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Systems analysis in cell biology: From the phenomenological description towards a computer model of the intracellular signal transduction network

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Abstract. In this paper we introduce a systematic approach for the modelling of complex biological systems which is especially useful for the analysis of signal transduction mechanisms in cell biology. It is shown that systems analysis in form of top-down levelled dataflow diagrams provides a powerful tool for the mathematical modelling of the system in terms of a stochastic formulation. Due to the exact formulation, the consistency of the model with the experimental results can be tested by means of a computer simulation. The method termed Structured Biological Modelling (SBM) is illustrated by modelling some aspects of the second messenger network which regulates cell proliferation. As an example for the straightforward development of a mathematical description a stochastic computer model for intracellular Ca^{2+} oscillations is presented.
Key words. Structured analysis; mathematical modelling; stochastic simulation; cell cycle control; tumor biology; calcium oscillations; signal transduction; Structured Biological Modelling.

1 Introduction

In biology, complex signal and information processing networks are widely known. The examples range from macrosystems in ecology like population dynamics to neural networks and cellular microsystems. Especially in the last few years a vast amount of genetic and biochemical data yielded much insight into the intracellular signal transduction network which regulates and controls cellular functions including proliferation[1]. Unfortunately, most of these data are not suitable for the direct prediction of the physiological functions of the system under consideration. This missing link causes important clinical consequences. For example, the carefully directed design of antimitotic drugs for cancer treatment could be drastically improved if a realistic model of the mitogenic signal network in neoplastic cells would be available.

What are the difficulties which complicate the development of such a model? a) Through variable feedback mechanisms and a diversity of cooperative reaction steps biological and especially biochemical systems display a highly non-linear kinetic. Without a precise mathematical formulation this non-linearity makes it impossible to predict the dynamics of the system over a longer time scale. b) Biological systems are dissipa-tive structures operating far from thermodynamical equilibrium. Therefore, a permanent flux of energy and/or material through the system must be taken into account. c) The high compartmentation of cellular systems, especially demonstrated by electron microscopy (fig. 1), is responsible for the generation of many quasi-autonomous subsystems. Often the dynamics of these subsystems is regulated by second messengers in a very sophisticated manner. Through the cellular compartmentation also the requirements for complex spatial patterns are met.

As early noticed by mathematical biologists many of the mentioned problems can be circumvented by a mathematical description using the tools of the theory of non-equilibrium systems[2, 3]. Besides theoretical insights into biological mechanisms, a following computer simulation can generate an experimentally testable hypothesis. This check of the logical consistence of a model is impossible if the system has only been described verbally. With these mathematical methods it was possible to model e.g. the formation of complex patterns during morphogenesis[4, 5], many aspects of the evolution[6–9], and also the behavior of biochemical systems on the cellular level[4, 10], to mention only a few. Most of the models presented so far have been formulated by deterministic differential equations. When applied to highly non-linear and multidimensional systems (as the cellular signal transduction network) the deterministic methods often fail because of the stiffness of the differential equations. In this case alternative approaches using stochastic methods are appropriate[11].

Especially adapted to the requirements of cellular biology we have developed a new computer-based modelling technique termed *Structured Biological Modelling* (SBM). The SBM-cycle consists of three essential steps (fig. 2): *Step1:* Structured analysis, which has been originally developed to model and design complex man-made systems in economics and computer science. For this technique the contributions of DeMarco[12], Gane and Sarson[13], Martin and McClure[14], and Yourdon[15] have set milestones. Supported by new types of com-

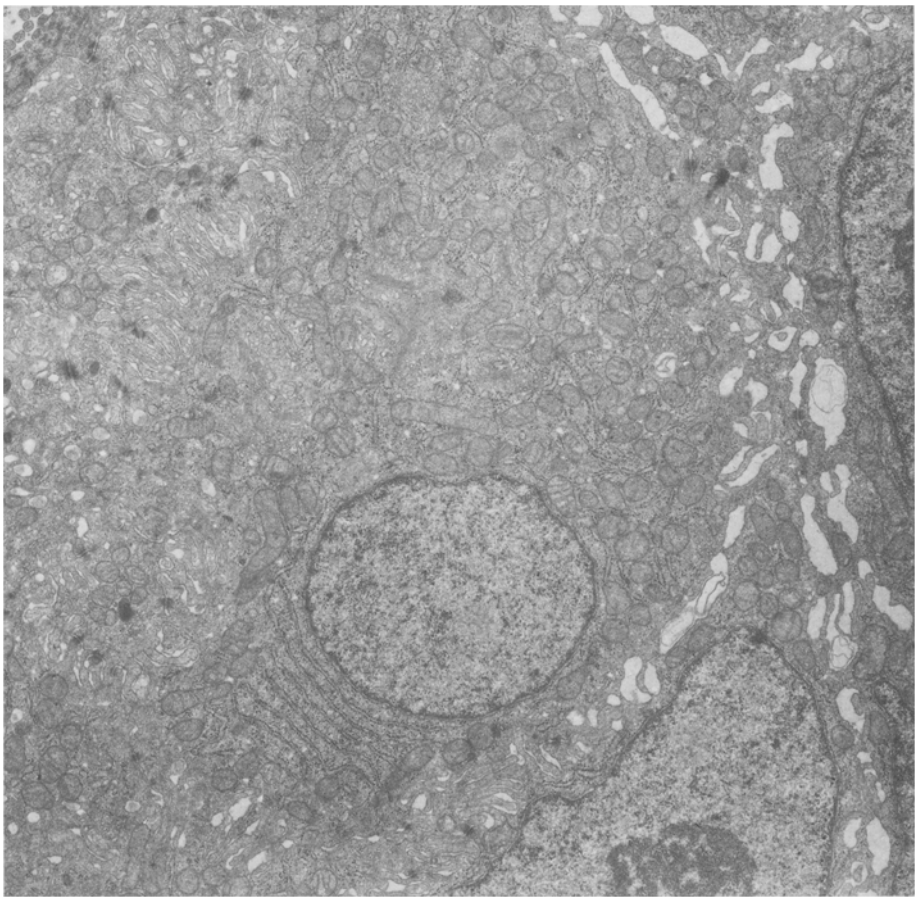

Figure 1. Electron microscopic image of an epithelial cell from colon tissue demonstrating the high amount of compartmentation (magnification × 11250).

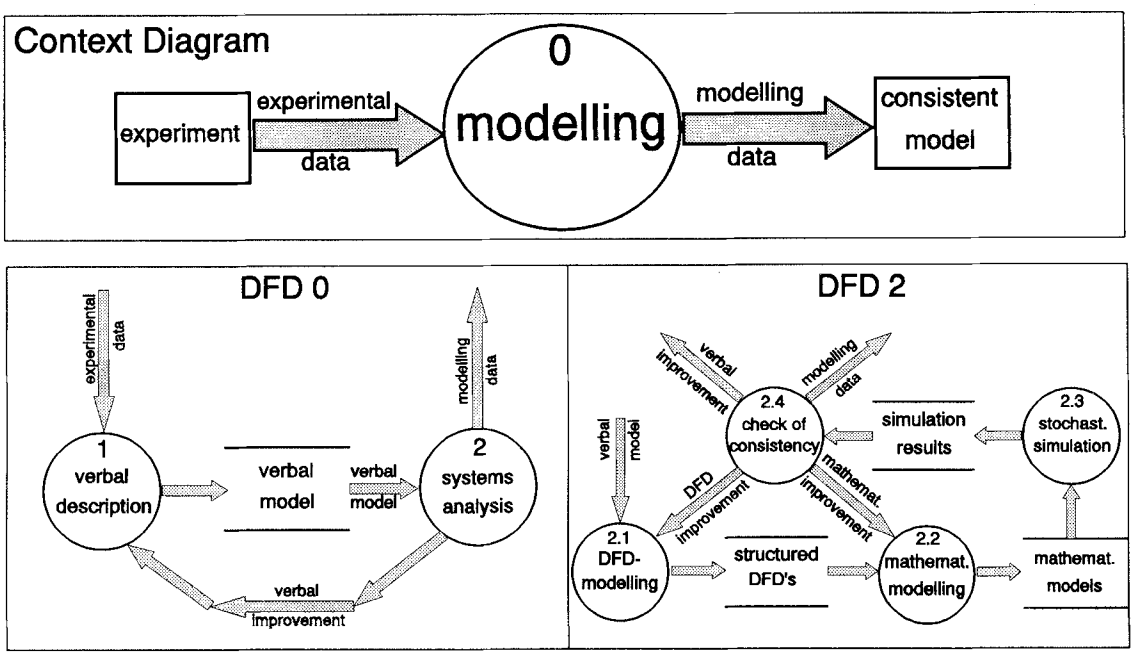

Figure 2. Top-down levelled DFD describing the SBM-cycle. The notation, which is intended to be as intuitive as possible, will be discussed in subsection 2.1. The context diagram models the interfaces of the system *0 modelling* with its environment. Process *0 modelling*, is decomposed into the processes *1 verbal description* and *2 systems analysis*. At last, DFD 2 illustrates the details of the process *2 systems analysis*. If necessary, each process in the DFD's, which itself models a subsystem, can be further analyzed in a lower-level diagram. Note, that for simplicity the data stores, dataflows, and external agents are not numbered.

puter programs, the so-called CASE-tools (computer-aided software engineering), structured analysis is the state-of-the-art method for the analysis phase of modern software engineering. As we will demonstrate below, CASE-tools can also be used for the analysis of complex systems in cell biology (see figs 4–8). The resulting model of this first phase of the SBM-cycle consists of a set of top-down levelled dataflow diagrams (DFD's). *Step 2:* The DFD- model serves as starting point for the mathematical modelling. With the rules given below the DFD-model can be translated into a master equation of the system. *Step 3:* As a last step the mathematical model is simulated on a computer. Now, verbally postulated and real properties of the model can be compared. By the direct stochastic simulation of the Markov process underlying the master equation, we circumvent the problems arising from the explicit solution of the master equation. As a prerequisite, SBM requires the foregoing quantitative numerical recording of the experimental results. Adapted to the modular style of the model's architecture only such experimental methods should be used which minimize the disintegration of the subsystem under consideration. In cell biology this *holistic* approach is ideally realized when using analytical light microscopical techniques (e.g. fluorescence monitoring of intracellular ion concentrations[16]) for low magnifications and analytical electron microscopical methods for higher resolution of subcellular structures[17–19]. In addition, NMR microscopy reveals insight into the chemical composition of the subsystem with spatial resolution[20]. Finally, for the investigation of molecular subsystems biochemical methods are appropriate.

Our paper is organized as follows: In section 2 an introduction into SBM is given. First, we modify the methods of structured analysis in a way that they satisfy the practical requirements for the analysis of biological systems and the interpretation of experimental data. Then the rules for the mathematical interpretation of the DFD-model are explained. At last, the stochastic algorithm is shortly described. In section 3 a systems analytical approach for the analysis and modelling of the intracellular signal transduction network is proposed. It is demonstrated that the handling of the complexity of this system is markedly facilitated through the application of SBM. As a simple example for the SBM-cycle a stochastic model for intracellular Ca^{2+} oscillations, based on the two-pool-oscillator[21, 22], is presented. Although the model of this cellular subsystem is simplified it displays many properties observed experimentally. Finally, in section 4 we give a brief summary and point out future perspectives of SBM in cell biology.

2 Structured analysis and mathematical modelling in cell biology: The SBM-cycle

Systems analysis in biology deals with the problem of how to handle an immense amount of experimental information about biological systems and how to reveal the major mechanisms which regulate these systems. Understanding of a system means to have a consistent model of it which determines the main regulatory processes. As a systematic and straightforward modelling technique we suggest using SBM. Its components will be introduced in the following subsections.

2.1. Step 1 in the SBM-cycle: Structured analysis

One main problem in systems analysis is that of determining what is part of the system and what is not. Whatever system has to be analyzed, it will be part of an even larger system. Thus, the first step is the development of a model which defines the interfaces between the system and its environment. This first model is known as the *environmental model*[15]. In this model the system itself is regarded as a black box. The critical aspect of the environmental model is that of identifying the events occurring in the environment to which the system must respond. The model of the inside of the system is called *behavioral model*[15] and models *how* the system reacts on external stimuli.

We propose in this paper to utilize the tools and systematics known from structured analysis of commercial systems for biological systems, too. We choose the method '*modern structured analysis*' as defined by E. Yourdon[15] because it is the current state-of-the-art method of systems analysis. Especially, we suggest to use top-down levelled DFD's for structuring and modelling complex systems. Some reinterpretations have to be done, however, to arrive at a model which is useful for mathematical description and simulation.

2.1.1 The elements. Table 1 shows the elements used in DFD's together with a short description of their significance.

Each element in a model has to have assigned a name, except for those dataflows which originate or terminate at a data store (where the name of the data store is assumed to be the name of the dataflow). We recommend to define naming-conventions before beginning with systems analysis. For example E-num-name naming external agents, F-num-name naming dataflows, and S-num-name for data stores. We shall shortly return to the naming conventions for processes.

Further, a short textual definition of each element should be given. The dictionary of these definitions can be further extended to include (for example) references to the literature relevant for the element under consideration.

2.1.2 The context diagram. As mentioned above, the first step in systems analysis is to identify the extent of the system and the interfaces to its environment. The conceptual result is the so-called environmental model and the method to visualize it is called *context diagram*. It consists of one single process (namely the system), plus all external agents with the dataflows connecting the system with the external agents.

Table 1. Elements of data flow diagrams

Symbol	Name(s)	Description (interpretation in biology)
E-l **EXTERNAL AGENT**	External agent, External partner, Terminator	Originator or receiver of information needed by or produced by the system. Not part of the system! (External concentrations, physical parameters)
S-k **DATA STORE**	Data store, Store	(Temporary) Store of information needed by the system for asynchronous processing of information. (Internal concentration)
j **PROCESS**	Data process, Process ("Bubble")	Transformation of information. Transforms incoming dataflows in outgoing dataflows.
F-i **DATAFLOW**	Dataflow Flow	Flow of information (not of material!).
	Junction	Point in the information flow where related data are grouped together or separated into single data.
F-i **DATAFLOW**	Input dataflow reference	Reference to an incoming dataflow from a higher-level DFD (analogous for output dataflows).

We mention at this point, that for completing the environmental model a so-called *event list* (namely a list of stimuli on which the system has to react) and a *statement of purpose* have to be included. The event list is not evident from the context diagram because it is not obvious, whether the initiative for a flow comes from the outside (stimulus) or from the inside (information reference) of the system.

2.1.3 Dataflow diagrams (DFD's). A DFD models the flow of information in a system. For this purpose, it contains dataflows, processes, data stores, junctions, and references to dataflows originating from higher-level DFD's. There are some rules for composing DFD's which will be explained now:

Rule 1: The only sinks and sources of information allowed in DFD's are external partners, which, in turn, have to be placed in the context diagram. Therefore, each process and also each data store has to have at least one incoming and one outgoing dataflow.

Rule 2: Dataflows can begin or end at either of the following: Process, data store, junction, or reference to higher-level DFD's. Only in the context diagram dataflows may be connected to external agents.

Each process – in turn – can be described by a lower-level DFD. Using this procedure leads to a hierarchical structure of the DFD's describing the system. Rule 1 for the DFD's implies automatically two other rules for hierarchical (or levelled) DFD's:

Rule 3: Each dataflow attached to a process has to be represented by a reference to a higher-level DFD in the lower-level DFD representing that process.

Rule 4: Data stores have to be introduced on the highest level of DFD's where more than one process is connected to that data store.

To make the levelling of the resulting DFD's transparent, we suggest the following naming conventions for processes: The processes in the top-level DFD (which is usually called 'DFD 0' and describes the process 'system' of the context diagram) are numbered beginning with 1, 2, and so on. The processes in the DFD describing process j in DFD i are numbered by i.j ($j = 1, 2, \ldots$) and so on.

On the lowest level of DFD's, processes are described by so-called process specifications. If we are looking for a mathematical model of our system, the process specifications are given by a mathematical description of the process under consideration. The rules for writing down these mathematical specifications differ depending on

the way we describe the data stores. In this paper we characterize the data stores by integer-valued components of a state vector which means, that the process specifications have to be given as transition probabilities between the states represented by the data stores. The next section describes this method in detail.

2.2 Step 2 in the SBM-cycle: The mathematical transcription of the DFD-model

We propose to regard the time evolution of the system as a stochastic process which is governed by a master equation

$$\frac{dp_n(t)}{dt} = \sum_{n'} [w_{nn'} p_{n'}(t) - w_{n'n} p_n(t)] \qquad (1)$$

(for a comprehensive review of stochastic processes in biology see Goel and Richter-Dyn[11]). The master equation is a differential equation for the time evolution of the probability $p_n(t)$ that there will be $n = (n_1, \ldots, n_k, \ldots, n_N)$ molecules of class k ($1 \leq k \leq N$) in the volume V at time t. The quantity $w_{nn'}$ denotes the transition probability from state n′ into state n. The knowledge of $p_n(t)$ for all times t would evidently provide a complete characterization of the system but requires the explicit solution of the master equation which is mostly very difficult[23]. In contrast, the Markov process underlying the master equation can be easily simulated on a computer as depicted in the next subsection. By this procedure possible realizations of the stochastic process are simulated. Hence by this method individual 'experiments' are simulated. Finally, if one repeats the simulation of the stochastic process many times, the statistical averages of the quantities of interest can be obtained.

With the following fundamental rules the Markovian stochastic description of the system can be derived directly from the DFD's with a minimum of additional assumptions. For this purpose we start at the lowest-level DFD's which should be levelled in a way that each dataflow F-i which is connected with a process j starts or ends at a data store S-k or at a reference to an external agent E-l. To translate the content of the data stores into mathematical terms we

A) identify the number of molecules (per volume V) in the store S-k at time t with $n_k(t)$ and construct the state vector $\mathbf{n}(t) = (n_1(t), \ldots, n_k(t), \ldots, n_N(t))$, where N is the number of stores in the lowest-level DFD's.

If known for all times t the state vector n would completely characterize one realization of the stochastic process described by the master equation (1).

In the stochastic formulation the dynamics of the system is set up by a sequence of transitions between different states which occur at discrete times t. Given that the system is in the state n at time t a transition into a state n′ during the time interval dt occurs with a certain transition probability $w_{n'n}dt$. How can this transition probability be determined from the DFD-model? In the DFD model the information stored in a data store is changed by the processes which write to this store. Therefore,

B) each process j characterizes a set of possible state transitions of the system, whereby the corresponding transition probability $w_{n'n} \equiv w^j$ depends on both, the intrinsic properties of process j and the input dataflows which characterize the initial state n. Accordingly, it is

$$w^j = w^j(\text{input dataflows, intrinsic characteristics}). \qquad (2)$$

The final state n′ is only determined by the output dataflows of process j.

Especially for the intracellular signal transduction network the determination of the w^j is mostly very difficult (or yet impossible) because often kinetic data of important receptors, enzymes and second messengers are lacking. In this case w^j has to be constructed with the help of plausibility arguments from the verbal specification of the process j.

The last step in the SBM-cycle is the computer simulation of the mathematical model derived by the above described procedure.

2.3 Step 3 in the SBM-cycle: The computer simulation

To circumvent the problems arising with the explicit solution of the master equation (1) we directly simulate the underlying Markov process. For this purpose a powerful, computer-oriented Monte Carlo algorithm has been introduced by Gillespie[24,25]. This algorithm is particularly useful for simulating the behavior of complex systems in conditions of instability where fluctuations and correlations give rise to transitions between non-equilibrium steady states. An overview over the simulation method described here (and possible alternatives) can be found in Honerkamp[23], and a proof that this method is equivalent to the formulation in terms of a master equation is given in Gillespie[24,25].

The aim of the simulation algorithm is the generation of a trajectory n(t) which is a possible realization of the stochastic process described by the master equation (1). To do this we start with a given initial state $n(t = 0) = n_0$, that means

$$p_n(t = 0) = \delta_{n,n_0}. \qquad (3)$$

All we have to do now is to answer two questions:

1) When will the next state transition occur?
2) Which transition takes place (or equivalently, which process will be activated) at that time?

Given the total transition probability

$$u_0(n) = \sum_{n' \neq n} w_{n'n} = \sum_j w^j \qquad (4)$$

with w^j computed according to equation (2), a simple calculation (see ref. 23) reveals that the next state transition occurs after the time interval

$$\Delta t = -\frac{1}{u_0(n)} \ln(\xi_1) \tag{5}$$

where ξ_1 is a random number uniformly distributed over the interval [0, 1].

The selection of the transition works through a stochastic switch where the probability for the selection of a particular process α, which mediates the selected state transition, is proportional to its w^α. With a second random number ξ_2, which is also uniformly distributed over the interval [0, 1], α can be determined by

$$\sum_{j=1}^{\alpha-1} \frac{w^j}{u_0(n)} \leq \xi_2 < \sum_{j=1}^{\alpha} \frac{w^j}{u_0(n)}. \tag{6}$$

The state vector $n(t + \Delta t)$ differs from $n(t)$ only in those components whose corresponding data stores are attached to the output dataflow of the executed process α. The two steps of the algorithm have to be repeated until the final time is reached.

In contrast to deterministic simulation techniques[26] the stochastic algorithm used by SBM is also working when applied to multidimensional systems with a highly non-linear kinetics (see, e.g., Turner[27] for a review of simulation methods for chemical kinetics). Particularly, this holds for the intracellular signal transduction network which consists of a large number of reactants interacting in a highly non-linear manner.

Probably the computer simulation suggests some improvements of the DFD-model and its mathematical interpretation. In this case the iterative application of the SBM-cycle should lead to the desired consistency of the model (see fig. 2).

In the next section we will apply SBM to the modelling of important aspects of the intracellular signal transduction network.

3 Modelling of intracellular second messenger networks

One of the most challenging questions in biology is the determination of the mechanisms which control the cell cycle. An immense amount of experimental data indicates that cellular proliferation is regulated by a complex network of second messengers (for review see refs 1, 28). The induction of proliferation is regulated by at least three interconnected second messenger pathways and is modulated by the intracellular ion concentration, where Ca^{2+} and H^+ play a key role (fig. 3). In cancer cells this network has characteristic defects. Quite apart from the fact that some cellular subsystems are yet well characterized, a thorough understanding of the cell cycle regulation and the differences in the signalling network between cancer and normal cells is still missing[29a]. As we will demonstrate in the remainder of this paper this lack can be gradually removed by the application of systems analytical methods like SBM. Before we do this, some preliminary remarks about self-organization in the cell cycle and in neoplastic growth shall emphasize our intention.

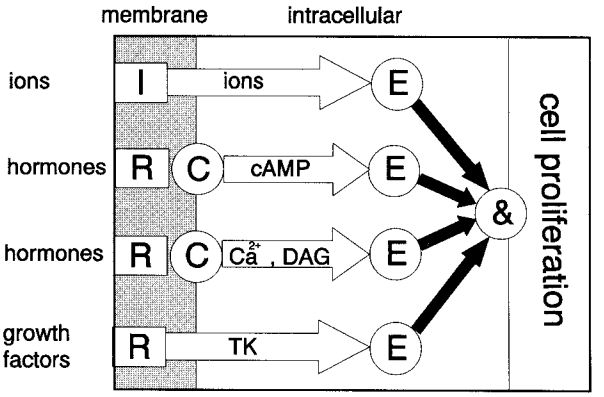

Figure 3. Schematic, highly simplified illustration of the second messenger pathways which regulate cell proliferation. Ions, hormones and growth factors from the extracellular medium are linked to intracellular effector systems (E) through the activation of second messenger pathways. Ions, most important Ca^{2+} and H^+, are transmitted into the cytosol through ion channels (I). In the cytosol they regulate effectors by cooperative activation or inhibition. The response following the opening of plasma membrane ion channels is short termed. Another possibility of intracellular signal transduction is initiated by hormones that bind to specific receptors (R) on the plasma membrane. As a next step in the signal processing cascade the hormone signal becomes encoded by G proteins (coding system, C), which also integrate hormone signals from different receptor systems. Downstream, G proteins then regulate enzymes which produce second messengers. There are at least two hormone activated second messenger pathways which modulate proliferation control. The first signal track transmits his signals through the second messenger cyclic adenosine monophosphate (cAMP). As an effector cAMP activates protein kinase A (PKA). Through distinct G proteins the other important hormone coupled signalling pathway mediates the regulation of the second messengers Ca^{2+} and DAG. As an effector, this pathway activates protein kinase C (PKC). Both, PKA and PKC phosphorylate target proteins on serine and threonine residues. Long termed responses, due to the phosphorylation of target proteins on tyrosyl residues, are mediated by a further signalling pathway: Growth factors bind to specific transmembrane receptors with an intrinsic tyrosine kinase activity (TK) at the cytoplasmic side. The balance of phosphorylation is regulated by certain phosphatases which can also be activated by the binding of growth modulating factors to specific receptors. Not implemented in the simplified schema are the close interconnections between the distinct signalling pathways as well as the diverse positive and negative feedback mechanisms which control the cellular signalling network. Also the signals mediated by cyclic guanosine monophosphate (cGMP) are not shown. As indicated by the dotted arrows, the signals from the different effector systems (E) are possibly summed up by a yet unknown mechanism (&) which also may weigh and integrate the incoming signals. Presumably, cell proliferation becomes induced when a critical level of the integrated signal is achieved.

3.1 Phase transitions in the cell cycle and in neoplastic growth?

There is evidence that the events determining cell proliferation take place in the G_1-phase of the cell cycle. It has been postulated that there is a so-called *restriction point*[30] at the end of G_1 at which cells become arrested for some time depending on the extracellular conditions. After this point has been passed, the cell cycle becomes independent of extracellular factors. Then all cellular functions are directed towards proliferation. Cancer cells can be characterized by a defect in the regulation of this G_1 arrest[31]. For example, some products of oncogenes[32–35] function as signal transducers which produce a short-circuit in the signalling network (e.g. *ras*, which codes for an activated G protein with a reduced GTPase activity[36, 37]). Other oncogenes reduce the dependence of the cell proliferation on extracellular factors (e.g. *erb-B*, which codes for an activated EGF receptor with no need for a ligand[34]).

As we have demonstrated in a number of optical and analytical electron microscopical investigations, these changes in the intracellular signal transduction network go along with characteristic and tumor stage specific alterations of the cellular ultrastructure and chemical composition[38–43].

These data indicate, that neoplastic growth is paralleled by a loss of many cellular degrees of freedom. It seems that in malignant cells the main activity is directed towards proliferation. Therefore, a cell has no further need for a well-balanced signalling network. We assume that in malignant cells one central signalling pathway dominates about the whole intracellular signalling network. In terms of the synergetics[44] neoplastic growth could be interpreted as a phase transition (like the transition of water from the liquid state into the solid state at the freezing-point) between the normal signalling mode of non-malignant cells and that of malignant ones, whereby the dominant mode slaves all other pathways.

To confirm the hypothesis it will be necessary to model the intracellular signalling network in mathematical terms. In this way the stability of the signalling network can be investigated by well-known mathematical methods[3, 44]. In addition, the dynamics of the system can be predicted by a computer simulation using the methods discussed above.

As a future perspective the phase transition hypothesis has important clinical consequences: a) Possibly, some therapeutic approaches which do not influence critical components in the cancer cell signalling network have to be dropped. b) Especially the independence of cancer cells of extracellular factors complicates the possibility of a directed attack on cancer cells. c) On the other hand, alterations of specific cellular parameters (now interpreted as variables of state which characterize the dominant signalling mode), e.g. revealed by methods like image analysis, EELS or NMR-microscopy which do not disintegrate the cell, can be directly correlated with defects of the signalling network. This would evidently increase the validity of prognosis and the chance for a therapeutic intervention.

3.2 Simplified SBM-model of the cell cycle regulation

Despite the lack of important data it is not possible in the scope of this article to describe the development of an approximately comprehensive model of the intracellular signalling network for reasons of space. Instead, we will give an idea of how (and that!) SBM functions when applied to this complex cellular system. As an example for the mathematical interpretation of the DFD-model an important subsystem which is responsible for the generation of intracellular Ca^{2+} oscillations is modelled and afterwards simulated on a computer.

In the DFD-model the whole intracellular signalling network is represented by process *0 CELLULAR SIGNALLING*. As modelled in the context diagram (fig. 4) this system regulates the onset of the S-phase of the cell cycle (denoted by the output dataflow *F-6 SPO* which ends at the external agent *S-PHASE ONSET*). After the onset of the S-phase, which requires the passage of the restriction point, the cell becomes nearly independent of extracellular factors and the time which elapses during the remaining S, G_2, and M phase of the cell cycle is almost invariable[31]. Also all important cell cycle events like the regulation of the transition into a quiescent G_0-state and the control of differentiation occur before the passage of the restriction point.

Which are the external factors that determine the regulation of the cellular network? This question is answered by the input dataflows of process 0 in the context diagram (fig. 4). First, the cell can be stimulated by hormones in the extracellular medium (the external agents *E-1 HORMONES CLASS-1*, H1, and *E-2 HORMONES CLASS-2*, H2, denote such stimuli which regulate receptors which are coupled to the adenylate cyclase, or respectively, to the phosphatidyl inositol system). Secondly, a variety of growth modulating factors, *GMF*[33, 45], influences the intracellular signalling through receptors with an intrinsic tyrosine kinase activity. In addition, *IONS*, which determine the cell membrane potential and modulate the functions of intracellular enzymes, channels and pumps, are exchanged between the intra- and the extracellular room. For simplicity, other inputs like the requirement of energy and material in form of nutrients and the influence of additional signal mediators (e.g. nitric oxide, and steroid hormones) have been neglected.

While in the context diagram the whole system *CELLULAR SIGNALLING* is regarded as a black box, DFD 0 (fig. 5) depicts the main subsystems of it and their interrelations. The individual subsystems (represented by the processes) are connected by dataflows

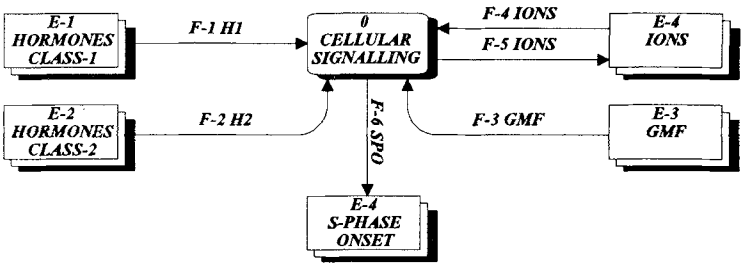

Figure 4. Context diagram of the system *CELLULAR SIGNALLING* which describes the intracellular network which regulates cellular proliferation. For the development of the DFD's shown in figures 4–8 a modern CASE-tool has been used (*A*pplication *D*evelopment *W*orkbench/*A*nalysis *W*orkstation: ADW/AWS™ from Knowledge Ware™). GMF: *G*rowth *M*odulating *F*actors; H1: Hormones which regulate the adenylate cyclase system; H2: Hormones which activate phospholipase C; SPO *S*-*P*hase *O*nset.

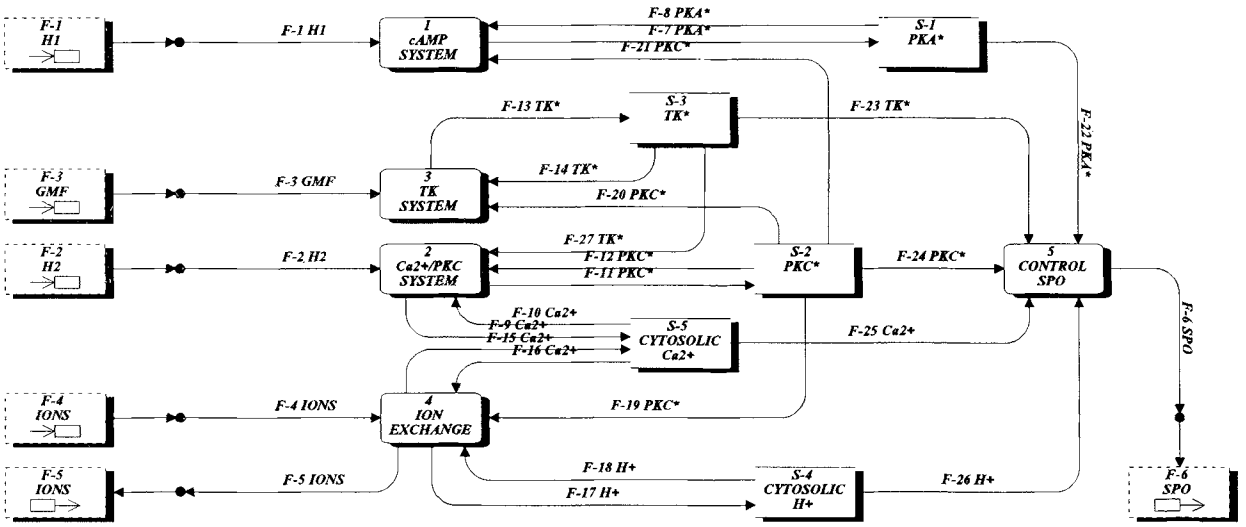

Figure 5. DFD 0 models process *0 CELLULAR SIGNALLING* on the highest level. If a deeper resolution of the system is desired each process of DFD 0 can be decomposed in further lower-level systems. PKA: *P*rotein *K*inase *A*; PKC: *P*rotein *K*inase *C*; TK: *T*yrosine *K*inase; *denotes an activated kinase.

which model the flow of information between them. In the data stores information is stored (e.g. the concentrations of ions or enzymes), which is required by more than one process. In the simplified model presented here, process 0 is decomposed into 5 subsystems.

Process *1 cAMP SYSTEM* models how the activation of H1-receptors (e.g. β-adrenergic receptors) leads to the activation or inhibition of adenylate cyclase via stimulatory G_s, or respectively, inhibitory G_i proteins. The enzyme adenylate cyclase catalyzes the generation of the second messenger cAMP[46] which then activates protein kinase A (in the DFD's activated enzymes are characterized by a star, *). The activated protein kinase A (PKA*) mediates many cellular functions[47]. For instance, it modulates the regulation of the SPO (process 5, see below). PKA* also induces the heterologous desensitization of H1-receptors by receptor phosphorylation[48]. As marked by the dataflow *F-21 PKC** the cAMP system is also modulated by activated protein kinase C (PKC*) which e.g. phosphorylates the β-adrenergic receptor[48,49]. For reasons of space DFD 1, which models process *1 cAMP SYSTEM*, is not shown here.

Nevertheless, some general characteristics of the intracellular signalling system should be mentioned here: a) Positive signals that induce an activation of a signalling pathway are usually followed by immediate feedback control which desensitizes the pathway to prevent an over-response. Therefore, a cell responds mainly to (rapid) changes of the extracellular conditions. b) Protein phosphorylation (by specific kinases) and dephosphorylation (by phosphatases) is the basis for the regulation of the intracellular signalling network[50]. The balance between phosphorylation and dephosphorylation is controlled by just a few second messengers. c) Different signalling pathways are not independent. On the contrary, there is cross-talk and synergistic action between different pathways. d) Small concentrations of hormones or growth factors become drastically amplified by an intracellular signalling cascade (e.g. receptors → G proteins → G protein effectors → second messengers → enzyme cascades).

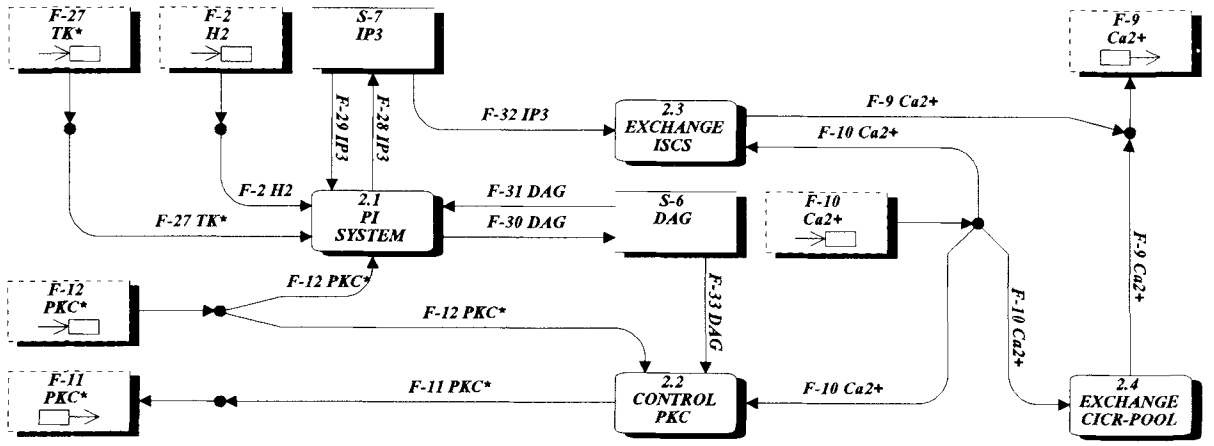

Figure 6. DFD 2 models process *2 Ca^{2+}/PKC SYSTEM* in more detail. DAG: diacylglycerol; IP3: inositol 1,4,5 trisphosphate; PI: phosphatidyl inositol.

The activation of protein kinase C and the regulation of the cytosolic Ca^{2+} level is modelled by process *2 Ca^{2+}/PKC SYSTEM*. As shown in DFD 2 (fig. 6) hormones of class 2 (dataflow *F-2 H2*) serve as an input for the regulation of the phosphatidyl inositol metabolism, characterized by process *2.1 PI SYSTEM*. The H2-receptors are coupled to distinct G proteins which regulate the rate of hydrolysis of phosphatidyl inositol 4,5-bisphosphate by phosphoinositide-specific phospholipase C (PLC). This pathway leads to the generation of two second messengers: diacylglycerol (DAG) which is together with Ca^{2+} necessary for the activation of PKC (process *2.2 CONTROL PKC*) and inositol 1,4,5 trisphosphate (IP_3) which controls the exchange of cytosolic Ca^{2+} with IP_3-sensitive Ca^{2+} stores (ISCS, process *2.3 EXCHANGE ISCS*).

Again, the activation of the effector enzyme PKC leads to the desensitization of the signalling pathway by the phosphorylation of H2-receptors[48,49] or G proteins[49,51]. Also cross-talk with the tyrosine kinase pathway (process 3, fig. 5) takes place: The activation of certain growth factor receptors with cytoplasmic tyrosine kinase (TK) activity leads to the activation of PLC (denoted by dataflow *F-27 TK**) through a yet not completely characterized mechanism[35]. This cross-talk has important consequences in transformed cells where the possibility of a down-regulation of the TK receptor system is missing. Another important example of cross-talk is the PKC-induced activation of the Na^+/H^+ antiporter[49]. Because many cellular enzymes and ion pumps are highly sensitive to pH-shifts, small alterations of the intracellular H^+ concentration can cause considerable effects.

Process *3 TK SYSTEM* converts extracellular growth factor and growth inhibitory signals into intracellular signals by the regulation of certain protein tyrosine kinases. The balance between phosphorylation and dephosphorylation is regulated by phosphatases which are possibly activated by the binding of growth modulating factors to specific receptors[52]. While the phosphorylation of target proteins on serine and threonine residues by PKA* and PKC* mediates mainly short-termed responses (see Cohen[50] for review), long-termed responses are mediated by the phosphorylation of target proteins on tyrosyl residues[53]. Today, the cAMP and Ca^{2+}/PKC pathways are well characterized, but much data about the key elements of the important tyrosine kinase pathway and their functions are missing. Remarkably, many oncogenes found in human tumors code for tyrosine kinases (e.g. *erb-B, neu, kit, src, abl*) or elements related to their regulation (e.g. *ras*)[33].

A further important subsystem is process *4 ION EXCHANGE*, which describes how ions are exchanged between the extra- and the intracellular room. While Na^+ and K^+ are mainly responsible for the maintenance of the cell membrane potential, Ca^{2+} and H^+ play important roles in the cooperative regulation of intracellular key enzymes[54].

As indicated by process *5 CONTROL SPO*, the onset of the S-phase of the cell cycle is regulated by protein kinases (in concert with phosphatases), Ca^{2+} and H^+. The detailed mechanisms by which the SPO is induced are yet unknown. But several elements linking the inputs of process 5 to its output are determined. For example, the cAMP response element-binding protein (CREB), which is phosphorylated by PKA* and Ca^{2+}-calmodulin dependent protein kinases, was demonstrated to function as a transcription factor which integrates Ca^{2+} and cAMP signals generated by processes 1, 2 and 4[55].

Each process shown in the DFD-model could be decomposed into lower level systems. Principally, this procedure could be carried on down to the systems resolution desired (e.g. the molecular level). Compared with non-levelled styles of presentation, the modular architecture of the DFD-model guarantees that the dia-

grams are always relatively easy to understand. In the second place, only single modules have to be changed if one wants to discuss alternative mechanisms. In this way experts can develop submodels revealing more detail of the system without any loss of information, while other people, who only want to have a general view over the system, can inspect higher-level DFD's without becoming confused by details. Through the use of a CASE-tool the formal consistency of the DFD-model according to the rules in subsection 2.1 is guaranteed.

As an example for the mathematical interpretation of the DFD-model we will now develop a simple computer model for intracellular Ca^{2+} oscillations which can be regarded as an important subsystem of the DFD-model depicted above.

3.3 Stochastic model for intracellular Ca^{2+} oscillations

Intracellular free calcium is a very important second messenger in regulating the cell cycle as well as in modulating many cellular functions including metabolism, secretion, contraction, growth and differentiation[56,57]. Analytical electron microscopical investigations, using the technique of electron energy loss spectroscopy (EELS)[19,42], have revealed that neoplastic growth of gastric and lung tissues is correlated with stadium specific alterations in the level of intracellular calcium[39,58]. These findings and other experiments of our group (B. Wolf: unpublished data) support that calcium is also important in carcinogenesis, tumor progression and therapeutic behavior of tumor cells in relation to resistance phenomena after treatment with antimitotic drugs (see also refs 59, 60).

Over the last few years it has been demonstrated in lots of experiments that intracellular Ca^{2+} is oscillating (see refs 61, 62 for review). To test the mechanisms underlying this phenomenon, intracellular Ca^{2+} oscillators have been modelled in a mathematically precise way (see refs 63, 64 for review). In addition, intracellular Ca^{2+} often propagates through the cell in form of non-dissipative oscillatory waves (see, e.g., Kraus and Wolf[65] for a mathematical model). A hypothetical function of Ca^{2+} oscillations and waves may be the frequency encoded signal transduction from the cell membrane to the nucleus.

Formally, cytosolic Ca^{2+} oscillator models can be divided into two categories[66]: Second messenger-controlled models try to describe Ca^{2+} oscillations at a constant level of IP_3. On the other hand, receptor-controlled models propose that Ca^{2+} oscillations can be switched on by oscillations in IP_3 through various feedback interactions between both second messengers. There is strong experimental evidence supporting the second messenger-controlled mechanism[67,68].

Berridge and Irvine[21] proposed a two-pool model in which Ca^{2+}-induced Ca^{2+} release (CICR) is the key mechanism responsible for the generation of Ca^{2+} oscillations and waves. CICR is a special kind of an autocatalytic reaction where cytosolic Ca^{2+} catalyzes the rapid elevation of cytosolic Ca^{2+} by inducing a Ca^{2+}-efflux from intracellular Ca^{2+} stores. According to their model, IP_3 generated through the receptor-mediated

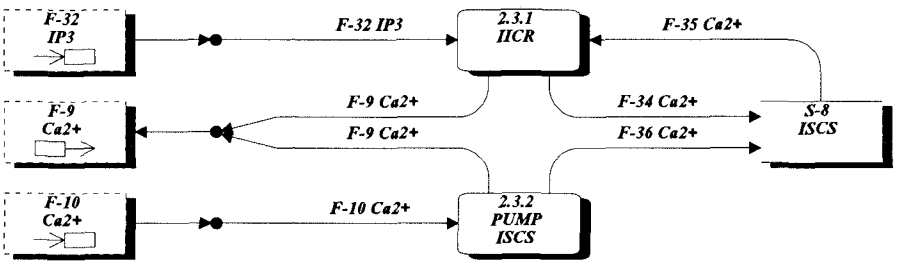

Figure 7. DFD 2.3 models how Ca^{2+} can be exchanged with IP_3-sensitive Ca^{2+} stores (ISCS's). IICR: *I*P_3-*I*nduced *C*a^{2+} *R*elease.

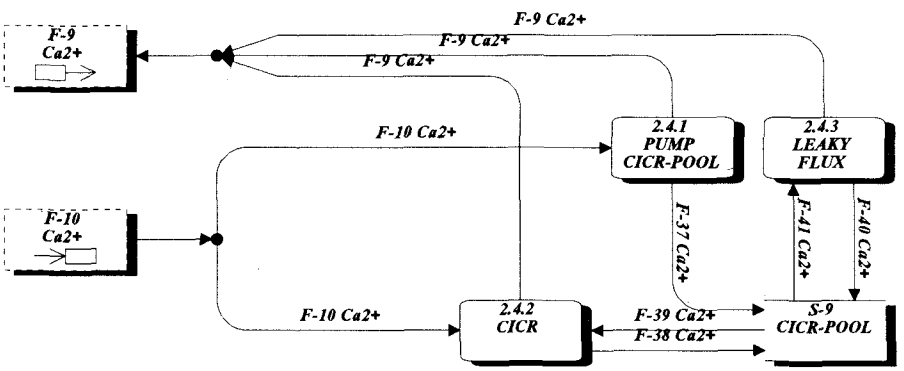

Figure 8. DFD 2.4 models how Ca^{2+} can be exchanged with the CICR-pools. CICR: *C*a^{2+}-*I*nduced *C*a^{2+} *R*elease.

Table 2. Process specifications and translation into the stochastic formulation of the subsystem describing intracellular Ca^{2+} oscillations (see figs 4–8). According to Goldbeter et al.[22] it is assumed, that the number of calcium ions in the IP_3-sensitive Ca^{2+} store (*S-8 ISCS*) remains constant. Therefore, the system can be characterized by the number of calcium ions in the cytosol (*S-5 CYTOSOLIC Ca^{2+}*), denoted by N_x, and the number of Ca^{2+} in the CICR-pools (*S-9 CICR-POOL*), N_Y. It is $n(t) = (N_x(t), N_Y(t))$. For more details on the stochastic two-pool model we refer to Kraus and Wolf[64].

Process j	Described reaction	Transition probability w^j	State transition
2.3.1 IICR	$Ca^{2+}_{ISCS} \xrightarrow{k_1\beta} Ca^{2+}_{cyt}$	$k_1 Ca^{2+}_{ISCS}\beta \equiv v_1\beta$	$N_X \to N_X + 1$ $N_Y = \text{const}$
2.3.2 PUMP ISCS	$Ca^{2+}_{cyt} \xrightarrow{k_2} Ca^{2+}_{ISCS}$	$k_2 N_x$	$N_X \to N_X - 1$ $N_Y = \text{const}$
2.4.1 PUMP CICR-POOL	$nCa^{2+}_{cyt} \xrightarrow{v_2} nCa^{2+}_{CICR}$	$v_2 = V_{M2}\dfrac{N_X^n}{K_2^n + N_X^n}$	$N_X \to N_X - n$ $N_Y \to N_Y + n$
2.4.2 CICR	$pCa^{2+}_{cyt} + mCa^{2+}_{CICR} \xrightarrow{v_3} (m+p)Ca^{2+}_{cyt}$	$v_3 = V_{M3}\dfrac{N_Y^m}{K_R^m + N_Y^m}\dfrac{N_X^p}{K_A^p + N_X^p}$	$N_X \to N_X + m$ $N_Y \to N_Y - m$
2.4.3 LEAKY FLUX	$Ca^{2+}_{CICR} \xrightarrow{k_f} Ca^{2+}_{cyt}$	$k_f N_Y$	$N_X \to N_X + 1$ $N_Y \to N_Y - 1$
4.1 Ca^{2+} INFLUX	$Ca^{2+}_{ex} \xrightarrow{k_3} Ca^{2+}_{cyt}$	$k_3 Ca^{2+}_{ex} \equiv v_0$	$N_X \to N_X + 1$ $N_Y = \text{const}$
4.2 Ca^{2+} EXTRUSION	$Ca^{2+}_{cyt} \xrightarrow{k_4} Ca^{2+}_{ex}$	$k_4 N_X$	$N_X \to N_X - 1$ $N_Y = \text{const}$

activation of PLC (process 2.1 in fig. 6) releases Ca^{2+} from ISCS's. In addition, IP_3 (or its metabolite inositol 1,3,4,5 tetrakisphosphate) causes presumably a higher Ca^{2+}-influx through the plasma membrane. This additional IP_3-induced Ca^{2+} influx will then charge up the second class of Ca^{2+} pools that release their Ca^{2+} by CICR. In the two-pool model the net influx of Ca^{2+} into the cytosol either from ISCS's or from the extracellular medium is one critical parameter determining whether cytosolic Ca^{2+} is oscillating or not.

To test the two-pool model, Goldbeter, Dupont and Berridge have formulated it mathematically in form of deterministic rate equations[22]. As we will demonstrate now, a stochastic formulation of the two-pool model can also be derived directly from the DFD-model described above. For this purpose we have to characterize the main mechanisms by which cytosolic Ca^{2+} (data store S-5) may be altered. First, Ca^{2+} can be exchanged with the extracellular medium as modelled by process *4 ION EXCHANGE* (DFD 4 not shown). As subsystems this process includes the extrusion of cytosolic Ca^{2+} through a Ca^{2+}-ATPase (process *4.2 Ca^{2+} EXTRUSION*) and the influx of extracellular Ca^{2+} through membrane channels (process *4.1 Ca^{2+} INFLUX*). Secondly, cytosolic Ca^{2+} can be exchanged with ISCS's as modelled in DFD 2.3 (fig. 7).

As a third possibility (fig. 8), Ca^{2+} is sequestered into CICR-pool (process *2.4.1 PUMP CICR-POOL*) from where it can be released by CICR (process *2.4.2 CICR*). In addition, a small leaky flux of Ca^{2+} stored in the CICR-pools takes place into the cytosol (process *2.4.3 LEAKY FLUX*).

After the characterization of the Ca^{2+} oscillator through the DFD-model the second step of the SBM-cycle could be performed. To do this, we specify the processes of the lowest-level DFD's by a stochastic description according to the rules given in subsection 2.2, whereby we use some key elements from the Goldbeter et al. model[22]. The result of the mathematical transcription is shown in table 2.

Using the simulation algorithm described in subsection 2.3 the stochastic model can be directly simulated on a computer (fig. 9). Thereby, one main problem is the choice of appropriate simulation parameters.

Although the stochastic two-pool model is relatively

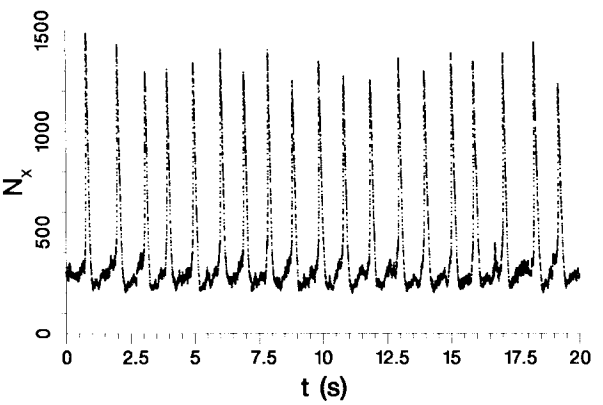

Figure 9. Stochastic simulation of the cytosolic Ca^{2+} level. The mechanisms by which the content of the data store *S-5 CYTOSOLIC Ca^{2+}* is changed are described mathematically in table 2. The plot shows the number of Ca^{2+} in the cytosol, N_x, versus time t (s). The following parameter values have been chosen: $\beta = 0.4$, $n = m = 2$, $p = 4$, $v_0 = 1000\ s^{-1}$, $v_1 = 7300\ s^{-1}$, $k_2 = k_4 = 5\ s^{-1}$, $k_f = 1\ s^{-1}$, $K_2 = 1000$, $V_{M2} = 32500\ s^{-1}$, $K_r = 2000$, $K_A = 900$ and $V_{M3} = 2.5 \cdot 10^5\ s^{-1}$, $N_x(t = 0\ s) = 300$, $N_y(t = 0\ s) = 1500$.

simple, it reproduces many of the facts observed experimentally. Most important, it explains how frequency encoded Ca^{2+} oscillations can emerge. Similar as in experiments, at low agonist doses no oscillations occur and the Ca^{2+} concentration in the cytosol is fluctuating around a low steady state level. At medium agonist doses oscillations occur whose frequency increases with increasing receptor stimulation. Finally, at high agonist doses cytosolic Ca^{2+} is fluctuating around a high steady state Ca^{2+} level without any oscillations. With a spatiotemporal extension of the stochastic two-pool oscillator it is also possible to model oscillatory intracellular Ca^{2+} waves[69].

4 Summary and future perspectives

In this paper we have introduced SBM as a systematic and flexible method for the modelling of complex biological systems. Beginning with a structured analysis of the system, a DFD-model is developed which models the processing of information through the system. The DFD-modelling can be considerably accelerated and advanced by modern CASE-tools. Because the DFD-model only consists of a few easy readable elements it can function as an interface between scientists working in different disciplines. As demonstrated on a simplified model for the cell cycle regulation, the modular style of the model's architecture allows the visualization of complex facts and facilitates the integration of substructural and biochemical data into a functional model of a cellular system.

In a second modelling phase of the SBM-cycle the DFD-model is translated into mathematical terms. Using a powerful simulation algorithm, the dynamics of the system can be simulated on a computer. Through this procedure verbally postulated and actual properties of the model can be compared. In addition, cheap computer experiments can be performed which produce an experimentally testable hypothesis.

As mentioned above, today too much data are lacking for the development of a comprehensive model of the cell cycle regulation especially in case of neoplastic growth. For this reason, flexible modelling techniques like SBM are necessary which allow, that future results can be incorporated into the model without any problems. On the other hand, SBM makes it also possible to model only single subsystems in a mathematically precise way, as demonstrated on intracellular Ca^{2+} oscillations.

We conclude that *Structured Biological Modelling* opens new fields for the structured analysis and simulation of complex biological systems, helps to reveal insight into fundamental biological mechanisms and provides the possibility to evaluate competing models.

Abbreviations. cAMP: cyclic adenosine monophosphate; CASE: computer-aided software engineering; CICR: Ca^{2+}-induced Ca^{2+} release; DAG: diacylglycerol; DFD: dataflow diagram; GMF: growth modulating factors; IICR: IP_3-induced Ca^{2+} release; ISCS: IP_3-sensitive Ca^{2+} store; PLC: phosphoinositide-specific phospholipase C; PKA: protein kinase A; PKC: protein kinase C; IP_3: inositol 1,4,5 trisphosphate; PI: phosphatidyl inositol; SPO: S-phase onset; TK: tyrosine kinase.

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1 Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D., Molekularbiologie der Zelle, 2nd edn. VCH, 1990.
2 Glansdorff, P., and Prigogine, I., Thermodynamic Theory of Structure, Stability and Fluctuations. Wiley, New York 1971.
3 Nicolis, G., and Prigogine, I., Self-organization in Non-equilibrium Systems. Wiley, New York 1977.
4 Murray, J. D., Mathematical Biology. Springer, New York, Heidelberg, Berlin, Tokyo 1989.
5 Gierer, A., and Meinhardt, H., Kybernetik *12* (1972) 30.
6 Eigen, M., Naturwissenschaften *58* (1971) 465.
7 Eigen, M., and Schuster, P., Naturwissenschaften *64* (1977) 541.
8 Eigen, M., and Schuster, P., Naturwissenschaften *65* (1978) 7.
9 Eigen, M., and Schuster, P., Naturwissenschaften *65* (1978) 341.
10 Goodwin, B. C., Temporal Organization in Cells. A Dynamic Theory of Cellular Control Processes. Academic Press, London 1963.
11 Goel, N. S., and Richter-Dyn, N., Stochastic Models in Biology. Academic Press, New York 1974.
12 DeMarco, T., Structured Analysis and Systems Specification. Prentice Hall, Englewood Cliffs, New Jersey 1979.
13 Gane, C., and Sarson, T., Structured Systems Analysis: Tools and Techniques. Prentice-Hall, Inc., Englewood Cliffs, New Jersey 1979.
14 Martin, J., and McClure, C., Structured Techniques – the Basis for CASE, Prentice Hall, Englewood Cliffs 1988.
15 Yourdon, E., Modern Structured Analysis. Prentice Hall, Englewood Cliffs 1989.
16 Moore, E. D. W., Becker, P. L., Fogarty, K. E., Williams, D. A., and Fay, F. S., Cell Calcium *11* (1990) 157.
17 Wolf, B., Lezius, C., and Schwinde, A., Micron. Microsc. Acta *18* (1987) 1.
18 Wolf, B., and Schwinde, A., Micron Microsc. Acta *19* (1988) 147.
19 Wolf, B., and Bessler, W. G., Naturwissenschaften *77* (1990) 110.
20 Kuhn, H., Angew. Chem. Int. Ed. Engl. *29* (1990) 1.
21 Berridge, M. J., and Irvine, R. F., Nature *341* (1989) 197.
22 Goldbeter, A., Dupont, G., and Berridge, M. J., Proc. natl Acad. Sci. USA *87* (1990) 1461.
23 Honerkamp, J., Stochastische Dynamische Systeme: Konzepte, numerische Methoden, Datenanalysen. VCH, Weinheim 1990.
24 Gillespie, D. T., J. Comput. Phys. *22* (1976) 403.
25 Gillespie, D. T., J. Phys. Chem. *81* (1977) 2340.
26 Press, W. H., Flannery, B. F., Teukolsky, S. A., and Vetterling, W. T., Numerical Recipes: The Art of Scientific Computing. Cambridge Univ. Press, Cambridge 1986.
27 Turner, J. S., J. Phys. Chem. *81* (1977) 2379.
28 Hofmann, F., Chemie in unserer Zeit *21* (1987) 186.
29 Wagner-Roos, L., Einblick: Zeitschrift des Deutschen Krebsforschungszentrums. (1/1992) 25.
29a Franke, W. W., Der heutige Kenntnisstand läßt noch kein durchgängiges Konzept zur Verhinderung und Therapie von Krebserkrankungen erkennen, in: Wagner-Roos[29].
30 Pardee, A. B., Science *246* (1989) 603.
31 Prescott, D. M., and Flexer, A. S., Krebs: Fehlsteuerung von Zellen: Ursachen und Konsequenzen. Spektrum-Verlag, Heidelberg 1990.
32 Bishop, J. M., Science *235* (1987) 305.

33 Sorrentino, V., Anticancer Res. *9* (1989) 1925.
34 Suarez, H. G., Anticancer Res. *9* (1989) 1331.
35 Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., and Soltoff, S., Cell *64* (1991) 281.
36 Bar-Sagi, D., Anticancer Res. *9* (1989) 1427.
37 Downward, J., TIBS *15* (1990) 469.
38 Wolf, B., Müller, E., and Schwinde, A., Virchows Arch. [Pathol. Anat.] *407* (1985) 209.
39 Wolf, B., Bischoff, E., and Schwinde, A., Virchows Arch. [Pathol. Anat.] *408* (1986) 665.
40 Wolf, B., Schwinde, A., and Wittekind, C., in: Pathology of Neoplastic and Endocrine Induced Diseases of the Breast, p. 101. Eds R. Bässler and K. Hübner. Gustav Fischer Verlag, Stuttgart. New York 1986.
41 Wolf, B., Lezius, C., and Schwinde, A., Anticancer Res. *7* (1987) 369.
42 Wolf, B., Hauschildt, S., Dinger, V., and Lämmlin, C., Eur. Microsc. Analysis *9* (1991) 17.
43 Gebhardt-Urbanek, I., Wittekind, C., and Wolf, B., Dtsch. Zschr. Onkol. *24* (1992) 85.
44 Haken, H., Synergetics. An introduction, 3rd edn. Springer, New York, Heidelberg, Berlin, Tokyo 1983.
45 Deuel, T. F., A. Rev. Cell Biol. *3* (1987) 443.
46 Schramm, M., and Selinger, Z., Science *225* (1984) 1350.
47 Taylor, S. S., Buechler, J. A., and Yonemoto, W., A. Rev. Biochem. *59* (1990) 971.
48 Sibley, D. R., Benovic, J. L., Caron, M. G., and Lefkowitz, R. F., Cell *48* (1987) 913.
49 Nishizuka, Y., Science *233* (1986) 305.
50 Cohen, P., Proc. R. Soc. Lond. B *234* (1988) 115.
51 Sagi-Eisenberg, R., TIBS *14* (1989) 355.
52 Tonks, N. K., and Charbonneau, H., TIBS *14* (1989) 497.
53 Yarden, Y., and Ullrich, A., A. Rev. Biochem. *57* (1988) 443.
54 Stryer, L., Biochemie. Spektrum-der-Wissenschaft-Verlagsgesellschaft, Heidelberg 1990.
55 Sheng, M., Thompson, M. A., and Greenberg, M. E., Science *252* (1991) 1427.
56 Rasmussen, H., and Rasmussen, J. E., Curr. Top. Cell. Regul. *31* (1990) 1.
57 Carafoli, E., A. Rev. Biochem. *56* (1987) 395.
58 Lämmlin, C., Elektronenoptische Untersuchungen zum Kerngrading an benignen und malignen Bronchial- und Magengeweben. Thesis, Freiburg University 1990.
59 Kaye, S. B., Cancer Treatment Rev. *17* (1990) 37.
60 Mickisch, G. H., Kossig, J., Keilhauer, G., Schilk, E., Tschada, R. K., and Alken, P. M., Cancer Res. *50* (1990) 3670.
61 Berridge, M. J., J. biol. Chem. *265* (1990) 9583.
62 Meyer, T., and Stryer, L., A. Rev. Biophys. biophys. Chem. *20* (1991) 153.
63 Kraus, M., and Wolf, B., Naturwissenschaften *79* (1992) 289.
64 Kraus, M., and Wolf, B., Cytosolic calcium oscillators: Critical discussion and stochastic modelling. Biol. Signals (1993), in press.
65 Kraus, M., and Wolf, B., Biol. Signals *1* (1992) 101.
66 Berridge, M. J., and Galione, A., FASEB J. *2* (1988) 3074.
67 Wakui, M., Potter, B. V. L., and Petersen, O. H., Nature *339* (1989) 317.
68 Wakui, M., Osipchuk, Y. V., and Petersen, O. H., Cell *63* (1990) 1025.
69 Kraus, M., Lais, P., and Wolf, B., BioSystems *27* (1992) 145.