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Systems modelling methodology for the analysis of apoptosis signal transduction and cell death decisions

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ABSTRACT

Systems biology and systems medicine, i.e. the application of systems biology in a clinical context, is becoming of increasing importance in biology, drug discovery and health care. Systems biology incorporates knowledge and methods that are applied in mathematics, physics and engineering, but may not be part of classical training in biology. We here provide an introduction to basic concepts and methods relevant to the construction and application of systems models for apoptosis research. We present the key methods relevant to the representation of biochemical processes in signal transduction models, with a particular reference to apoptotic processes. We demonstrate how such models enable a quantitative and temporal analysis of changes in molecular entities in response to an apoptosis-inducing stimulus, and provide information on cell survival and cell death decisions. We introduce methods for analyzing the spatial propagation of cell death signals, and discuss the concepts of sensitivity analyses that enable a prediction of network responses to disturbances of single or multiple parameters.

1. Introduction

A system consists of a defined set of components and, importantly, their interactions. Mathematically modelling and analysing the dynamic behaviour of systems is of fundamental importance in the natural sciences of physics and chemistry, and their applied engineering disciplines. Even though systems modelling of biological processes on the cellular and subcellular level has a successful history in the areas of electrophysiology and metabolic pathway control that predates the molecular biology revolution, research strategies specifically exploiting the potential of predictive systems modelling in the life sciences have re-gained wider acceptance only within the past decade.

Systems modelling can assist in identifying and understanding systems features emanating from multi-gene and –protein networks (that is functions which cannot be assigned to a single system component). These include, for example, molecular switches, rheostats (used to adjust network behaviour to alterations in signalling input), and response thresholds. Particular significant progress has been made in recent years in the area of systems biology of apoptosis. This is largely due to three reasons. Firstly, compared to other continuously active biological signalling processes that constantly interact with and flexibly adapt to external conditions, apoptosis signalling networks, especially the execution phase, can be considered to be largely dormant when not specifically triggered. And, once initiated, apoptosis signalling is reasonably well isolated from other cellular signalling processes. Secondly, the vast amount of literature in the field of cell death research allows to construct reasonably well defined pathway models and provides ample opportunity to extract qualitative and quantitative data on individual system components and their interactions. Thirdly, the methodological progress made in measuring multiple parameters quantitatively and in parallel, not only in cell populations but also in individual cells, has provided opportunities to investigate signalling networks and their signalling characteristics in time and space directly within the complex environment of the living cell. This allows to train mathematical models to optimally reflect biological signalling behaviour, and, more importantly, to test systems-based research hypotheses and model-generated predictions on signalling behaviour of the respective (sub-) systems under investigation. Indeed, systems modelling of apoptosis has considerably advanced our understanding of cell fate decisions between life and death in recent years.

Models of biological "reality" are at the centre of all biological research. In their most basic form, such models are static and

Abbreviations: MOMP, mitochondrial outer membrane permeabilization; ODE, ordinary differential equation; PDE, partial differential equation.

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descriptive, such as text-based descriptions of research findings or the drawing of pathway diagrams that reflect key relationships between system components. Mathematical modelling extends static models by introducing the possibility to interactively test signalling systems *in silico* for their intrinsic properties, for example their kinetic behaviour in the dimensions of time and space, qualitatively or quantitatively. In the following we will briefly cover different modelling strategies, and more specifically describe methodology to model apoptosis signal transduction by ordinary and partial differential equations. Such models can be developed to closely reflect basic cellular signalling processes, and gain particular power through appropriate parameterisation with experimental data. We also introduce reference studies that used the methodology described herein and finally outline how to parameterise systems models with biologically meaningful parameters.

2. Mathematical modelling approaches and their application towards apoptosis signalling

From a biologist's perspective, models that are mechanistically close to the biological processes under investigation, for example a representation of protein–protein interactions during apoptosis signalling, seem to be most desirable and well accepted. Indeed, carefully parameterised models that build on the mathematical representation of individual reactions or core processes have proven to be of exquisite predictive value and had significant impact in apoptosis research in the past years [1–3]. We will therefore describe the key steps for the mathematical implementation of such models. However, before doing so, we will give a brief overview of related or alternative modelling approaches that can be helpful when a detailed parameterisation is impossible or not required.

Probably the most basic mathematical progression from static pathway models are Boolean models. In their simplest form, processes represented in the model, e.g. interactions, active states, transport processes etc., are described as binaries. For example, rather than assigning a specific activity to an enzyme, its state is changed from off (zero) to on (one) upon activation. The reverse applies for inactivation. The development of a network coded purely by off/on states (and derivative models that also allow intermediate steps) already can give useful qualitative information on which experimental outcomes are possible or likely based on the underlying signalling network topology and starting conditions. Indeed, such models were successfully applied in apoptosis research and validated qualitatively against experimental data in order to investigate the interplay of extrinsic and intrinsic apoptosis signalling and the competition between external pro-survival and pro-death signals [4,5]. At the other end of the spectrum lie models that are generated by statistical approaches. Rather than building models bottom-up from a detailed mechanistic understanding of the network, initially large data sets, often integrated from multiple experimental approaches, are used to identify underlying patterns from which data–driven models are then derived [6]. Typically, the rich data sets are first reduced in their dimensionality by identifying correlations in the investigated variables. Subsequently, *in silico* cross-validations and perturbations are conducted to validate statistical models against experimental data. Such models were, for example, successfully generated from high-throughput datasets to gain insight into the molecular basis of cytokine-induced apoptosis and the apoptosis sensitization through sequential drug administration schemes [7,8]. We previously gave broader overviews of the various modelling strategies used in apoptosis systems biology, and the interested reader may consult these sources for more comprehensive information [9,10].

3. Modelling apoptosis signalling kinetics in time using ordinary differential equations

As mentioned in Section 2, significant mechanistic and quantitative understanding of apoptosis signalling can be obtained from systems models that are comprised of components which reflect the central underlying biological processes. When restricting ourselves to protein signalling networks in the following, two central processes fundamentally dictate the behaviour of cellular signalling pathways. These are (i) the binding/dissociation of two (or more) interaction partners, and (ii) reactions that alter and transform a target, for example the enzymatically-catalysed posttranslational modification or cleavage of a protein. The velocities of these reactions can be described using basic biochemical information, and from the velocities a list of ordinary differential equations (ODEs) can be generated. These ODEs then describe the dynamics of all proteins/protein complexes of the system *in silico*. Since experimental approaches are naturally limited in their capacity to observe multiple parameters in parallel, such models, once parameterised with the starting concentrations of the proteins involved (see Section 8), therefore significantly expand the insight into the system under investigation.

Optimally, it should be possible to link a model to a parallel experimental approach that is suitable to validate model predictions. Such a link can be achieved when model and experiment share common inputs (e.g. clearly defined processes such as drug administration or a measurable upstream signalling event) and quantifiable outputs (e.g. cell death readings or caspase activities) [11]. To name examples, we previously linked a model of apoptosis execution directly to experiments by using mitochondrial outer membrane permeabilisation (MOMP) and substrate cleavage by effector caspases as measureable inputs and outputs of both model and experiments [3]. Likewise, we recently coupled death ligand TRAIL addition and initiator caspase-8 activation as input/output parameters to a quantitative model of apoptosis initiation [12].

3.1. Representing basic biochemical processes by ordinary differential equations

To generate an ODE model, a list of all reactions occurring in the system of choice is needed. Using this list, a simple procedure can be followed to obtain the equations required for the model. In the following we describe this process for a simple system of only two reactions. Let reaction R_1 be the binding/dissociation between two proteins A and B, and R_2 be the cleavage of protein A by a protease C.

$$\text{Binding/Dissociation}\, R_1 : \mathrm{A} + \mathrm{B} \leftrightarrow \mathrm{AB}$$

$$\text{Irreversible cleavage}\, R_2 : \mathrm{C} + \mathrm{A} \rightarrow \mathrm{C} + \mathrm{A}^{I} + \mathrm{A}^{II}$$

The first reaction is reversible and indicates that a balance between free and bound fractions exists. Quantitative information on the binding and dissociation kinetics of binding partners involved in apoptosis signaling can often be obtained from the rich pool of literature in the form of k_{on} and k_{off} rates (see Section 8). The second reaction instead can be considered to be irreversible. Here, protein A is cleaved into two fragments ($A^I + A^{II}$) by enzyme C. This reaction can be described by mass-action kinetics, and requires knowledge on the catalytic constant k_{cat}, which, for example for caspases, likewise often can be obtained from the literature (see Section 8). Where values have not been reported yet, justifiable estimations can be used or, alternatively, mathematical fitting pro-