TOWARDS A VIRTUAL BIOLOGICAL LABORATORY

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For a system-level understanding of living cells, a quantitative representation of these systems involving mathematical models and corresponding computer tools is required. Our approach focuses on a modeling concept which relies upon modular structuring of cellular systems oriented strongly at the biomolecular structure of these systems. Mathematical submodels for functional units comprising metabolism and regulation can be aggregated in a hierarchical way to obtain more complex modules. In the Virtual Biological Laboratory, the process modeling tool PROMOT contains an object-oriented knowledge base with reusable modeling entities and enables a purely symbolical model building process via a graphical user interface. The simulation environment DIVA then uses the model library for dynamic simulation, parameter estimation and model analysis. Two examples for models of complex regulatory networks in *Escherichia coli* and in *Saccharomyces cerevisiae* are given to demonstrate the usefulness of the approach. It can provide a framework for straightforward development of virtual representations for cellular systems.

Introduction

Although being one of the most important challenges in modern biology, a system-level understanding of how cells and organisms function is very rudimentary. This results mainly from two reasons: The overwhelming part of experimental investigations can be characterized as qualitative and descriptive, directed towards understanding of biomolecular details. The concomitant lack of quantitative data will certainly be reduced by further development and wider application of massively parallel experimental methods in functional genomics and proteomics [20, 12]. Furthermore, due to the complexity of cellular systems even the (nearly) complete measurement of the systems' states *per se* will not enable an integrated understanding of all relevant functional connections and their influence on the observable behaviour [4].

Recent efforts for a system-level understanding in biology rely on interdisciplinary approaches combining concepts from biology, information sciences and systems engineering. They especially stress the importance of mathematical modeling of complex biological systems in order to come to a virtual representation of cells and organisms. In the end, this representation should allow for computer experiments similar to experiments with real biological systems. Thus systematic testing of biological hypotheses as well as purpose-driven design of cellular functionality are perspectives of these approaches [4, 18].

The use of mathematical models including the development of computer tools for model formulation and simulation has been demonstrated by e.g. Tomita *et al.* [19] who were able to establish a hypothetical cell comprising 127 genes. Schaff *et al.* [14] follow comparable approaches in the development of a "Virtual Cell" . However, two major challenges for application of mathematical concepts in the life sciences still have to be resolved: (i) the work on a conceptional framework promoting interdisciplinary research in this direction by finding a "common", non-mathematical language and (ii) a clearly defined modeling concept adapted to cellular systems that allows for easy model development and interpretation [18].

Focusing on the internal structure of cellular systems, one central, increasingly accepted notion is that these systems are composed of 'functional units' or 'modules'. In this respect, biological systems are more closely related to synthetic, engineered systems than to e.g. physical systems [4, 8]. Therefore, a promising way to come to a system-level understanding of cells and organisms is to extend successful theoretical concepts established for the analysis and synthesis of complex technical systems [2] to biological systems.

On this basis we are currently developing a system- and signal-orientated modeling concept for cellular systems. It relies on the modular structuring of these systems and a systematic representation of biomolecular components in modeling objects. The modeling concept will be outlined in the following section. Afterwards we provide a short sketch of the nature of interdisciplinary research to be carried out to establish a "Virtual Biological Laboratory". The usefulness and validity of the approach will be demonstrated by two examples of cellular functional units: the system controlling catabolite repression in *Escherichia coli* and aspects of a complex regulatory network involved in cell cycle regulation in budding yeast.

Modular modeling concept

The notion of a living cell being composed of subunits of limited autonomy (functional units) plays a prominent role for the modular modeling concept. For the mathematical modeling of cellular systems, this modular structure raises the possibility to independently develop mathematical models for each of the functional units. Submodels as entities in the "model world" hence correspond to functional units in the "real world". The submodels can afterwards be connected to obtain a description at the system-level. As this approach depends on the identification of functional units, one important question is how to demarcate these units, i.e. how to decompose a complex cellular biochemical network.

At a very abstract level, a cell can be divided into two general subnetworks, a regulatory network and a metabolic network [7] as shown in Fig. 1. These networks possess very different characteristics: The metabolic network is mainly occupied with substance transformation e.g. to provide metabolites and cellular structures. In many cases it involves fast biochemical reactions. The regulatory network's main task is information processing in order to e.g. adjust enzyme concentrations to the requirements of variable internal and external conditions. This network involves the use of genetic information. Compared to information flow, mass flow only plays a subordinated role in the regulatory network. In this sense, the regulatory network is superimposed to the metabolic network, fulfilling functions analogous to a controller in a technical process. The interaction between both networks is necessarily bound to substance exchange due to the requirements for precursors and proteins. However, the main connections consist in directed signal flow, i.e. sensor signals (e.g. generation of second messengers) and control action (e.g. adjustment of enzyme concentrations).

One important feature of the regulatory network is its hierarchical structure, which has to be considered for the system-level description of living cells. As shown in Fig. 1 for transcriptional regulation in budding yeast, the system's possible behaviour on a lower level is constrained by regulation at higher levels. The presence of RNA-Polymerase e.g. offers a wide variety of different gene expression patterns, but the actual gene expression is adjusted by combinatorial control involving associated factors and specific transcription factors. Including these components in the modeling process hence results in detailed (and more complex) mathematical models. It also explicitly considers system-wide coupling of cellular regulation and enables the exploitation of hierarchical network structures for model reduction [7].

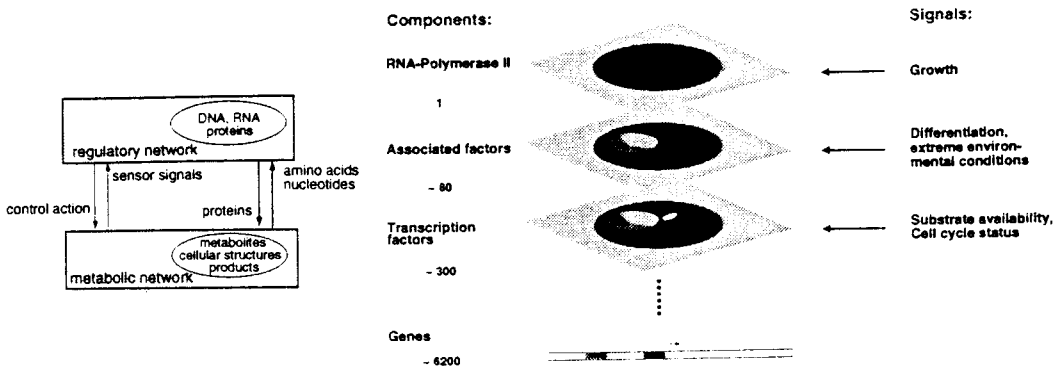

Fig. 1: Structural decomposition of cellular systems (I): Regulatory network and metabolic network (left) and hierarchical structure of the regulatory network exemplified for transcriptional regulation in budding yeast (right).

Each functional unit has to be composed of a part of the metabolic network and a corresponding part of the regulatory network. For the demarcation of functional units (or modules), we use a preliminary set of three, biologically motivated criteria. To be (relatively) self-contained, the modules have to (i) perform a common physiological task as e.g. represent a linear pathway for amino acid synthesis, (ii) to be controlled at the genetic level by a common regulatory network and (iii) to posses a common information processing (signal transduction) network. The essential feature of the approach is the combination of classical concepts in the analysis of metabolic systems with a signal-oriented perspective to cellular regulation. Distinct to our approach, several authors adressed the question of demarcation in a more quantitative, flux-oriented way regarding either metabolic pathways [13, 16, 15] or intracellular signal processing networks [5, 17]. As systematic investigations on larger modular systems like the work by van der Gugten *et al.* [21] are only at the beginning we use this heuristic way of demarcating functional units. Further work on these theoretical questions will clearly be necessary to come to a more stringent formulation of the above cited criteria.

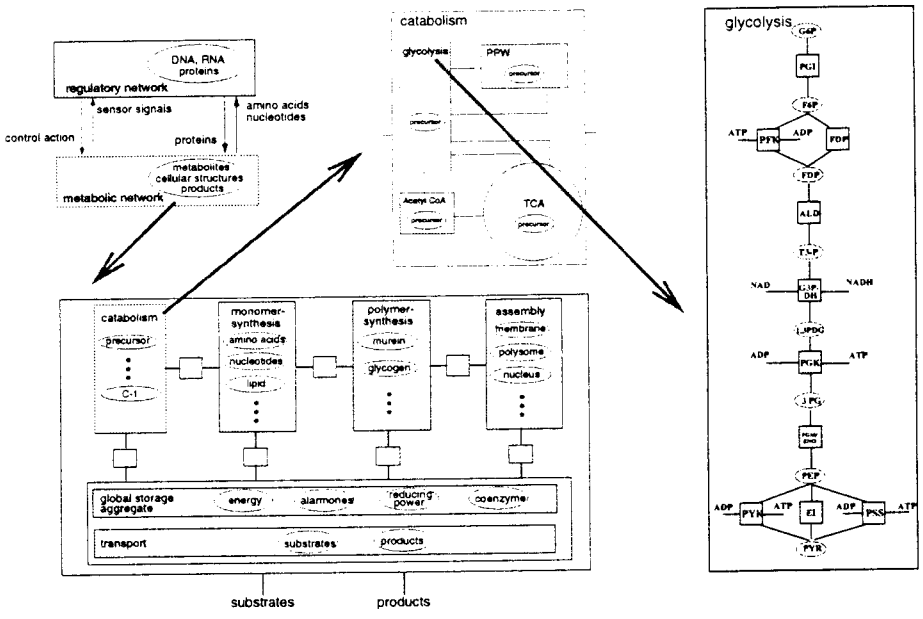

Fig. 2: Structural decomposition of cellular systems (II): Example for hierarchical nesting of modules

The application of these criteria enables to structure an entire cellular system and therefore means a holistic approach to cellular function. Depending on the desired degree of resolution of subsystems, it offers a flexible description of hierarchically nested modules (Fig. 2). An enzymatic reaction in glycolysis belongs accordingly to the functional unit "glycolysis" which in turn is part of the larger unit "catabolism".

Our modular modeling approach involves the systematic representation of the above identified biological functional units in submodels (modeling objects). These modeling objects are characterized along two coordinates: They have structural properties representing the number and types of inputs and outputs. Additionally, they are assigned behavioural properties, i.e. mathematical equations describing the dynamic behaviour. Depending on the modeling objectives these equations include e.g. algebraic equations, ordinary or partial differential equations (ODEs / PDEs). As the modeling approach aims at a consistent link between macroscopic and microscopic dynamics, special attention is given to provide a realistic description of the molecular interactions governing each of the regulatory mechanisms under consideration. Often the mathematical equations as the "core" of each modeling object are therefore derived from elementary chemical reaction networks applying chemical kinetic theory (detailed models). To allow for an adjustable degree of model accuracy as well as for efficient simulation, model reduction e.g. via quasi steady-state assumptions is carried out where appropriate. Furthermore, each modeling object is assigned a specific symbolic representation which guarantees a high degree of biological transparency by its modular structure. This is especially important to facilitate interdisciplinary discussions on the underlying biological structures and mechanisms.

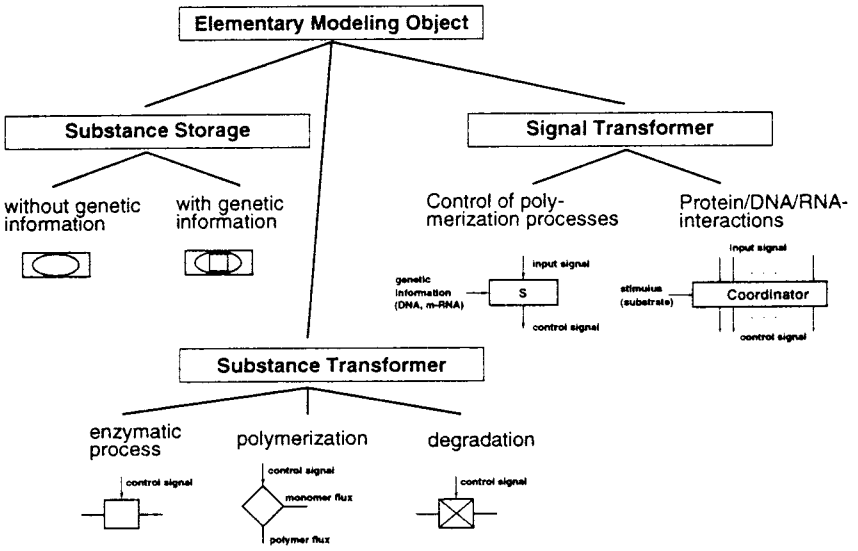

Fig. 3: Hierarchy of elementary modeling objects for cellular systems. Substance exchange is marked by bold lines whereas arrowheads are used to indicate signal connections.

At the most fundamental level, a finite and disjunct set of so-called "elementary modeling objects" (Fig. 3) has been defined. They are used to represent substance formation, degradation and storage as well as the corresponding signal transformation processes as in the control of transcription initiation via specific DNA-protein interactions. The organization of these modeling objects in an object-oriented class hierarchy lays the basis for computer-aided model development as described in the next section. Elementary modeling objects can subsequently be interconnected to form higher aggregated structures. A mode-

ling object for gene expression e.g. comprises transcription and translation. In summary, this approach enables to progressively obtain a holistic description of more complex functional units.

The Virtual Biological Laboratory: An Outline

One main purpose of the Virtual Biological Laboratory is to enable computer experiments with cellular systems in analogy to experiments carried out with real biological systems in the laboratory. Applications include the quantitative and qualitative analysis of overall behaviour, systematic design of functional units by genetic modifications and the systematic planning of real laboratory experiments. The Virtual Biological Laboratory has to integrate mathematical models with a sound biological background and methods for data storage, computer-aided modeling, simulation and model analysis in a software tool (Fig. 4). Accordingly, the development of such a tool requires the close cooperation of biologists, information scientists and system scientists.

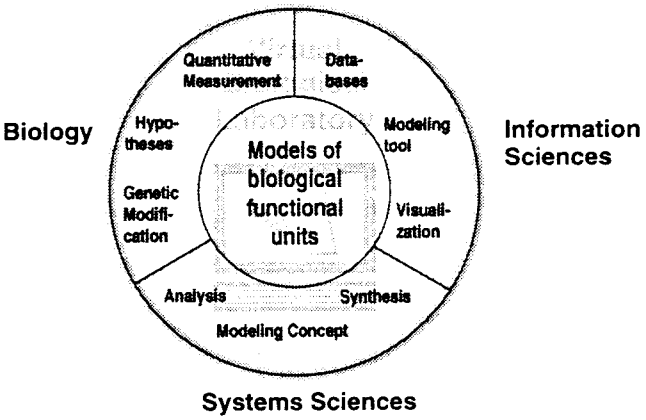

Fig. 4: Elements to be integrated into a Virtual Biological Laboratory.

The necessity of contributions by each of the three disciplines arises also from the fact that model development has to be understood as an iterative process leading to a maximal convergence of "model world" and "real world". It always requires a careful evaluation of all hypotheses and assumptions by comparison with experimental data. To provide these data and to develop methods for specific perturbation of cellular processes are two of the main tasks of biology. Information science is needed for database design including a systematic representation of experimental and kinetic data, the development of computer-based modeling tools and finally the implementation of visualization techniques. The system sciences primarily have to provide theoretical methods for e.g. demarcation of network structures, system-level analysis and synthesis.

The Virtual Biological Laboratory is currently under development and major parts of it have already been established: The process modeling tool PROMOT, originally designed for application in chemical engineering, allows for the computer-aided development and implementation of mathematical models for living systems [3]. The model building process supported by PROMOT consists of the hierarchical aggregation of structural and behavioural modeling entities according to the modeling concept described in the previous section. The differentiation of modeling objects into structural modeling entities and behavioral modeling entities allows for systematic model-building in a two-step process. Model structure and e.g. kinetic equations can hence be handled independently. PROMOT also enables the implementation of a flexible, object-oriented knowledge base containing reusable modeling entities. Using the

knowledge base, model formulation means the selection and linking of pre-defined modeling objects via a graphical user interface. In this respect the Virtual Biological Laboratory differs significantly from other biological simulation environments like *E-Cell* [19]. Speed and easiness of model development - even without knowing exactly about the underlying mathematical formulations - are thus greatly increased.

Mathematical models generated using PROMOT can be directly added to the model library of the simulation environment DIVA [9, and refs. therein]. This simulation tool has been designed especially for dealing with large-scale dynamical (differential-algebraic) systems, which arise in chemical engineering, but also in the mathematical modeling of complex cellular networks. State-of-the-art numerical methods integrated in DIVA include dynamic and steady state simulation, model optimization, parameter estimation and numerical analysis e.g. by methods of nonlinear dynamics.

The combination of PROMOT and DIVA is therefore well suited to form the core of the "electronic infrastructure" of a Virtual Biological Laboratory. Examples for the content of the Virtual Biological Laboratory will be given in the following two sections. We present signal-oriented models for catabolite repression in *E. coli* and for aspects of cell cycle regulation in budding yeast, respectively.

Example: Catabolite repression in *E. coli*

In bacteria the expression of carbohydrate uptake systems and metabolizing enzymes is very well controlled in order to avoid the useless expression of proteins. For growth, some carbohydrates are preferred to others, resulting in the sequential use of different carbohydrates in mixed cultures. The best examined example of this phenomenon is the diauxic growth of *E. coli* in cultures with glucose and lactose [11]. Different regulatory proteins contribute in controlling the expression of the corresponding operons and the activity of carbohydrate uptake systems. Being extensively studied over the past years, glucose-lactose diauxie of *E. coli* is a perfect model system of complex regulatory networks.

The regulatory proteins involved in glucose-lactose diauxie in *E. coli* influence the expression of the lactose metabolizing enzymes. The lactose repressor, LacI, is able to bind to a control sequence in front of the *lac* operon in the absence of lactose, thereby inhibiting transcription from *lacZp*. This repression is relieved in the presence of allolactose, the natural molecular inducer of the *lac* operon. In a side reaction to the cleavage of lactose into glucose and galactose, allolactose is produced by β-galactosidase, encoded by the gene *lacZ*. This specific control hinders the expression of the lactose metabolizing enzymes in the absence of their substrate lactose. Additional control is exerted by the Crp protein. This protein is active in the regulation of a number of operons, most involved in the quest for food. The Crp protein is able to form a complex with cAMP, that acts as a transcriptional activator for the *lac* operon as well as for the other members of the *crp* modulon.

The concentration of the alarmone cAMP inside the cell is regulated by complex mechanisms. These mechanisms are basically understood, but despite many well-established details some questions remain. Central in its regulation is the action of the phosphoenolpyruvate-dependent phosphotransferase systems (PTSs), especially the glucose PTS. If the PTSs are not active in the uptake of substrates, the PTS proteins including Crr which acts as the EIIA in glucose transport accumulate in their phosphorylated form. Crr-P is needed for the activation of the enzyme adenylate cyclase (CyaA) that converts ATP into cAMP. Only in the absence of PTS substrates or their transport respectively an activation of CyaA is hence possible. This leads to an increased level of cAMP inside the cell and in the formation of the cAMP.Crp activator complex. As a result operons like the *lac* operon that depend on the Crp.cAMP complex for transcription can only be expressed if no PTS-substrates are present. Vice versa PTS-substrates

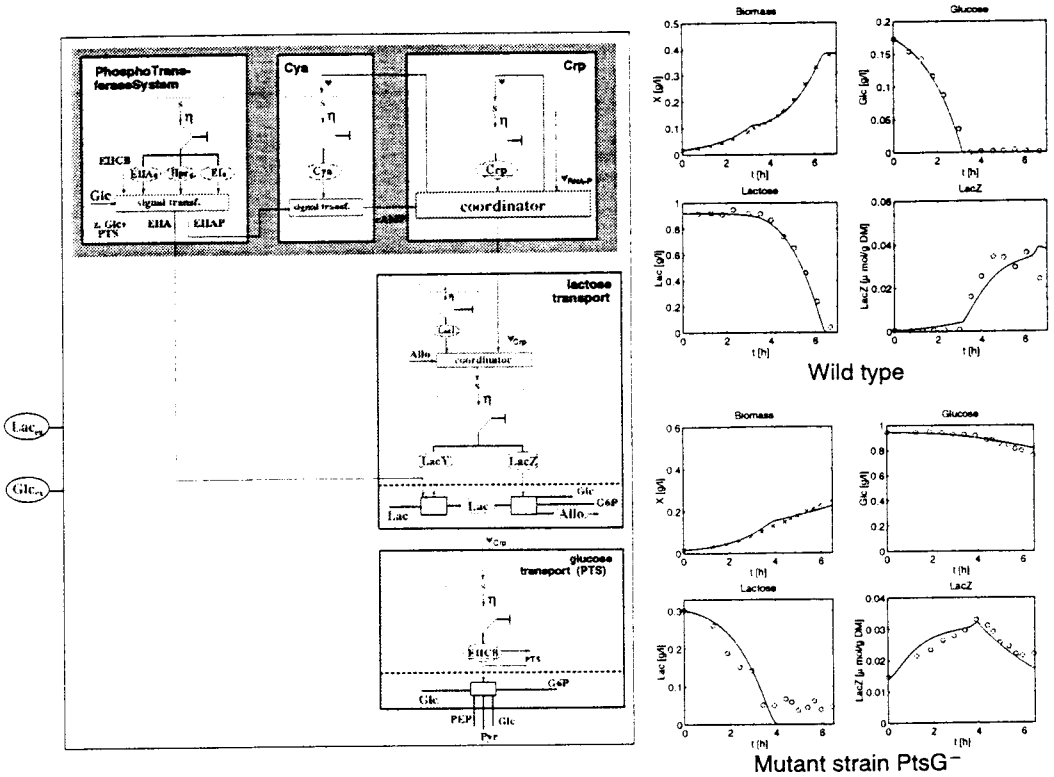

Fig. 5: Catabolite repression in *E. coli* : Model structure (left) and simulation results for wild type and mutant strain (right).

in the medium repress transcription of the members of the *crp* modulon. This regulation has therefore been termed catabolite repression. The glucose PTS is also active in regulating the activity of the lactose permease, LacY, another important factor in the formation of diauxie. The unphosphorylated form of Crr is able to form a complex with LacY thereby inhibiting uptake of lactose from the culture medium. This interaction prevents the entry of lactose into the cell and the formation of allolactose. This phenomenon is referred to as inducer exclusion.

A mathematical model describing carbon catabolite repression was developed and validated with a set of experiments (Fig. 5). Here, isogenic mutants, i.e. strains derived from one wild type strain with a defined mutation in the signal transduction pathways, were constructed [6]. In the experiments the media composition as well as the preculture conditions were varied. After parameter analysis and estimation, the time course of the simulation and the experimental data agree very well.

Example: Cell cycle regulation in budding yeast

In all eukaryotic cells the cell division cycle is characterized by a fixed sequence of cell cycle phases (Fig. 6), during which the main cellular tasks are switched from simple mass growth (G1 phase) to DNA replication (S phase) and finally to chromosome separation and cytokinesis (G2 / M phase). In response to multiple internal and external signals, the sequence is mainly controlled by cyclin dependent kinases (CDKs). They are activated by phase-specific cyclins forming distinct kinase complexes with different functionality. Even in the relatively simple yeast *S. cerevisiae*, one catalytic subunit (Cdc28) and nine

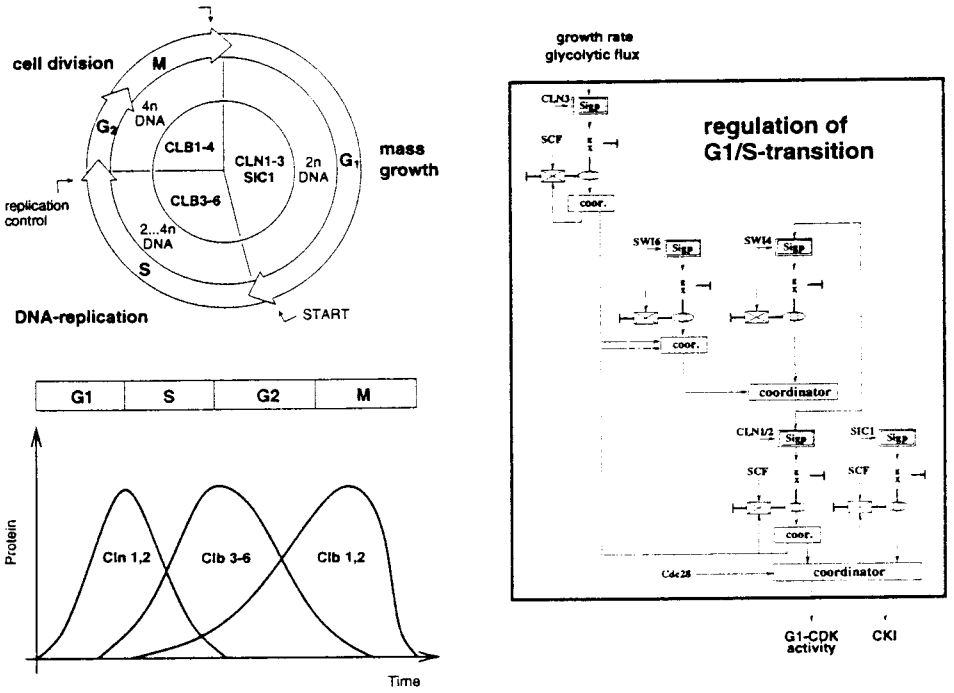

Fig. 6: Cell cycle regulation in *Saccharomyces cerevisiae* : Main cellular tasks in specific cell cycle phases, checkpoints, DNA-content, cell cycle regulators (upper left), corresponding cyclin concentrations (lower left) and model structure for description of the G1 / S-transition (right)

cyclins (Cln1-3 / Clb1-6) are involved in cell cycle regulation [10]. The phase-specific cyclin fluctuation in this organism relies upon such diverse processes as regulated transcription of cyclin genes, constitutive or controlled protein degradation and specific inactivation of Clb-CDKs via the CDK inhibitor Sic1. All regulators are embedded in a highly interconnected network including positive and negative feedback loops [10]. Additionally, cell cycle regulation in budding yeast does not only serve as an example for a complex regulatory network. It also involves many of the known regulatory mechanisms at the DNA, mRNA and protein levels which generally have to be accounted for during model development.

In the cell cycle, the G1/S-transition plays a crucial role, because at this boundary – via the associated checkpoint called "START" – the cells ultimately have to decide whether to undergo a new round of replication and division or not. The accumulation of sufficient cellular material i.e. the attainment of a critical cell size constitutes the major prerequisite for this transition [10]. At the molecular level, the transition is governed by an approximately constant level of Cln3, which surprisingly results in the sudden activation of a transcription factor composed of Swi4 / Swi6. In this way the production of G1 cyclins Cln1/2 induces the transition to S phase. Whereas these regulatory mechanisms are well established, finding a consistent explanation for the sudden appearance of G1 cyclins as a function of cellular growth is complicated [10]. To quantitatively analyze the system's dynamics, a submodel was formulated according to the modeling concept outlined above. Its structure, which is based solely on the known regulatory mechanisms, is shown in Fig. 6. Special attention was given to incorporate the interaction between regulatory processes at the DNA as well as at the protein level.

Several conclusions regarding the character of the G1 / S transition can be drawn from the simulation results (Fig. 7): Although being held at approximately constant concentration, Cln3 is able to drive the

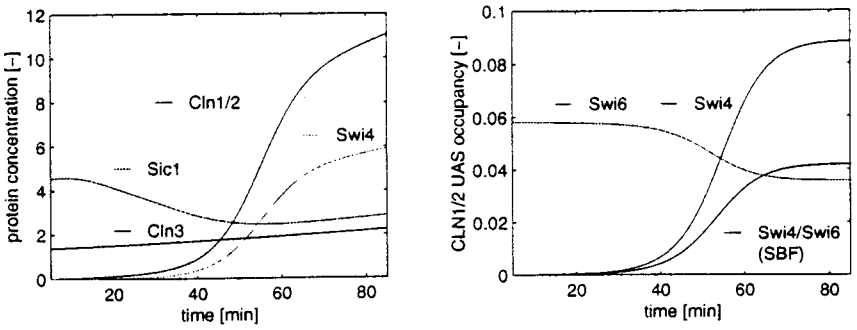

Fig. 7: Simulation results for regulation of the G1 / S transition: Protein concentrations of G1 cyclins, CDK inhibitor and transcription factor Swi4 (left) and transcriptional regulation of the *CLN1/2* genes (right).

transition as a function of cellular growth. Mechanistically, the control of *CLN1/2* transcription via Swi4/6 plays a prominent role in this process. Due to several positive and negative feedback loops, the system behaves as a switch function as soon as a Cln3 threshold is reached. The known regulatory mechanisms therefore sufficiently explain the behaviour observed *in vivo*. Differing from a published mathematical model of cell cycle regulation in budding yeast [1], our (partial) model is based on the deduction of dynamic properties from a more detailed description of regulatory mechanisms: Without implementing e.g. an ultra-sensitive switch function for the activation of the transcription factor SBF, this behaviour results from an interplay of regulated gene expression, phosphorylation / dephosphorylation reactions and cooperative binding to multiple sites on the DNA.

Conclusions

Finding concepts to deal with the complexity of living systems represents the major challenge on the way to a system-level understanding of cells and organisms. In this contribution, we present a framework which is derived from concepts in engineering science and systems theory. It essentially relies upon the modular mathematical modeling of the overall behaviour of cellular functional units. The decomposition of cells into such units is oriented at the modular biomolecular structure of cellular systems. This demarcation also represents the most crucial aspect of the modeling concept as mainly heuristic criteria are applied at the moment. In the future, theoretical work in this area will be intensified taking into account recently published concepts by other groups. Finally, the modeling concept should guarantee a high degree of biological transparency and promote the interdisciplinary cooperation between biologists and system engineers.

A long-term perspective of our work is the establishment of a Virtual Biological Laboratory combining mathematical models of cellular systems with tools for their efficient development, simulation and analysis. The purpose of this laboratory is to enable computer experiments with cellular systems similar to the analysis and design of cellular systems in the "real" world. One milestone in reaching this aim is the development of process modeling and simulation tools forming the "core" of the Virtual Biological Laboratory. As we have shown, computational tools like PROMOT and DIVA are already available and allow for a straight-forward realization of the modular modeling concept outlined in this contribution. For two small example systems, we showed a systematic formulation of mathematical models based on (structural) biological knowledge. This can lead to an adequate description of experimentally observable cellular behaviour and to new insight into how cellular biochemical networks operate.

For the system-level description of more complex systems, even of a simple bacterium like *E. coli*, an intensified cooperation of biology, information sciences and systems sciences will be essential. However,

regarding the modular structure of cellular systems, there exists a realistic perspective of coming to a virtual representation of real cellular systems by cooperation and division of labour.

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