# **Systems Biology**

# Towards an Understanding of the Dynamics of Life



gain greater insight into the mechanism that determine the functions of genes and cells, and ultimately their consequences at physiological or phenotypic level. In systems biology, this is achieved through mathematical modelling and simulation. The emergence of systems biology signals a shift of focus away from molecular characterisation of the components in the cell to an understanding of functional activity through temporal changes and dynamic interactions in cells. Pathways (i. e., regulated networks of biochemical reactions) are the conceptual framework of molecular and cell biology, employed to describe inter- and intra-cellular dynamics.

#### **Cell Signalling**

Fig. 1 provides a (dramatic) simplification of the dynamic processes involved in cells signalling. Signal transduction pathways commonly consist of a large number cascaded modules between receptor and genome. There can be numerous intermediate steps before the

Ligands

ver the last decade, the areas of bioinformatics and genomics have identified the nuts and bolts of the machinery that make up a living cell. Scientists are now embarking on an endevour to discover how the many components interact to create the complex behaviour that underlies development and disease.

#### Systems Biology

A principal challenge for the life sciences is to understand the "organisation" and "dynamics" of those components that make up a living system, i. e., to investigate the spatio-temporal relationships between the elements that give rise to cause and effect in living systems. A major problem is that networks of cellular processes are regulated through complex interactions among a large number of genes, proteins, and other molecules. From these considerations, the fundamental goal of systems biology is to understand the nature of this regulation in



Fig. 1: Cell signaling (signal transduction): The expression of information in the genome can be affected by extracellular signals. Receptors spanning the cell membrane receive signals (by binding with ligands) and transmit the information to activate intracellular proteins (the response regulator), which can lead to changes in the gene expression programme. In the figure, the response regulator binds to the operator region of a gene and prevents the RNA polymerase from reading the adjacent gene. Disruptions or errors of the signal transfer can have catastrophic consequences and may lead to diseases, including cancer. signal transduction process ends, often with a change in the gene expression program of the cell.

In addition to crosstalk between pathways, negative feedback loops are a key regulatory mechanism. Mathematical modeling and simulation in this field has the purpose to help and guide the biologist in detecting feedback loops, nonlinear (and thus counter-intuitive) interactions. It further helps in designing experiments, suggesting which variables should be measured and why. Applications of dynamic pathway modeling (i.e. the simulation of dynamic interactions among proteins) are in drug target identification and validation.

# Dynamic Pathway Modelling and Simulation

We argued that it is dynamic interactions of gene products and other molecules, not the genome in itself, that gives rise to biological function, regulation and control. For our experimental designs this means that instead of trying to identify genes as causal agents for some change, function or phenotype, we should relate these observations to sequences of events.

Conducting time course experiments that investigate signal transfer and transient behaviour form the basis for dynamic pathway modelling. The aim is then to identify the direction and strength of relationships between variables in a pathway. The fact that most of these relationships are nonlinear, and that feedback connections exist, makes this is a non-trivial problem. Nonlinearity implies a breakdown of the superposition principle (i.e. the whole is more than the sum of its parts). As a consequence, observations often do not match an intuitive or expected response (see Fig. 3). Feedback loops provide a different challenge in that their existence is often a key aspect of the biological investigation but mathematical formalisms to detect and quantify them are in short supply.

As an example, we consider the Ras/Raf-1/MEK/ERK module that conveys signals from the cell membrane to the nucleus. This kinase cascade is spatially organized in a signalling complex nucleated by Ras proteins. The small G protein Ras is activated by many growth factor receptors and binds to the Raf-1 kinase with high affinity when activated. This induces the recruitment of Raf-1 from the cytosol to the cell membrane. Activated Raf-1 then phosphorylates and activates MAPK/ERK Kinase (MEK), a kinase that in turn phosphorylates and



Fig. 2: Graphical representation of a mathematical model that consists of sixteen nonlinear ordinary differential equations with 17 parameters. The model investigates the influence RKIP has on the Ras/Raf-1/MEK/ERK pathway. The figure is read from left to right: from growth factor stimulation of the receptors, recruitment of Raf-1 and binding to Ras, followed by the activation of Raf-1 and MEK, to the activation and translocation of ERK. These graphical representations are translated into mathematical equations. Subsequent simulation studies of this model (see Figure 3) help the experimental scientist in formulating hyptheses and design his experiments.

activates Extracellular signal Regulated Kinase (ERK), the prototypic Mitogen-Activated Protein Kinase (MAPK). Activated ERKs can translocate to the nucleus and regulate gene expression by the phosphorylation of transcription factors. This kinase cascade controls the proliferation and differentiation of different cell types. The specific biological effects are crucially dependent on the amplitude and kinetics of ERK activity – hence the need for mathematical modelling of the dynamics.

Walter Kolch from the Cancer Research UK, Beatson Laboratories in Glasgow, identified a novel protein (RKIP) that affects the Ras/Raf-1/MEK/ERK module (see Fig. 2). Western blot time course experiments were conducted and a mathematical model constructed. The entire pathway is thereby composed of template modules that allow a graph representation, and each of which is associated with a set of four nonlinear ordinary differential equations. Once a model structure is established and the model parameters are determined, simulations of the mathematical model for different initial concentrations of RKIP can be conducted very easily as shown in Fig. 3.

While, at present, we usually cannot generate complete and accurate data sets for parameter estimation, the value of such simulations leis largely in the generation of hypotheses and in supporting experimental design. Simulating different model structures or conducting a sensitivity analysis to identify key variables of a system can be done within minutes on a computer, helping us to decide which variables to measure and why.



Fig. 3: The two figures show the dynamic response of phosphorylated Ras (left) and double phosphorylated ERK (right) as a function of increasing initial concentrations of RKIP. The plot on the left illustrates a nonlinear relationship: as the initial concentration of RKIP increases, the concentration profiles of Ras\* appears to be lower than expected. However, as the initial concentration of RKIP increases further, the level of Ras\* increases again. The plot on the right confirms the experimental finding that increasing levels of RKIP determine the Raf-1 available and the subsequent strong surpression of ERK through high levels of RKIP.

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#### References

For further information on the research referred to in this article, publications, detailed references, and activities of the research groups, see www.sbi.uni-rostock.de.

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He holds the chair in Systems Biology & Bioinformatics at Rostock University since July 2003. Before moving to Rostock, he worked for over nine years at UMIST in the U.K. The research group covers a wide spectrum of bioinformatics techniques that are based on statistical data



analysis and mathematical modelling; including sequence-, mass-spec-, microarray-, and proteomicdata analysis. Dynamic pathway modelling and systems biology, as described in this article, are emergent areas of research that are a primary focus of the group.

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#### Box 1: Cell Signalling

Biological cells are not running a program, but rather continually sensing their environment and making decisions on the basis of that information. To determine how cells act and interact within the context of the organism, we need to understand how information is transferred between and within cells. Cell signaling, or "signal transduction", is the study of mechanisms by which this transfer of biological information comes about. Signaling impinges on all aspects of biology, from development to disease. Many diseases, such as cancer, involve malfunction of signal transduction pathways.

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#### Walter Kolch

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search focus on MAPK and the role of protein interactions in the regulation of signalling pathways. A current aim is to understand signalling networks by using proteomics, real time imaging and mathematical modeling.

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