SMP: Protein

Project: Dynamic Modelling and Simulation of Signal Transduction Pathways

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Introduction

Many neuro- and cancer-related diseases can be considered a failure of communication at the molecular level. Research in *cell signalling* is concerned with the investigation of pathways that transmit information from receptors to specific intracellular targets, most prominently transcription factors regulating gene activation. Such signal transduction pathways form complex networks that regulate cell differentiation, maturation, apoptosis etc., and therewith the development and health of an(y) organism.
As the above title suggests, this sub-project explores the theoretical approaches of mathematical modelling and computer simulation in the context of pathways that are relevant to other partners in the SMP Protein.

Rac/Rho GTPases in Cell Biology

The Ras-related Rac and Rho proteins are small GTPases that act as signals. One of their potential downstream targets is actin polymerisation. Rac and Rho have been found to regulate many cellular activities such as cytoskeleton and cell adhesion, endocytosis, vesicle trafficking and gene transcription (Etienne-Manneville and Hall 2002). Rac and Rho proteins also control the progression through the cell cycle and a variety of differentiation events, including cell fate specification, cell proliferation, polarity and migration. Inappropriate activation of Rac/Rho pathways can cause developmental defects and lead to tumor formation (oncogenesis). Because of this involvement in human cancers Rac/Rho signalling has become a field of increasing research interest.

Huntington's Disease and Htt

Huntington's disease, an inheritable neurodegenerative disorder, leads to the early decline of physical and intellectual capacities (i.e. dementia). One cardinal feature of the disease is that it affects neurons in the body and in the brain. Through apoptotic cell death, the brains of Huntington patients lose neurons but what activates the cell death pathway in diseased brains?
The mutation of the HD gene was found to be responsible for Huntington's disease. This gene was mapped to chromosome 4 in 1983 (Gusella et al. 1983) and cloned in 1993 (HD Collaborative Research Group 1993). It produces a 350k protein designated huntingtin (htt). A characteristic expansion of the nucleotide triplet repeat CAG in the huntingtin gene translates into a mutant huntingtin protein with an expanded N-terminal polyglutamine stretch (N-htt). The cleavage products of N-htt form aggregates in diseased brains but the question remains: how does mutant htt cause neuronal cell death (apoptosis)?
Biochemical and cell biological studies in mice point to a role for the proteins Hip-1 and Hippi in caspase-8 activation and the initiation of apoptosis during the pathogenesis of Huntington's disease (Gervais et al. 2002). The key seems to be in the regulation of the interaction of Hip-1 with its partners huntingtin and Hippi. The two compete for Hip-1, and N-htt has a lower affinity than normal huntingtin. The Hip-1/Hippi complex can activate caspases, the effector enzymes of apoptosis. This is maybe a mechanism by which aggregation of N-htt induces apoptosis.
Furthermore, huntingtin associates in vitro with microtubules and possibly interferes with the process of assembly and disassembly of microtubules. Both wild-type (htt) and mutant huntingtin (N-htt) can associate with microtubules to almost the same degree. This suggests that huntingtin has a role in intracellular organelle transport and axonal growth.
A scheme of the huntingtin network containing some of the known interactions is shown in Figure 1 below.

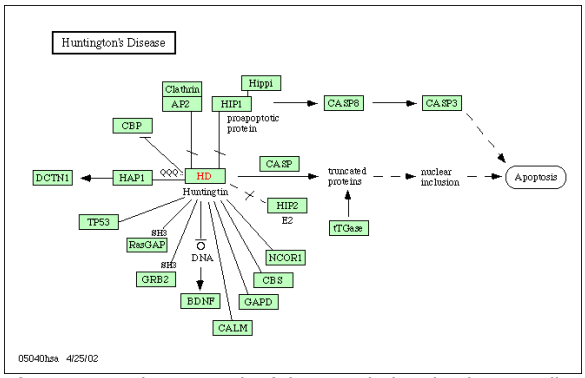

Fig 1: *Interaction network of the protein huntingtin according to the public KEGG database.*

Rac/Rho and Htt

Results from yeast two hybrid (Y2H) screens carried out as part of the SMP Protein (Goehler et al. 2004) suggest that there is a connection between the affects of Rac and Rho proteins and the concentration of huntingtin, the protein known to be accumulated in Huntington's disease. Both the protein interactions of Rac/Rho GTPases and the huntingtin-network are the focus of this theoretical project.
Mathematical modelling and simulations can facilitate the quest for the mechanisms of cellular control and regulation by signal transduction pathways. In this project, they are used especially in the analysis of huntingtin aggregation and its possible cross talk with other cell signalling pathways.

From Experiments to Models – and Back

The modelling and simulation of signalling pathways aims to make sense of experimental data. Herein, it is the data that is provided by the experimental groups (TP1.1-TP4.1) within the SMP Protein network. It is obtained employing different techniques and protein analytic methods, e.g. cDNA cloning, antibody production, protein arrays, yeast two hybrid screening, etc. The data (protein protein interaction networks, expression patterns, structural properties, etc.) is used to build the model, to refine it, and to further the modelling process.
In the inverse direction, the experimental groups can benefit from the theoretical modelling when its results lead to improved designs of experiments.
This sub-project is most closely related to experimental sub-projects through the analysis and modelling of their primary data, in particular with projects TP3.1 (protein protein interactions) and TP3.3 (protein complexes). There will also be cooperation with the other non-experimental sub-project TP5.1 (data integration).

Project Status

SInce the start of the sub-project (March 2005), we compiled a list of relevant proteins, i.e. proteins that need being considered for building the mathematical model and for designing the protein assay experiments that are to provide data which is of use for dynamic modelling (time series).

NGFN Nationales Genomforschungsnetz
GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

We also started building the model by using the information available through published research articles, database entries (from databases: Entrez Protein (NCBI), HGNC, GenomeCube (RZPD), BIND and KEGG) and data that was generated through the SMP Protein.

Modelling the Pathway

The function of huntingtin is still unknown but several huntingtin-associated proteins, e.g. HAP1, HIP-1, ubiquitin-conjugating enzyme (HIP-2) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH), have been reported (Harjes and Wanker 2003). Some were already mentioned in the introduction above (see also in Figure 1).
A key role seems to be played by the huntingtin interacting protein 1 (HIP-1). By immunostaining in cortical pyramidal neurons of mouse brain, it was shown that the interaction between Hip-1 and huntingtin on the one hand, and between Hip-1 and Hippi on the other, are mutually exclusive, and that Hip-1/Hippi complexes can activate caspases, the effector enzymes of apoptosis (Gervais et al. 2002).
Hip-1 interacts with huntingtin but the affinity of Hip-1 for mutant htt is much lower than its affinity for wild-type htt. So in diseased mouse brains, levels of the Hip-1/Hippi complex are relatively higher than the levels of the Hip-1/htt complex. The proenzyme procaspase-8 is recruited to Hip-1/Hippi complexes. This suggests that Hip-1 and Hippi cooperate to induce apoptosis in a caspase-8-dependent manner (dimerisation activates procaspase-8, thereby initiating the apoptotic cascade).
Figure 2 summarises these relations schematically.

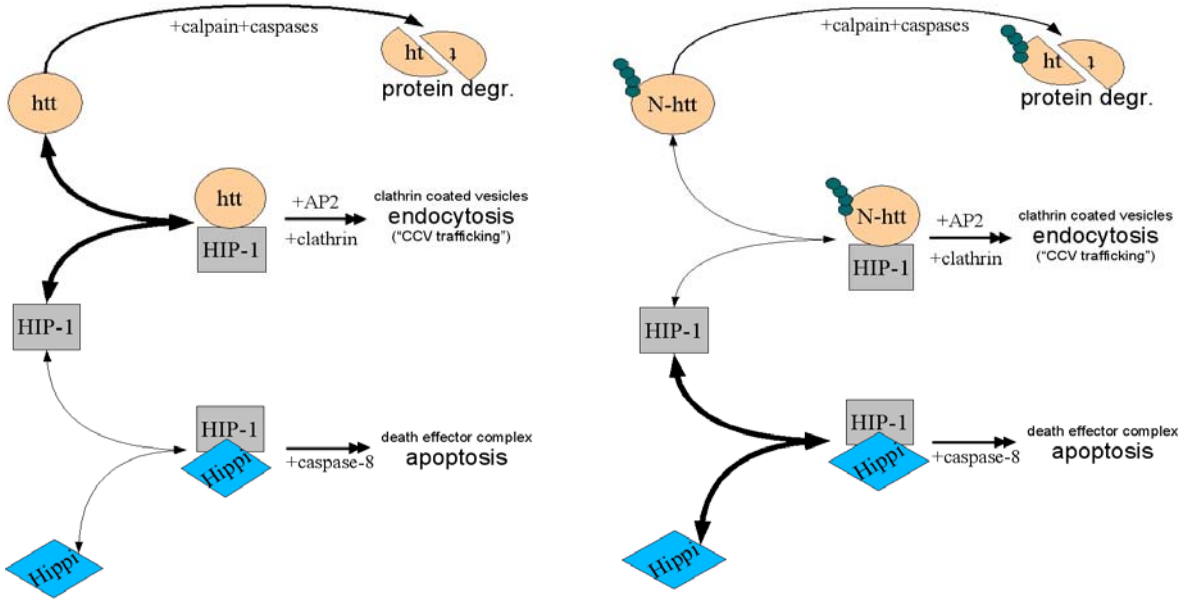

Fig 2: *A schematic representation of the interplay of the two forms of huntingtin (normal htt; mutant N-htt) with HIP-1 and Hippi. In humans, htt becomes N-htt if the length of the polyQ is greater than 36 amino acids.*

A mathematical model can facilitate the quest for intrinsic mechanisms of cellular control and regulation by signal transduction pathways. In such a model the dynamic interactions of the proteins that form the complex signalling network are encoded as non-linear ordinary differential equations (ODE's). This allows for the modelling of transient changes in protein concentrations (whereas a protein protein interaction network is a rather static representation).

Model Interrogation

For simulating and interrogating the model standard software is used (free and licensed, e.g. XPP, MatLab).
A mathematical model can be interrogated at any state of its development but the more refined it is the more predictive power it has. First preliminary results from the analysis and simulation of the model have to be investigated further, before conclusions can be drawn.

Disease-Relevant Proteins

A comparison of the list of proteins mentioned above with the proteins already used in the Y2H screenings and in the experiments of the other SMP Protein sub-projects is next in order to check the availability of clones, antibodies etc. If necessary (in the case of proteins not yet considered by the experimental groups), possibilities of acquisition have to be discussed.

Outlook

The robustness of the model against perturbations and its sensitivity to input signals will be analysed, and the emergence of multistability and oscillations by positive and negative feedback loops investigated. Further interrogation of the model and computer simulations will produce hypotheses and predictions that can be tested experimentally. This again allows to further improve, iteratively refine, and expand the model.

Lit.: ***1.*** *Etienne-Manneville S & Hall A. Rho GTPases in cell biology. Nature. 2002 Dec 12;420(6916):629-35.* ***2.*** *Gusella JF et al. A polymorphic DNA marker genetically linked to Huntington's disease. Nature. 1983 Nov 17;306:234-8.* ***3.*** *The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993 Mar 26;72(6):971-83.* ***4.*** *Gervais FG et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nature Cell Biology 2002 Jan 14;4:95-105.* ***5.*** *Goehler H et al. A Protein Interaction Network Links GIT1, an Enhancer of Huntingtin Aggregation, to Huntington's Disease. Mol Cell. 2004 Sep 24;15(6):853-65.* ***6.*** *Harjes P & Wanker EE. The hunt for huntingtin function: interaction partners tell many different stories. Trends Biochem Sci. 2003 Aug;28(8):425-33.*