial approaches to metabolic engineering. Metabolic Engineering VII, Puerto Vallarta, Mexico.
35. G.N. Vemuri 2008. Bakers yeast as a model for studying metabolic disorders. Yeast Systems Biology Workshop, International Conference on Systems Biology, Göteborg.

36. G.N.Vemuri, J. Nielsen. 2008. Why does yeast make ethanol? International Study Group on Systems Biology, Helsingør, Denmark.
37. Posters:
38. G.N. Vemuri, J. Nielsen, L. Olsson. 2006. Metabolic responses to redox perturbations in Saccharomyces cerevisiae. Metabolic Engineering VI, Noordwijkerhout, The Netherlands.
39. Beta-subunit of Snf1 Protein Kinase in Saccharomyces cerevisiae is Partially Redundant in glucose repression. The 27th International Specialized Symposium on Yeasts (ISSY27), Aug 26-29, 2009, France
40. Beta-subunit of Snf1 Protein Kinase in Saccharomyces cerevisiae is Partially Redundant in glucose repression The 9th International Conference on Systems Biology (ICSB2008), Aug 22-28, 2008, Sweden
41. Beta-subunit of Snf1 Protein Kinase in Saccharomyces cerevisiae is Partially Redundant in glucose repression The 8th International Conference on Systems Biology (ICSB2007), Oct 1-6, 2007, USA

5. Project website

The project website is running at (http://www.sbi.uni-rostock.de/ampkin).

6. Posters and flyers

Those have been delivered to the EC services.

Section 3 - Publishable results

AMPKIN has not produced exploitable results that are ready for publication.

3. Final management report

Form C Financial statement per activity for the contractual reporting period, to be completed by each contractor (see Appendices 5-11)

These items are attached to the report as separate items

Audit certificates
These items are attached to the report as separate items

4. Final report on the distribution of the Community's contribution

To be prepared to the coordinator following the final payment by the EC

5. Final science and society reporting questionnaire

Attached to the report as separate file.

6. Final reporting questionnaire on workforce statistics

Attached to the report as separate file.

7. Final socio-economic reporting questionnaire

Attached to the report as separate file.