Meeting report

Mathematical models in mammalian cell biology

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A report on the Conference on Systems Biology of Mammalian Cells, Dresden, Germany, 22-24 May 2008.

Systems biology has been defined in many ways, emphasizing various aspects of interdisciplinary research. At the recent symposium in Dresden on systems biology of mammalian cells, this emerging field was presented as a fruitful symbiosis of genomics, imaging, and high- and medium-throughput technologies with data analysis and mathematical modeling. Here we focus on selected applications of mathematical modeling in mammalian cell biology - a challenging but promising branch of systems biology.

Mathematically modeling the circadian clock

One application of mathematical models is in analyzing the workings of the mammalian circadian clock. About 20,000 synchronized neurons in the suprachiasmatic nucleus (SCN) control daily rhythms of physiology, metabolism and behavior. In addition, almost all peripheral tissues of mammals and even cell lines contain cellular clocks, in which endogenous oscillations of mRNA and protein abundance rhythms with a period of about 24 hours are driven by intracellular feedback loops involving clock genes such as *Per1-2*, *Cry1-2*, *Clock* and *Bmal1*. Post-translational events such as phosphorylation of clock proteins contribute to the delay in negative feedback and thus are crucial for the dynamics of circadian rhythms.

One of us (HH) reported that a combination of experiments in the lab of Achim Kramer and mathematical modeling led to a deeper understanding of the molecular mechanisms underlying human circadian behavior. In the first example, it was reported that the molecular explanation for a human behavioral disorder called familial advanced sleep phase syndrome (FASPS), which leads to a 4-hour advance in sleep and wakefulness, can be attributed to a point mutation in the circadian *Per2* gene. This mutation leads to a phosphorylation defect of the PER2 protein, changing its stability and subcellular localization in a cell culture model for FASPS. This cell culture model nicely recapitulates the 4-hour phase advance of human behavior by showing advanced rhythms of clock gene expression. Other phosphorylations of PER2, however, have partly opposite effects. Mathematical modeling integrated these experimental data and proposed a dynamical model with differential roles of PER2 phosphorylation sites for circadian dynamics.

In the second example, human skin fibroblasts from extreme chronotypes (that is, either 'night owls' or 'morning larks') have been used to characterize intrinsic circadian properties of these cells. Although for a large part of the subjects a good correlation between behavioral phase (that is, 'morningness' or 'eveningness' assessed by a questionnaire) and period of clock gene rhythms in skin fibroblasts (assayed by live-cell imaging using luciferase-based reporters) could be found, some subjects have normal circadian periods in their cells, but do display extreme behavioral phases. Computer models here helped to explain these phenotypes by suggesting that the amplitude and input sensitivity of the cellular oscillators should be experimentally investigated.

Quantifying apoptosis and signaling cascades

Another impressive example of mathematical modeling and quantitative experimentation going hand-in-hand is the analysis of apoptotic pathways. Heinrich Huber (Royal College of Surgeons, Dublin, Ireland) reported the monitoring of cytochrome *c* release during apoptosis at a resolution of seconds, using confocal and FRET-based imaging techniques. In this way the onset of mitochondrial outer membrane permeabilization in individual HeLa cells was monitored. Combining this imaging approach with mathematical modeling allowed the identification of two separate kinetic phases: an 'ignition phase' during which

the mitochondria were not yet fully permeabilized, and a second phase of cytochrome *c* redistribution.

A highlight of the conference was the opening lecture by Douglas Lauffenburger (Massachusetts Institute of Technology, Cambridge, USA). He asked how information about extracellular cues is encoded in the intracellular signaling network and causes a specific cellular response - in this case apoptosis. His group stimulated cells with different levels of tumor necrosis factor α, insulin, and epidermal growth factor (EGF), and measured phosphoprotein levels distributed across five kinase pathways as well as four apoptotic outputs. This impressive dataset showed that the response is not encoded in a single pathway, but that the information is distributed over the signaling network. It also enabled a comparison of different modeling strategies, including principal component analysis (PCA), fuzzy logic, and differential equations. The combination of these approaches led to interesting insights into the time-dependent role of the kinase IKK in the NF-κB pathway in inducing the apoptotic response and cross-talk mediated via autocrine loops involving transforming growth factor α and interleukin 1.

The question posed by Lauffenburger as to how information about extracellular cues is encoded in intracellular signaling pathways was addressed throughout the conference. Takashi Naka (RIKEN, Yokohama, Japan) presented data from breast cancer cell lines showing that EGF and heregulin induce broadly overlapping immediate-early gene expression patterns despite triggering different cellular responses. Naka speculated that the specificity of response is generated during late-phase signaling by feed-forward loop duration decoding through transcription factors such as c-Fos, which generate waves of expression of secondary, more specific transcriptional regulators depending on the length of the signal. In his closing talk, Hans Westerhoff (Free University, Amsterdam, The Netherlands) pointed out that the length of the signal in a pathway, and thereby the biological response, can be selectively regulated by the expression of phosphatases. He illustrated this with experimental data from growth-factor stimulated mammalian cells, where inhibition of the kinase in the MAP kinase (MAPK) pathway leads to smaller amplitudes of the signal, whereas inhibition of the phosphatases leads to an increase in the durations. This behavior has been previously postulated by Reinhard Heinrich using mathematical models of kinase cascades.

Leonidas Alexopoulos (Massachusetts Institute of Technology, Cambridge, USA) presented data from large-scale experiments on the response of the signaling network of primary and transformed hepatocytes following treatment with various cytokines and small-molecule inhibitors, alone and in combination. Using linear regression, he could work out alterations in the signaling network that were caused by oncogenic transformation. Again, these alterations were distributed in the network and not confined to a single pathway. As a consequence of the distributed information in the network, conventional single-target therapies could have limited efficacy. In this regard, Westerhoff pointed out that targeting the signaling network would require novel therapeutic agendas, such as targeting multiple nodes in the network, and that these targets might not necessarily be close to the original mutation in the network. Responses to such combinatorial inhibitor treatment can also be used to reverse-engineer the network, as demonstrated by Sven Nelander (Memorial Sloan-Kettering Cancer Center, New York, USA). He and colleagues applied combinations of pharmacological inhibitors to perturb mitogenic and pro-apoptotic signaling pathways in breast cancer cells and measured the activity of the pathways' components. From these data they were able to infer feedbacks within the pathway, and the use of combinatorial inhibition allowed for the inference of nonlinear interactions ('synergies') in the pathways. The method may be applicable to the design of targeted combination therapies for cancer.

Mathematical models in pattern formation

There is a long history of applying partial differential equations to spatio-temporal pattern formation in morphogenesis, as pioneered by Alan Turing and Hans Meinhardt. Advanced imaging techniques such as fluorescence recovery after photobleaching (FRAP) now allow the quantification of diffusion-reaction dynamics. Frank Jülicher (Max Planck Institute of the Physics of Complex Systems, Dresden, Germany) has combined modeling with experimental data to describe morphogen gradients in the wing imaginal disc of the fruit fly. He reported that quantitative studies reveal that morphogen transport in the tissue is coupled to cellular kinetic processes such as ligand-receptor binding, endocytosis of ligand-receptor pairs and the recycling of ligands to the cell surface. The resulting nonlinear transport equations guarantee a robust gradient profile to regulate the expression of genes in a manner that depends on the distance to the source of the morphogen.

The classical 'clock and wavefront model' proposed by Erik Christopher Zeeman in 1976 has been applied by Oliver Pourquié (Howard Hughes Medical Institute, Kansas City, USA) to study the vertebrate segmentation clock. This periodic pattern is established in the embryo during the formation of the somites. Somitogenesis involves an oscillator driving the dynamic expression of genes in the presomitic mesoderm from which the somites are derived. Microarray data indicated that the mutually exclusive activation of Notch/FGF and Wnt pathways coordinate the oscillator. The FGF pathway controls the positioning of the wavefront of gene expression and couples the spatio-temporal activation of segmentation to the posterior elongation of the embryo.

How useful are mathematical models?

For small subsystems, dynamical models can be fitted directly to experimental time-course data. But despite extensive microarray, proteomics and imaging data, larger systems such as the mammalian cell-cycle machinery cannot yet be described in quantitative detail by mathematical models. In these cases, however, statistical approaches such as PCA, Bayesian networks and fuzzy logic help to extract useful information.

Presentations at the conference impressively illustrated that complex processes such as apoptosis or morphogenesis can be tackled with a combination of quantitative spatio-temporal data and modeling, thus giving hope that we might be able to make dynamic models of other more complex processes in future as quantitative data becomes available. The conference nicely illustrated that the collaboration of experimentalists and theoreticians helps to design appropriate experimental strategies for the analysis of mammalian cells. Therefore, we believe that the time is ripe to apply systems biology more widely in mammalian cells. Although many models will be semi-quantitative for the near future, they will help guide our experimental design and integrate our data.

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