Inna N. Lavrik *Editor*

Systems Biology of Apoptosis

Springer

Systems Biology of Apoptosis

Inna N. Lavrik
Editor

Systems Biology of Apoptosis

Springer

Editor
Inna N. Lavrik
Department of Translational Inflammation Research
Institute of Experimental Internal Medicine
Otto von Guericke University
Magdeburg, Germany

ISBN 978-1-4614-4008-6 ISBN 978-1-4614-4009-3 (eBook)
DOI 10.1007/978-1-4614-4009-3
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012943946

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Programmed cell death is a fascinating process common to all multicellular organisms. Programmed cell death results in the elimination of cells via a complex but a highly defined programme. Defects in the regulation of programmed cell death are associated with serious diseases such as cancer, autoimmunity, AIDS, and neurodegeneration.

Apoptosis has been the best studied type of programmed cell death so far. Cells that undergo apoptosis are characterized by chromatin condensation, nuclear fragmentation, membrane blebbing, cell shrinkage, and formation of apoptotic bodies.

The central role in apoptosis execution belongs to cysteine-specific aspartate proteases (caspases). Caspases are enzymes that orchestrate apoptosis via cleavage of cellular substrates.

There are two major pathways of apoptosis: intrinsic and extrinsic. The intrinsic pathway is triggered via chemotherapeutic drugs, irradiation, and growth factor withdrawal. These stimuli lead to mitochondrial outer membrane permeabilization (MOMP), which results in cytochrome *C* release and caspase activation. In the extrinsic apoptotic pathway, the caspase cascade is triggered by signals emanating from the cell-surface death receptors (DR) triggered by death ligands (DL) (TNF, CD95L/FasL, TRAIL). The DR stimulation results in the formation of the death-inducing signaling complex (DISC) and subsequent caspase activation.

Despite the fact that signaling pathways of apoptosis have been described with an impressive level of detail, the understanding of apoptosis regulation in quantitative terms has been missing until recently. There were many unclear points: when does a cell decide that it has to die, what are the rate-limiting steps in apoptosis, is there a point of no return, how can cell death be accelerated or blocked, and many others. From another side the years of apoptosis research resulted in a profound understanding of how signaling in apoptosis occurs. All major apoptotic complexes have been identified from the DISC to the apoptosome, including the death receptors and adaptors and the most important enzymes and their inhibitors. Therefore, apoptosis was an ideal system to go into quantitative studies using the emerging field of systems biology.

Systems biology combines mathematical modeling with experimental approaches in a closed loop cycle (Fig. 1). On the modeling side there are a number of mathematical formalisms, e.g., Ordinary Differential Equations (ODEs), Boolean models, etc., that allow to address different biological questions. Experimental work for systems biology of apoptosis involves the generation of quantitative data using different apoptotic assays.

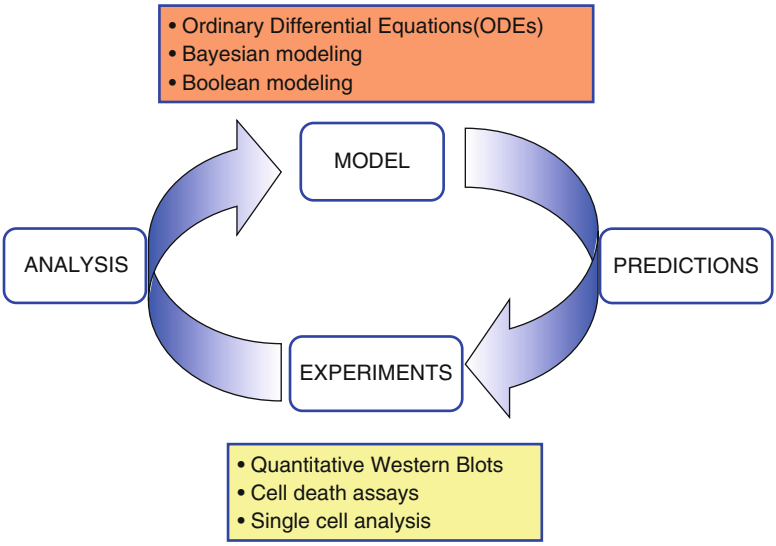

Fig. 1 Systems biology of apoptosis. Schematic view of systems biology of apoptosis

The development of this field in the recent years is fascinating. Studies of apoptosis using systems biology have provided novel insights into the quantitative regulation of cell death. In this book we describe contemporary systems biology studies devoted to cell death signaling both from experimental and modeling sides and focus on the question how systems biology helps to understand life/death decisions made in the cell and how to develop new approaches to rational treatment strategies.

Chapter 1 starts with an overview of the major types of mathematical modeling used in apoptosis and cell death. A simple minimalistic model of CD95/Fas-induced apoptosis is designed to introduce the most commonly used mathematical formalism, ordinary differential equations (ODEs). Besides ODEs, other modeling approaches are discussed in depth as well.

In Chap. 2 we focus on the biology of the extrinsic apoptotic pathway and its modeling by Ordinary Differential Equations (ODEs). We discuss new insights in the extrinsic death signaling which have been obtained using modeling.

Chapter 3 is devoted to model reduction approaches and uses the extrinsic apoptotic signaling as an example. This chapter provides a beautiful example of

how complex biological signaling can be simplified using mathematical modeling and how a simplified model can provide new insights in complex biological questions. The first three chapters provide a major insight into the modeling of extrinsic pathways.

Chapter 4 covers the molecular mechanisms of the mitochondrial apoptotic pathway and the major models describing this pathway. An emerging question in the field how bioenergetics influence the cell death pathway is also addressed in detail.

Chapter 5 further addresses the molecular mechanisms of extrinsic and intrinsic apoptosis in the context of modeling hepatocytes. Notably, an enormous progress has been recently made in modeling the signaling pathways in the liver and, in particular, cell death in the liver. This work is essential to define new therapeutic strategies for liver regeneration and liver disease.

Chapter 6 explores other forms of cell death, e.g., necrosis, autophagy, their cross talk with apoptosis, as well as the way to model cross talk between different cell types using Boolean modeling.

Chapter 7 describes a single cell analysis. Single cell analysis is compared to bulk approaches and the importance to follow a single cell rather than a cell population is discussed.

Chapter 8 discusses a systems-level understanding of cytokine–cytokine cross talk, namely how the cross talk between different cytokine pathways could be modeled on intracellular and extracellular levels. The importance of this cross talk for development and disease is also highlighted.

Chapter 9 deals with an important question in the field: the importance of searching for new components of cell death networks using different screening techniques. The unraveling of new components versus the investigation of dynamic models, which include all known components of the network, is a highly discussed question.

Taken together, the different chapters of the book describe in detail the remarkable progress which was made in recent years in systems biology of apoptosis and show new challenges in this field that can provide even more exciting insights into cell death regulation.

Contents

Contributors

Christoph Borner Institute of Molecular Medicine and Cell Research, Albert Ludwigs University Freiburg, Freiburg, Germany

Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University Freiburg, Freiburg, Germany

Bioss – Centre for Biological Signaling Studies, Albert Ludwigs University Freiburg, Freiburg, Germany

Michael Boutros German Cancer Research Center, Division of Signaling and Functional Genomics, Heidelberg University, Department of Cell and Molecular Biology, Medical Faculty Mannheim, Heidelberg, Germany

Laurence Calzone Institut Curie, Paris, France

Ecole des Mines ParisTech, Paris, France

INSERM U900, Paris, France

Christopher D. Deppmann Department of Biology, University of Virginia, Charlottesville, VA, USA

Michael Ederer Institute for System Dynamics, University of Stuttgart, Stuttgart, Germany

Christina Falschlehner Division of Signaling and Functional Genomics, German Cancer Research Center (DKFZ), University of Heidelberg, Heidelberg, Germany

Nicolai Fricker Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany

Division of Immunogenetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

Bioquant, Heidelberg, Germany

Heinrich J. Huber Department of Physiology and Medical Physics, Centre for Systems Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Kevin A. Janes Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA

Stefan Kallenberger Division of Theoretical Bioinformatics, DKFZ and BioQuant, Heidelberg, Germany

Inna N. Lavrik Department of Translational Inflammation Research, Institute of Experimental Internal Medicine, Otto von Guericke University, Magdeburg, Germany

Stefan Legewie Institute of Molecular Biology, Mainz, Germany

Irmgard Merfort Department of Pharmaceutical Biology and Biotechnology, Albert Ludwigs University Freiburg, Freiburg, Germany

Carla O'Connor Centre for Human-Systems Medicine, Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland

Jochen H.M. Prehn Department of Physiology and Medical Physics, Centre for Systems Medicine, Royal College of Surgeons in Ireland, Dublin 2, Ireland

Dennis Rickert Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany

Rebekka Schlatter Institute for System Dynamics, University of Stuttgart, Stuttgart, Germany

Kolja Schleich Division of Immunogenetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

Bioquant, Heidelberg, Germany

Kathrin Schmich Department of Pharmaceutical Biology and Biotechnology, Albert Ludwigs University Freiburg, Freiburg, Germany

Fabian J. Theis Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany

Boris Zhivotovsky Institute of Environmental Medicine, Stockholm, Sweden

Andrei Zinovyev Institut Curie, Paris, France

Ecole des Mines ParisTech, Paris, France

INSERM U900, Paris, France

Chapter 1
Modeling Formalisms in Systems Biology of Apoptosis

Stefan Kallenberger and Stefan Legewie

Abstract Apoptosis is a form of cellular suicide central to various aspects in biology including tissue homeostasis, embryonic development, carcinogenesis, and neurodegenerative disorders. Quantitative modeling approaches provided valuable insights into the digital and irreversible nature of apoptosis initiation. In this chapter, we summarize the mathematical formalisms used in systems biology of apoptosis. In addition, we give an overview of apoptosis-related research questions that can be addressed by modeling. Moreover, we review top-down and bottom-up modeling approaches applied to apoptosis, and particularly focus on ordinary differential equation (ODE) modeling. Basic concepts such as bistability and sensitivity analysis are introduced, and a review of apoptosis-related ODE models is provided. We describe bistability, temporal switching, crosstalk between death and survival, and also discuss approaches to model cell-to-cell variability.

1.1 Why Modeling Apoptosis?

Apoptosis is a phenomenologically easily observable process. However, understanding its mechanistic basis is challenging owing to complex interactions of a large number of signaling proteins and emergent behavior at the systems level. After applying a sufficiently strong death-inducing stimulus to a population of cells, irreversible signaling events are initiated leading to the characteristic appearance of an apoptotic cell: Membrane blebbing proceeds, the cell shrinks, and organelles disintegrate. Apoptosis occurs for extrinsic stimuli on a timescale of hours and for intrinsic stimuli of days, and is accessible to several experimental techniques

S. Kallenberger
Division of Theoretical Bioinformatics, DKFZ and BioQuant, Heidelberg, Germany

S. Legewie (✉)
Institute of Molecular Biology, Mainz, Germany
e-mail: s.legewie@imb-mainz.de

I.N. Lavrik (ed.), *Systems Biology of Apoptosis*, DOI 10.1007/978-1-4614-4009-3_1,

allowing for the acquisition of quantitative data. The classical techniques of Western blotting and immunoprecipitation enable coincidental acquisition of coarsely time-resolved population data for proteins and their intermediate processing stages. Fluorescence-based flow cytometry techniques allow measuring the protein concentrations at the single-cell level. A major disadvantage of flow cytometry is the inability of tracking time-dependent behavior of individual cells. This problem is overcome by fluorescence-based microscopic methods that were developed to obtain quantitative data of single cells with high temporal resolution: The activity of caspases can be monitored with FRET reporters or smart probes that harbor caspase cleavage sites. Moreover, the mitochondrial pathway of apoptosis can be monitored by measuring Bax translocation, outer membrane permeabilization, and Smac release. The wide range of available experimental techniques and the detailed knowledge about molecular events render apoptosis a system suitable for modeling analyses. Apoptosis induced by death ligands is one of the few cell fate decisions known to proceed by purely posttranscriptional mechanisms, thus further simplifying the formulation of mathematical models.

Even though individual steps of the apoptotic signal transduction cascades are well understood, we lack insights into the system properties and the dynamics of the death decision. Questions to be addressed in apoptosis by systems biology approaches include:

1. How do cells ensure that apoptosis robustly occurs in all-or-none manner? What is the "point-of-no-return" representing irreversibility in apoptosis? Which signaling motifs are responsible for such digital and history-dependent behavior? As detailed below, mechanisms proposed using kinetic modeling include bistability due to positive feedback and sigmoidal responses arising from competitive inhibition.
2. How is specificity in the apoptosis vs. survival responses ensured? A topic of particular interest for apoptosis modeling is that apoptotic stimuli trigger survival or death signaling depending on initial conditions and the stimulus strength. At least in some cases, the inhibitory crosstalk between survival and cell death signaling pathways appears to be mutually exclusive at the single-cell level (Nair et al. 2004), implying that death and survival represent different attractor states for the cell. Modeling can be employed to identify critical nodes of signaling crosstalk that tip the balance between cell death and survival. Furthermore, the interlocked regulation of cell cycle is a topic followed by modelers. In this context the characterization of attractors, fixed points, and limit cycles is of interest.
3. What are the principles underlying cell-to-cell variability in the apoptosis response of a cell population? Why do cell types differ in their sensitivity to death-inducing stimuli? Currently, several therapeutic applications are tested to stimulate apoptosis in cancer cells, to decelerate tumor growth, or to prevent cells, preferentially neurons or cardiomyocytes, from undergoing programmed cell death. Modeling approaches could help to plan therapies and to predict the outcome on a population of cells. Particularly by distinguishing cell death

kinetics and the behavioral heterogeneity of different cell types, and predicting drug sensitization by cotreatments, modeling could be a valuable tool. We will describe and review strategies to predict cell death kinetics of single cells and of heterogeneous cell populations.

First we will give an overview of the basics of mathematical formalisms and then review successful application of ODE apoptosis models to resolve biological questions.

1.2 Overview of Mathematical Formalisms

Analyzing the cell on a systems view can be done by top-down and bottom-up approaches. Detailed mechanistic mathematical models constructed from the molecular characteristics of individual proteins ("bottom-up models") have only been developed for metabolic and signaling networks. In contrast, transcriptional regulatory networks, and the link between signaling networks and ultimate cellular decisions are best tackled by statistical methods which integrate huge amounts of data but are mostly phenomenological ("top-down modeling").

Top-down approaches examine the cell on a global level, treating individual regulatory modules as black boxes that are not analyzed mechanistically but only characterized with respect to input–output behavior. Thus, top-down methods typically do not require much prior knowledge about the system, so that many signaling and/or metabolic pathways can be studied at once. Most top-down approaches are solely data-driven and rely on high throughput screens of cellular behavior (gene expression profiling, proteomics, siRNA screening, sequencing, and affinity assays). Typically, the ultimate goal of top-down approaches is to identify biologically relevant patterns and correlations to the data (e.g., disease marker gene identification) or to predict new molecular interactions (e.g., reverse engineering algorithms).

Bottom-up approaches focus on well-characterized parts of the biochemical regulatory network, and are typically based on the assumption that the properties of these subnetworks (or "modules") can be studied in isolation. Based on prior knowledge and on time-resolved experimental data, mechanistic mathematical models describing the interactions of individual proteins in the module are constructed (e.g., by using sets of coupled differential equations). The goal of bottom-up modeling is to identify physiologically relevant systems-level properties emerging from complex interactions within the network (e.g., feedback).

Apoptosis-inducing signaling cascades, especially those induced by death ligands, were mainly studied using bottom-up modeling approaches, since (1) the molecular events are well characterized; (2) transcriptional events can be neglected; (3) the ultimate death decision often closely correlates with all-or-none activation of effector caspases, implying that statistical methods are not required to link signaling to cellular phenotypes. However, bottom-up approaches to apoptosis are diverse and the methodology of choice depends on the complexity

of the signaling network under study, the available experimental data, and the question to be addressed by modeling. Boolean approaches are typically employed to qualitatively analyze the (quasi-)static behavior of large apoptosis-survival crosstalk networks which comprise many molecular species. Ordinary differential equation (ODE) models allow for the quantitative description of network dynamics but typically require knowledge about many kinetic parameters which either limits the network size and/or requires huge amounts of experimental data. Standard ODE modeling may even not be sufficient if spatiotemporally resolved single-cell data is available (1) spatial gradients within the cell can be modeled using subcellular compartment ODE models or partial differential equations (PDEs). (2) Cell-to-cell variability may arise due to stochastic dynamics of the apoptotic signaling cascade ("intrinsic noise") or due to cell-to-cell variability in the expression of pathway components ("extrinsic noise"). While ODE models with randomly sampled initial protein concentrations can be employed to simulate extrinsic noise, stochastic simulation algorithms are required to understand intrinsic noise. In the following, we will give an overview of top-down and bottom-up modeling approaches applied to apoptosis signaling, before discussing applications of ODE models in more detail.

1.2.1 Linear Regression Models

To systematically analyze how the pro- and anti-apoptotic cytokines TNF, EGF, and insulin impinge on the cellular apoptosis decision, Janes et al. (2005) generated a compendium of costimulation measurements. Based on the assumption that simple linear combinations of signaling activity profiles account for apoptosis initiation, they employed a top-down modeling approach known as partial least-squares regression (PLSR) which does not require prior knowledge. PLSR modeling calculates super axes as an orthogonal set of "principal components," which contain linear combinations of the original signaling protein activities weighted by their contribution to the apoptotic outputs. Thereby, the dimension of the data matrix is reduced to a small set of informative super axes, which can be used to predict apoptosis initiation for any experimental condition, provided that measurements of signaling species used for model training are available. PLSR has been successfully applied to other large-scale apoptosis datasets, and provided insights into complex phenomena such as autocrine amplification loops (Janes et al. 2006). For a more detailed description, please, see the chapter by Deppmann and Janes. A major drawback of PLSR is the lack of mechanistic insights into (1) how signaling activity patterns are generated and (2) how signaling activities are integrated, e.g., at the level of caspases, to control the death decision. Therefore, the next section will be devoted to bottom-up approaches applied to apoptosis which take into account mechanisms of apoptosis initiation.

1.2.2 Boolean Models

Recent biomedical research revealed a plethora of protein–protein and enzymatic interactions, and thus extensively characterized the topology of the intracellular signaling network. However, quantitative information characterizing the affinity of protein–protein interactions or enzyme kinetic parameters is still scarce. Moreover, quantitative characterization is often performed using recombinant proteins in vitro, with questionable relevance to the in vivo situation. Simulations of large-scale networks is therefore often performed using Boolean or logic modeling, a qualitative approach that is based on network topology, but does not take into account quantitative features of individual reactions. Instead protein activities are represented by nodes which can either be on or off (activity 0 or 1), depending on the activities of upstream input nodes. Logic rules are applied at each iteration: For example, in a so-called *AND-gate*, the node *Z* will be activated if and only if *both* input nodes *X* and *Y* are active. In contrast, an *OR-gate* simply requires either *X* or *Y* to be active. Thus, Boolean rules can be used to qualitatively represent real biochemical mechanisms such as functional redundancy (*OR-gate*) or coincidence detection (*AND-gate*), the latter, arising from sequential processing by two distinct enzymes. Since logical rules are applied iteratively, the approach can be used to study temporal phenomena such as adaptation. Moreover, Boolean networks can exhibit nonlinear dynamic phenomena such as oscillations, and stable vs. unstable attractors. Please see chapters by Schlatter et al. and Calzone et al. for a detailed review on Boolean network dynamics.

A number of Boolean modeling studies have been presented in the context of apoptosis (Calzone et al. 2010; Mai and Liu 2009; Philippi et al. 2009; Schlatter et al. 2009; Zhang et al. 2008). All these studies analyzed the crosstalk of apoptosis signaling via caspases and survival pathways such as NF-κB signaling. The main goal was the identification of stable states in the systems, representing cell fates such as apoptosis, necrosis, and survival. Calzone et al. (2010) and Mai and Liu (2009) focused on signaling upon death receptor engagement. They showed that the stable states of the apoptosis network are robust and investigated the requirements for irreversibility in the apoptosis decision. Schlatter et al. (2009) and Philippi et al. (2009) took into account costimulation with prodeath and prosurvival ligands, and experimentally confirmed key model predictions. Zhang et al. (2008) analyzed antigen-induced survival signaling network in T cell large granular lymphocyte (T-LGL) leukemia cells including transcriptional induction of cytokines and autocrine stimulation events. Model predictions could be confirmed in leukemic cells isolated from patients, thus contributing to our understanding of signaling deregulation in the disease. Taken together, Boolean modeling approaches provided valuable insights into apoptosis at multiple timescales and for various experimental settings.

1.2.3 Quantitative Modeling Approaches

Boolean models are inherently limited in their capability of quantitatively describing the temporal dynamics of biochemical networks. In the context of perturbation analysis, Boolean approaches are restricted to the simulation of complete elimination of network nodes and/or reactions; thus, gradual phenomena such as dosage compensation cannot be studied. Moreover, the qualitative effects of perturbations as revealed by Boolean modeling are often intuitively clear. Thus, in many cases, nontrivial and experimentally testable predictions require quantitative modeling approaches such as ODE and PDE modeling, as well as stochastic simulations.

ODE approaches, described in detail below, assume that large numbers of signaling molecules are present within the cell, so that random fluctuations in reaction events can be neglected by averaging over the whole molecule population. Moreover, in ODE modeling it is assumed that the cell represents a well-stirred reactor, implying that diffusion effects do not matter. In apoptosis networks, these assumptions are likely to be fulfilled, as caspase and their regulators are typically expressed at the number of several hundred thousand molecules per cell (Svingen et al. 2004). Furthermore, the time scale of apoptosis induction (hours) is slow relative to the time scale of protein diffusion within a cell (milliseconds to seconds); therefore, spatial gradients of apoptosis signaling molecules are unlikely to play a decisive role in apoptosis initiation.

Nonetheless, reaction–diffusion models allowed investigating molecular mechanisms of apoptosis induction: Using live-cell imaging with high temporal resolution, Rehm and colleagues (2009) observed that cytochrome c release from mitochondria during apoptosis occurs in spatial waves that propagate from a subcellular mitochondrial pool to the remainder of the mitochondrial population. PDE modeling was employed to investigate the dynamics of nonsteady state diffusion. This approach revealed that localized release and diffusion of inducers of mitochondrial outer membrane permeabilization (MOMP) alone was insufficient to explain the data. However, then the authors took into account that MOMP inducers bind to mitochondria, and modeling indicated that this absorption shapes the dynamics of cytochrome c release, thus providing insights into molecular mechanisms controlling apoptosis induction.

Owing to low molecule numbers of Bcl-2 family members, stochastic simulations using cellular automaton approaches were performed by Chen et al. (2007a), Siehs et al. (2002), and Düssmann et al. (2010) to describe the dynamics of MOMP. Chen et al. (2007a) focused on bistability and concluded that the stochastic system attained two distinct stable states much like the deterministic case; thus robustness of switching towards molecular noise could be confirmed. Düssmann et al. (2010) compared their model to measurements in cells expressing Bax-FRET probes monitoring Bax oligomerization. Their model could provide an explanation for pore formation upon Bax accumulation and oligomerization in the outer mitochondria membrane.

Live cell imaging tools are increasingly important and allow the analysis of apoptosis at the single cell level or even with subcellular resolution. Thus, stochastic and reaction–diffusion modeling are likely to become central to apoptosis modeling. For example, death receptors are frequently expressed at low levels and form localized (nano-) clusters on the cell membrane (Dumitru and Gulbins 2006), implying that deterministic ODE approaches will fail, especially upon weak stimulation. Stochastic and reaction–diffusion modeling will reveal underlying mechanisms and, more importantly, predict strategies for intervention for testing the functional relevance of such phenomena.

1.3 Basic Concepts in ODE Modeling

In this section, we give an overview of the most important steps in ODE model that include implementation, optimization, and analysis.

1.3.1 Building Blocks of Biochemical Models

The kinetics of chemical reactions can be described with reaction rates dependent on the concentrations of educts and products. Specifically, one typically assumes that the number of product molecules synthesized in a certain time interval is linearly dependent on the concentrations of educt molecules (law of mass action). The net influx or efflux arising from all participating reactions determines the rate of change in each molecular species. Thus, ODE modeling is based on the assumption that the temporal derivatives of molecule concentrations equal the sum of all relevant reaction rates (Table 1.1). Larger biochemical signal transduction networks are therefore reflected using coupled ODEs.

Table 1.1 lists elementary reactions in biochemical networks: Most steps in ODE models are described as unimolecular reactions that could represent irreversible reactions representing processes as degradation or substrate cleavage (1.1) or reversible transitions between certain states of a protein (1.2). Other common elements are the reversible assembly of two proteins, such as ligand binding to a receptor (1.3), reversible dimerization of two monomers (1.4), and enzyme–catalyzed reactions (1.5). In many cases, the full enzyme catalysis mechanism (enzyme + substrate $\leftrightarrow$ enzyme–substrate complex $\rightarrow$ enzyme + product) can be described by a single overall reaction rate, e.g., by using the Michaelis–Menten approximation (see biophysical textbooks).

Table 1.1 Exemplary components of a model graph

Unimolecular irreversible reaction	$A \xrightarrow{k} B$	$\frac{dA}{dt} = -kA$	(1.1)
Unimolecular reversible reaction	$A_1 \underset{k_-}{\overset{k_+}{\rightleftarrows}} A_2$	$\frac{dA_2}{dt} = k_+A_1 - k_-A_2$	(1.2)
Reversible ligand–receptor binding	$R \overset{L}{\underset{k_-}{\overset{k_+}{\rightleftarrows}}} RL$	$\frac{dRL}{dt} = k_+R \cdot L - k_-RL$ $\frac{dRL}{dt} = 0 \Leftrightarrow K_b = \frac{k_+}{k_-} = \frac{RL}{R \cdot L}$	(1.3)
Dimerization	$2\,M \underset{k_-}{\overset{k_+}{\rightleftarrows}} D$	$\frac{dD}{dt} = k_+M^2$	(1.4)
Enzyme–catalyzed reaction	$A \xrightarrow[k]{E} B$	$\frac{dB}{dt} = kAE$ $E = \text{const.}$	(1.5)

1.3.2 Simulation

Based on such simple building blocks, mechanistic models of biochemical reaction networks can be implemented. As a demonstrative example, we constructed a model of caspase activation by death ligands (Fig. 1.1a), where each reaction is described by an equation similar to those in Table 1.1. Signaling is initiated by reversible ligand binding to the death receptor, followed by formation of the so-called death-inducing signaling complex (DISC), recruitment of procaspase-8 and procaspase-3 cleavage by active caspase-8. The model consists of the stimulus (L; assumed to have a constant concentration in the medium), eight dynamical variables (R, LR, $DISC$, $C8$, $DISC.C8$, $C8^*$, $C3$, $C3^*$), and seven kinetic parameters (k_{1+}, k_{1-}, k_2, k_{3+}, k_{3-}, k_4, k_5). The kinetic parameters and initial concentrations were taken from previous theoretical and experimental studies (Albeck et al. 2008b; Bentele et al. 2004; Neumann et al. 2010; Rehm et al. 2009; Stennicke et al. 1998).

Using numerical integration techniques, the temporal evolution of the model species to extracellular stimulation by death ligands can be simulated (Fig. 1.1b). The simplest numerical integration method, known as the Euler method, approximates the solution of the differential equation $dx/dt = f(x)$ iteratively by the discretization

$$x(t_{i+1}) = x(t_i) + f(x(t_i)) \cdot \Delta t, \tag{1.6}$$

where $x(t_i)$ is the solution at time point t_i and $\Delta t = t_{i+1} - t_i$. In practice, the solution of the differential equation at any time point is obtained by iteratively applying (1.6) starting from the initial conditions at $t = 0$. The smaller the time increment Δt is chosen, the more accurate solution might be obtained. However, in general, the numerical error of the Euler method increases with increasing number of

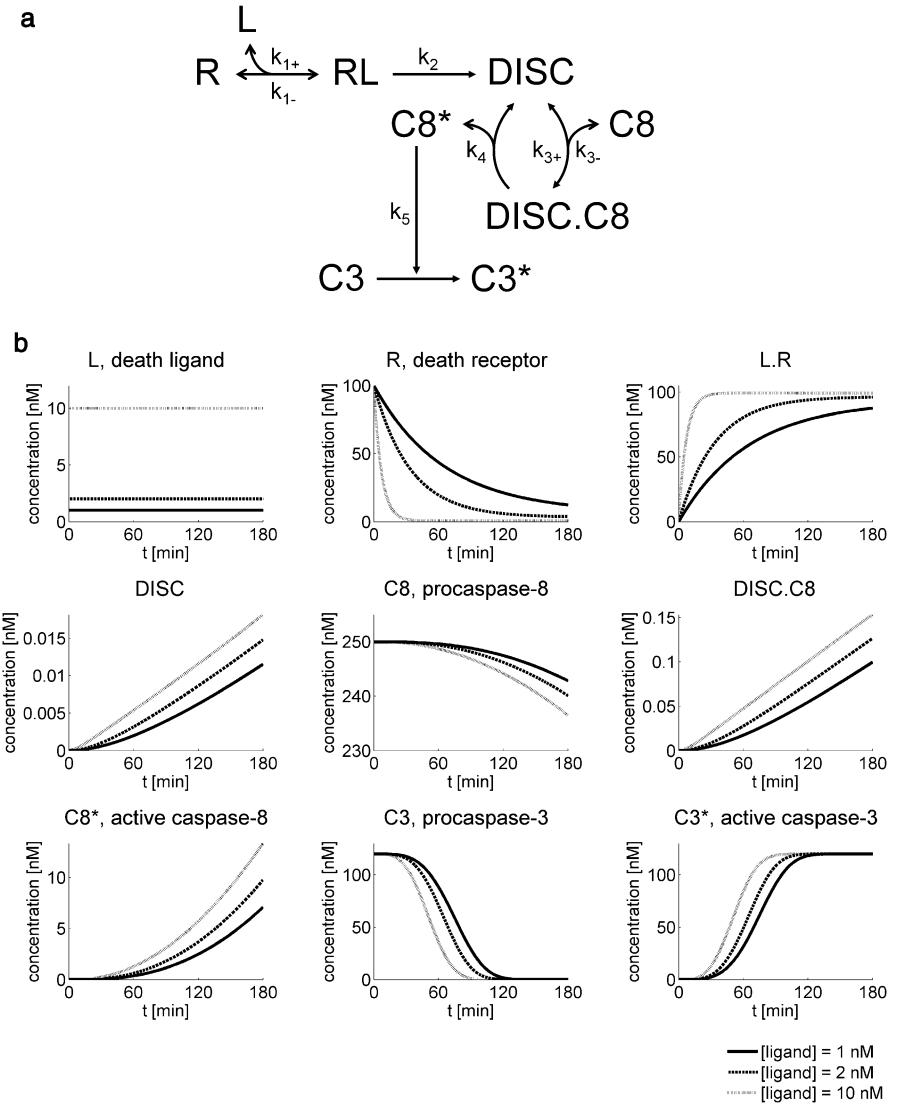

Fig. 1.1 Exemplary model of extrinsic apoptosis and predicted trajectories for its variables. (**a**) The model graph represents five reactions that are translated into a set of eight ODEs. The ligand in the medium is assumed to be present in excess, and is therefore not described by a differential equation, but considered to be constant. The model variables are receptor (*R* with $R_0 = 100$ nM), receptor ligand complexes (*RL*), DISCs, procaspase-8 (C8 with $C8_0 = 250$ nM), procaspase-8 bound to DISCs (*DISC.C8*), active caspase-8 (C8*), procaspase-3 (C3 with $C3_0 = 120$ nM), and active caspase-3 (C3*). (**b**) Simulated model trajectories for a step-like increase in the death ligand stimulus (see legend for ligand concentrations)