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Model validation of biological pathways using Petri nets—demonstrated for apoptosis

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Abstract

This paper demonstrates the first steps of a new integrating methodology to develop and analyse models of biological pathways in a systematic manner using well established Petri net technologies. The whole approach comprises step-wise modelling, animation, model validation as well as qualitative and quantitative analysis for behaviour prediction. In this paper, the first phase is addressed how to develop and validate a qualitative model, which might be extended afterwards to a quantitative model.

The example used in this paper is devoted to apoptosis, the genetically programmed cell death. Apoptosis is an essential part of normal physiology for most metazoan species. Disturbances in the apoptotic process could lead to several diseases. The signal transduction pathway of apoptosis includes highly complex mechanisms to control and execute programmed cell death. This paper explains how to model and validate this pathway using qualitative Petri nets. The results provide a mathematically unique and valid model enabling the confirmation of known properties as well as new insights in this pathway.

Keywords: Apoptosis; Signal transduction pathway; Model validation; Petri net; Transition invariant

1. Motivation

Systems biology is a major field of growing importance in current biology research. It is concerned with the modelling, simulation and analysis of biological processes in biological systems ranging in size from a single pathway to a whole cell. Obviously, it is of great interest for biology, medicine, and pharmaceutical industry to get a really deep understanding of the highly complex world of biological processes as well as to have sophisticated means to analyse these models thoroughly. With the rapidly increasing amount of produced genomic and proteomic data, new insights into special properties and the general behaviour of biological systems might be achieved, provided the handling and systematic exploration of these huge collected data sets will be mastered.

Because these biological networks tend to be very dense and large—far beyond the human skills, a crucial point seems to be their concise and unambiguous representation enabling us to handle computationally these highly integrated networks in an efficient manner.

Biological networks are studied and modelled at different description levels establishing different pathway types, e.g. there are metabolic pathways, describing the conversion of metabolites by enzyme-catalysed chemical reactions given by their stoichiometric equa-

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tions, such as main pathways of the energy household as glycolysis or pentose phosphate pathway. Another pathway type are signal transduction pathways, also known as information metabolism, explaining how cells receive, process, and respond to information from the environment. They are often formed by cascades of activated/deactivated proteins or protein complexes. Such signal transduction cascades may be seen as molecular circuits, which mediate the sensing and processing of stimuli. They detect, amplify, and integrate diverse external signals to generate responses, such as changes in enzyme activity, gene expression, or ion-channel activity.

Separation of concern is a well-known successful principle in computer engineering. But nevertheless, all these pathways interact actually together at any time to form complex mechanisms. Therefore, we are looking for an unifying modelling approach supporting diverse separate models for different description levels or aspects as well as their step-wise composition afterwards.

Moreover, our knowledge about a particular pathway is generally widely spread over various separate data bases and numerous papers using a quite large variety of different graphical schemes (for typical examples see Section 3). Usually, these schemes are—at least partly—open for interpretation; whereby even additional verbose explanations do not clarify sufficiently the intended meaning in an unambiguous manner.

To get a consistent view of the entire current state of knowledge about a particular pathway, there is no other way than to puzzle these pieces of distributed understanding together. For that purpose, a readable language with a formal, and hence unambiguous semantics would obviously be of great help as a common intermediate language in order to avoid the production of just larger patchwork, exposed to even more interpretation choices.

Independently of the given description level and the particular view extension, all pathways—and therefore their models, too—exhibit inherently very complex structures. These structures, reflecting the causal interplay of the basic actions, exploit all the patterns well-known in computer engineering, like sequence, branching, repetition, and concurrency, in any combination. But, in opposite to technical networks, natural networks tend to be very dense and apparently unstructured, making the understandability of the full network of interactions extremely difficult and therefore error-prone.

In the long-term, the actual purpose of modelling of biological networks is certainly a model-based behaviour prediction of the modelled real system. The crucial point of prediction is that it involves the readiness to believe—more or less blindly—what the results say. But are these results really trustworthy? Maybe there are some plausibility checks, but are they strong enough to get the predicted behaviour approved? It corresponds to common sense to confront a going-to-be oracle at first with questions, where the answers are known and well-understood. This step to establish a sufficient confidence in the correspondence of model and reality is usually called model validation.

Taking all these considerations into account, the most important usability criteria of a suitable model representation are on hand: (1) readability—to support understanding, and therefore enable fault avoidance in the model construction process, (2) executability (animation techniques)—to experience a model in order to get really familiar with it, (3) validation techniques—for consistency checks to ensure the model integrity and correspondence to reality, and (4) analysis techniques—for qualitative as well as quantitative behaviour prediction. Actually, each model under development should run through all four stages of usage, increasing step-by-step the model's confidence level. So the question arises, how many representations do we really need for these purposes?

In our opinion, mastering the given outstanding complexity of biological networks can be reached only by applying a fundamental engineering principle: step-wise model development by careful refinement as well as composition for all stages of confidence: animation via validation up to qualitative analysis, and finally quantitative analysis. That means at the same time an integration of model validation and behaviour prediction, resulting in one "all-purpose" model. With other words, our approach is to start with an animation model to get preliminary confidence in the model behaviour by its execution. Afterwards, the animation model is turned into a qualitative model for validation and qualitative analysis. Finally, the qualitative model is extended to a quantitative one by adding quantitative parameters like substance concentration, equilibrium constants and reaction rates, making it

ready for quantitative analysis, while preserving the model's confidence level gained in the former stages.

Comparable integrating approaches relying on Petri net technologies are well-known and have been proven to be successful in engineering of technical systems, see, e.g. Heiner et al. (1994). So, why not adapting this positive experience to the engineering of biological systems?

The idea to model biological systems by Petri nets has been introduced 10 years ago in Reddy et al. (1993) and has attracted in the meantime the attention by several research groups. But a closer look on the literature, compare Will and Heiner (2002), reveals that the majority of papers, applying Petri nets for modelling and analysis of biological systems, concentrate on quantitative aspects. Typical examples of used Petri net extensions are stochastic Petri nets, e.g. Narahari et al. (1989) and Peccoud (1998), and hybrid Petri nets, e.g. Matsuno et al. (2003a, 2003b) and Chen and Hofestaedt (2003) but also coloured Petri nets, e.g. Genrich et al. (2000), as well as discrete time extensions, e.g. Koch et al. (1999), have been employed for that purpose. Contrary, qualitative aspects are discussed only in a few papers, see e.g. Reddy et al. (1993), Heiner et al. (2001).

In this paper, we focus on model validation by means of qualitative models, because it is obviously necessary to check first a model for consistency and correctness of its biological interpretation before starting further analyses, aiming in the long-term at behaviour prediction by means of quantitative models. Doing so, we restrict ourselves here to the first two steps of the above mentioned technology aiming at the integration of qualitative and quantitative modelling and analysis. The expected result, justifying the additional expense of a preliminary model validation, consists in a concise, formal and therefore unambiguous model, which is provably self-consistent and corresponds to the modelled reality. As running example we employ in this paper the signal transduction pathways of apoptosis.

The paper is organised as follows: After a very short informal introduction into qualitative Petri nets, we apply them to model some of our current knowledge about apoptosis. First, we explain the biological background of the known major apoptotic pathways, and then we demonstrate our approach for a step-wise technique to model them. In the chapter afterwards we validate the developed model using a standard Petri net analysis technique and present the biological meaning of the analysis results. The final chapter gives a summary of the results reached up to now and an outlook on future research directions.

2. Introduction into Petri nets

Petri nets represent a modelling method, very well-known for its powerful combination of readability and analysability. They provide a generic description principle, applicable on any abstraction level. At the same time, they have a sound formal semantics, allowing thorough model evaluation.

In computer engineering, Petri nets have a quite long success history as a suitable intermediate language for a huge variety of different specification and programming languages, see e.g. Heiner et al. (1999). The well-established concept of a common intermediate language supports unification of model-based system validation and verification by means of complementing standard analysis techniques, working all on the same representation. As a result, reliable sophisticated tools for Petri net design and analysis are available now, mostly free of charge, encouraging the handling of increasing model sizes, which could not be mastered without tool support.

Following that line, we claim that some of the current problems in systems biology research (compare Section 1), could be resolved by taking advantage of related experience in computer engineering.

To make the paper self-contained, we shortly describe informally the basic ingredients, any Petri net model is made of. For a formal definition see, e.g. Reisig (1982).

(1) Petri nets are special graphs with two types of nodes, called places and transitions. Places, represented as circles, model usually "passive system elements" like conditions, states, or e.g. chemical compounds, while transitions, represented as boxes, stand for "active system elements" like events, actions, or e.g. chemical reactions.
(2) Arcs, always connecting only nodes of different type, describe the causal relation between active and passive elements. Generally, arcs describe which input compounds are transformed by a

chemical reaction into which output compounds (compare Fig. 1). In case of chemical reactions, given by their stoichiometric equations, the specific quantities of the involved compounds are reflected as the arcs' multiplicity. Otherwise, an arc simply states the fact of binary causal relation. In the binary case, arcs connect an event with its preconditions, which must be fulfilled to trigger this event, and with its postconditions, which will be fulfilled by the event if it takes place.

(3) All moving objects, like vehicles, work pieces, data, or, quantities of chemical compounds (e.g. number of molecules), are modelled by tokens residing in places. Principally, a place may carry any number of tokens. All the places together with their current amount of tokens describe the current system state, or a given distribution of chemical compounds, and are shortly called marking.

(4) A Petri net comes to live by the flow of tokens. The rules of the game to follow are as follows.

0.1. An action may happen (the transition may fire), if all preplaces are filled sufficiently, e.g. all input compounds are available at least in the required quantities specified by the related incoming arc weights.

0.2. If an action happens, then tokens are removed from all preplaces, e.g. input compounds, corresponding to the incoming arc weights, and tokens are added to all postplaces, e.g. output compounds, corresponding to the outgoing arc weights.

0.3. An action happens (a transition fires) atomically as well as time-less.

Fig. 1 shows two snapshots of a simple Petri net, modelling just one chemical reaction, given by its stoichiometric equation.

This modelling principle has been applied successfully to a variety of biological pathways, see Will and Heiner (2002) for a bibliography of related papers. The resulting metabolic Petri nets describe the set of all paths from the input to the output compounds respecting the given stoichiometric relations.

Moreover, the same modelling idea may be applied on a more abstract level, where stoichiometric details are not known or do not matter, resulting into a partial order description of binary causal relations of the basic (re-) actions involved. In the next chapter we are going to apply this concept to model some pathways of apoptotic signal transduction. For their readable representation we utilize two widely used short-hand notations:

- a test arc, represented as a bidirectional arc, stands shortly for two-directional arcs;
- logical nodes, given in grey, serve as connectors to fusion distributed net components.

3. Application to apoptosis

The term apoptosis, which stands in Greek for leaves falling from a tree in the autumn, was coined in Kerr et al. (1972) to describe a regulated intrinsic cell suicide program. Apoptosis is of central importance in the cells' life cycle. It allows the organism to control cell numbers and tissue size, and to protect itself from morbid cells. Cells which undergo apoptosis, exhibit chromatin condensation, nuclear fragmentation, plasma membrane blebbing, cell shrinkage, and ultimately shedding of membrane-delimited cell fragments, also known as apoptotic bodies Wyllie (1997).

Apoptosis plays an important role especially during neural development. It is estimated that at least half of the original cell population is removed as a result of apoptosis during developing the nervous system, see Oppenheim (1981), Burek and Oppenheim (1999). Neurodegenerative diseases, e.g. Alzheimer's,

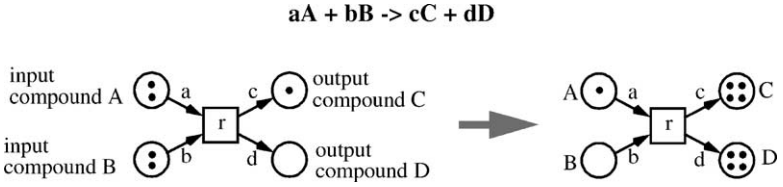

Fig. 1. Petri net model of a single chemical reaction, given by its stoichiometric equation, in two possible snapshots, whereby $a = 1$, $b = 2$, $c = 3$, and $d = 4$ has been assumed.

Huntington's and Parkinson's disease, amyotrophic lateral sclerosis Löffler and Petrides (1997), and other diseases as AIDS and cancer, exhibit often disturbances in apoptosis or its regulation.

3.1. Biology of apoptosis

Apoptosis is a special inducible process with increased RNA and protein biosynthesis. A variety of different cellular signals initiate activation of apoptosis on different ways in dependence of the various kinds and biological states of cells, as demonstrated in Fig. 2. Caspases (cysteinyl-aspartate-specific proteinases) are a class of cysteine proteases that includes several members involved in apoptosis. In living cells, caspases exist as inactive zymogens that are activated by proteolytic cleavage. The caspases convey the apoptotic signal in a proteolytic cascade, whereby caspases, cleave and activate other caspases which then degrade other cellular targets.

There are two relatively well-studied pathways that lead to caspase activation, the extrinsic (extracellular) and intrinsic (intracellular) pathway. The extrinsic pathway (see left sides in Figs. 2 and 3) is activated by different extracellular ligands that bind death receptors such as tumor necrosis factor receptor 1 (TNFR-1), glucocorticoid receptor, and Fas. These death receptors bind, in turn, to adaptor proteins such as Fas-associated death domain protein (FADD). The ligand for CD95 or Fas (CD95L or FasL) is a trimer, which promotes receptor trimerization by association with the receptor. The resulting intracellular clustering of parts of the receptor called death domain (DD) allows the adapter protein FADD to associate with the receptor through an interaction between homologous DD on the receptor and on FADD. FADD contains furthermore a death effector domain (DED) which allows binding of procapase-8 to the Fas–FADD complex. Procaspase-8—also known as FLICE—associates with FADD through its own DED, and upon recruitment by FADD is immediately cleaved to produce caspase-8, which then triggers activation of execution caspases such as caspase-9. Caspase-8 activation can be blocked by recruitment of the degenerate caspase homologue c-FLIP. The complex of proteins—Fas, FADD, and procaspase-8—is also known as death inducing signalling complex (DISC).

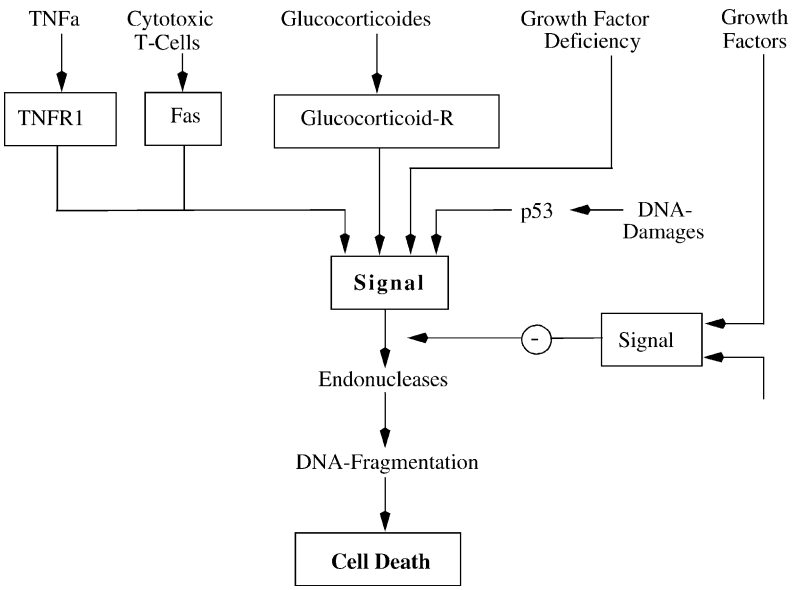

Fig. 2. Schematic overview of several apoptosis inducers, according to Löffler and Petrides (1997). TNFR-1, Fas and glucocorticoid-R belong to the extrinsic pathway, whereas growth factor deficiency and DNA damaging signals induce the intrinsic pathway. There are also other signals (e.g. growth factors), which can stop (inhibit) apoptosis. Apoptosis takes place through activation of endonucleases resulting in DNA fragmentation leading to the cell death.

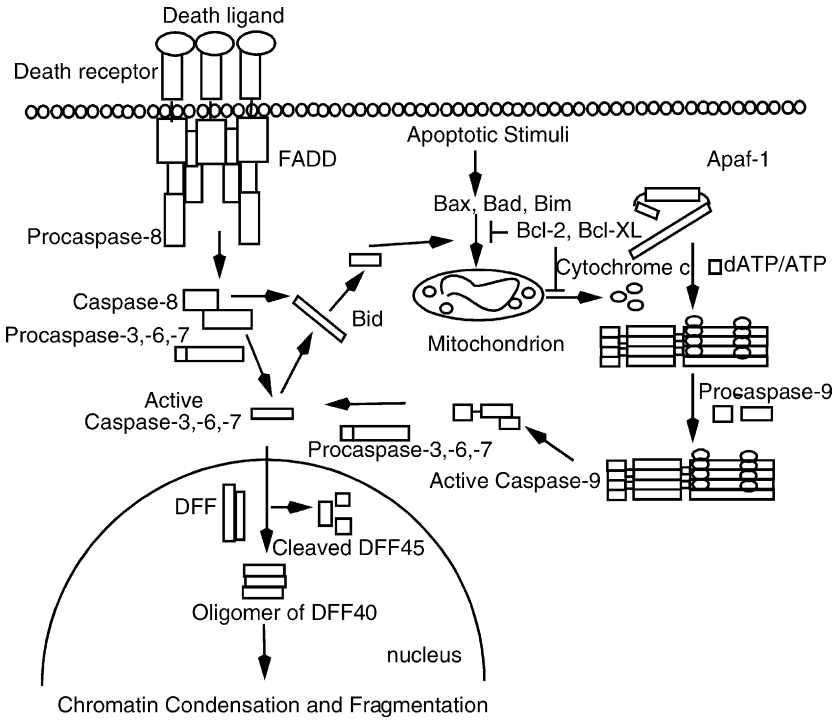

Fig. 3. Schematic overview of apoptosis induced by DNA damaging and Fas signals, resulting in DNA fragmentation, according to Nijhawan et al. (2000). Here, the death ligands are drawn as ellipses, the proteins as rectangles, and complexes consist of attached rectangles. The involved cell organelles mitochondrion and cell nucleus are represented by a large ellipse and semicircle, resp. Relations between proteins and complexes are indicated by arrows. The cell surface is drawn as a line of small circles. There the death ligands activate their receptors, which in turn recruit adapter molecules such as FADD, which then recruit procaspase-8 to the receptor complex within the cell and there undergoes autocatalytic activation. The inhibition of apoptosis by Bcl-2 and Bcl-X_L is indicated by crossbar arrowheads.

The intrinsic pathway (see right sides in Figs. 2 and 3) for caspase activation—also called mitochondrial pathway—is engaged in response to growth factor deficiency and deprivation, genotoxic injury (as DNA damage), hypoxia and many other insults. Following exposure of cells to many apoptotic stimuli, the outer membranes of mitochondrion undergo permeability changes that permit cytochrome c and other proteins normally sequestered in space between the inner and outer membranes of these organelles to leak out and enter the cytosol. There, cytochrome c binds a caspase-activating protein—Apaf-1 (apoptotic protease activating factor-1)—and then pro-caspase-9 to form the apoptosome, which activates caspase-3.

A large family of evolutionary conserved proteins, the Bcl-2 family, has been identified, which regulates the release of cytochrome c and other proteins from mitochondrion, see, e.g. Adams and Cory (1998), Reed (1998), Gross et al. (1999). The family includes both anti-apoptotic and pro-apoptotic signals. The major anti-apoptotic proteins are Bcl-2 and Bcl-X_L which are localised to the mitochondrial outer membrane. Pro-apoptotic proteins, Bax, Bad, Bim, and Bid, can shuttle between the cytosol and organelles. The cytosylic forms represent pools of inactive, but battle-ready proteins. Pro-apoptotic signals redirect these proteins to the mitochondrion, where they meet anti-apoptotic proteins to compete for the regulation of the cytochrome c exit. The occurrence of apoptosis depends on the interplay of pro-apoptotic and anti-apoptotic factors.

Fig. 3 comprises both the extrinsic pathway and the intrinsic pathway through DNA fragmentation induced by DNA damaging and Fas signals. Caspases are activated through three different pathways:

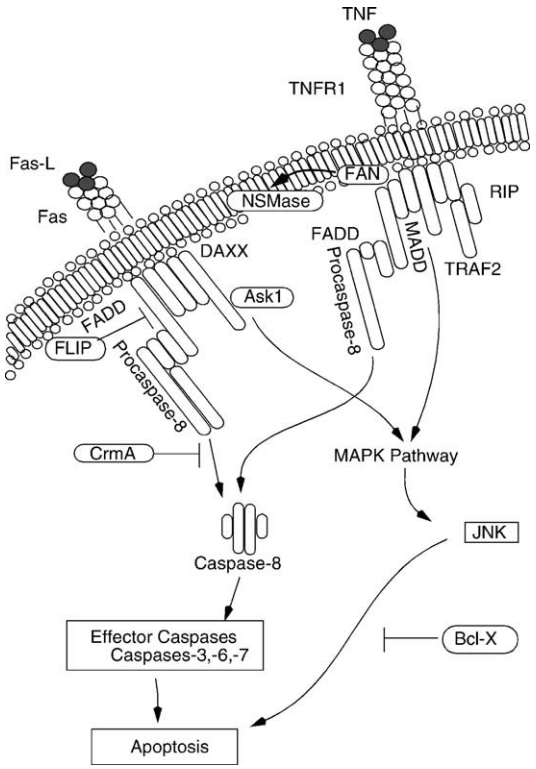

Fig. 4. Schematic representation of the extension of the model in Fig. 3 by the Fas-induced MAPK pathway and TNFR-1 receptor-induced pathways, according to Moldoff and Baudino (2002). Ligands and the cell surface are drawn in more details than in Fig. 3. The activation of the JNK pathway by DAXX through interaction with Ask1 and activation of the MAPK pathway induce apoptosis. The TNF induced pathway is represented by the formation of complexes as TRADD which cluster intercellular death domains.

(1) Fas/FADD/caspase-8/caspase-3, -6, -7;
(2) Fas/FADD/caspase-8/Bid/mitochondrion/cytochrome c/caspase-9/caspase-3, -6, -7;
(3) apoptotic stimuli/Bax, Bad, Bim/mitochondrion/cytochrome c/Apaf-1/caspase-9/caspase-3, -6, -7.

In order to get more detailed insight into caspase cascades we extend our model by the Fas-induced MAPK (mitogen-activated protein kinase) pathway and the TNFR-1 receptor-induced pathways, see Fig. 4. The receptor-associated protein Daxx (Fas death associated protein xx) can activate the Jun N-terminal kinase (JNK) pathway through interaction with Ask1 (apoptosis signal-regulating kinase-1) (Chan et al., 1998; Chen and Tan, 1999). TNF produced by T-cells and activated macrophages can have several effects by ligating TNFR-1. TNF binds to TNFR-1 to result in receptor trimerization and clustering of intracellular death domains. This allows binding to the intracellular adapter molecule TRADD (TNF receptor 1 associated death domain) through interaction between death domains. TRADD can recruit a number of different proteins, e.g. TRAF2 (TNF associated factor 2), to the activated receptor. TNFR-1 is also able to mediate apoptosis through recruitment of another adapter molecule RAIDD (RIP-associated Ich/CED homologous protein with a death domain), which associates with RIP (receptor interacting proteins) and can recruit caspase-2. Another DD protein—MAP kinase activating death domain (MADD) associates with the DD of TNFR-1 through its own C-terminal DD implicating MADD as a component of the TNFR-1 signalling complex.

The activation of both the Fas receptor and TNFR-1 receptor induces the JNK (Jun amino terminal kinase) pathway, the activation of caspase-2, and of main stream caspase-8, see Hengartner (2000), KEGG (2003), Moldoff and Baudino (2002), Reed (2002), Yuan and Yankner (2000). Then, caspase-8 activates effector caspase-3 directly, resulting in apoptosis. Caspase-8 triggers also the Bid controlled apoptotic pathway through cytochrome c release from the mitochondrion. Apoptotic stimuli like DNA damage induce this mitochondrial pathway, too.

3.2. Modelling of apoptosis

The graphical representations given in the former section (see Figs. 2–4) discuss not only apoptosis on different abstraction levels, but differ also in their focus. Therefore, they are not alternative representations, but complement each other. Furthermore, these figures are schematic and informal ones, because they need additional verbose explanations how to read them. To get a unifying as well as unambiguous knowledge representation allowing at the same time some consistency checks to get the unification approved, we have to apply representation techniques enjoying a formal and therefore unambiguous semantics.

For this purpose, monochromatic graph representations, i.e. graphs with just one node type, are quite common and widely accepted, see for example the KEGG data base KEGG (2003). However, a closer

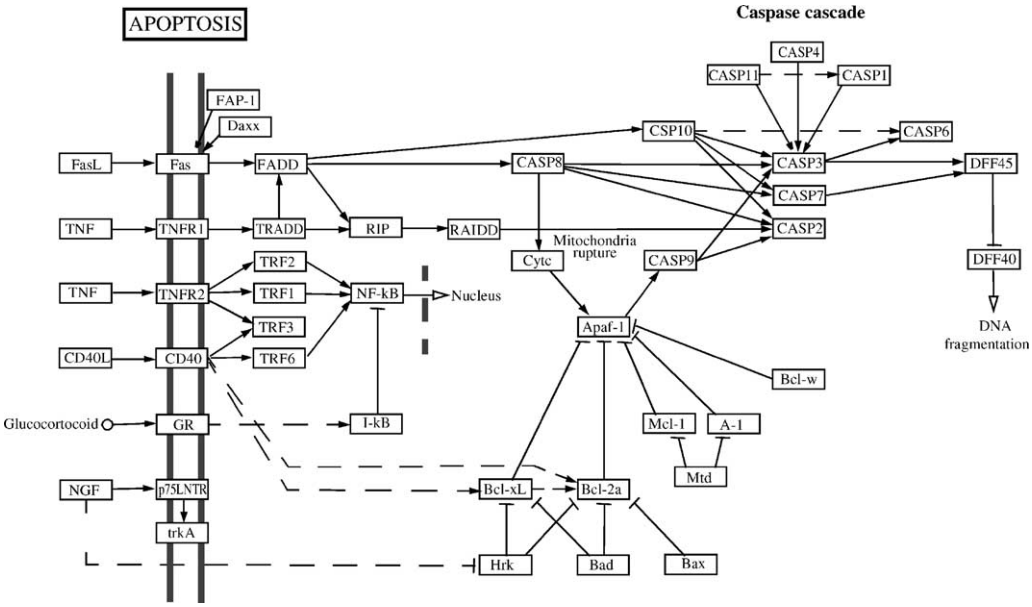

Fig. 5. The KEGG representation of apoptosis, according to KEGG (2003).

look on Fig. 5, which gives apoptotic pathways in a KEGG-style manner, reveals several serious problems. Besides the lack of an explicit specification of the differentarc types used (solid/dashed lines combined with filled/ hollow/crossbar arrowheads), the meaning of a given arc of a certain type seems to depend on the graphical orientation (e.g. compare the solid arc with filled arrowhead connecting Daxx and Fas with the one connecting CASP9 and CASP3, and try to figure out what they might mean). But even worse, the

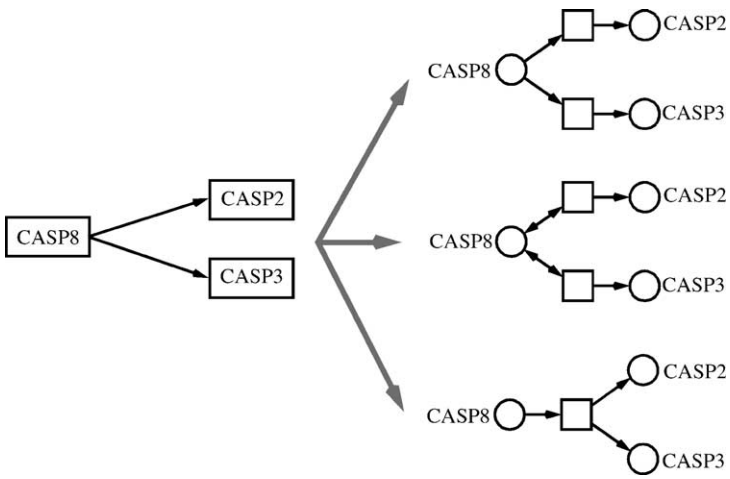

Fig. 6. Different interpretations of branching in a monochromatic graph representation. The given case has been taken from the KEGG representation of apoptosis, see Fig. 5. Possible interpretations of monochromatic branching: caspase-8 triggers the activation of either caspase-2 or caspase-3, i.e. both alternatives exclude each other (compare upper part); caspase-8 triggers the activation of caspase-2 as well as of caspase-3, but independently and in any order (compare middle part) caspase-8 triggers the simultaneous activation of caspase-2 as well as of caspase-3, i.e. both caspases are always activated at the same time (compare lower part).

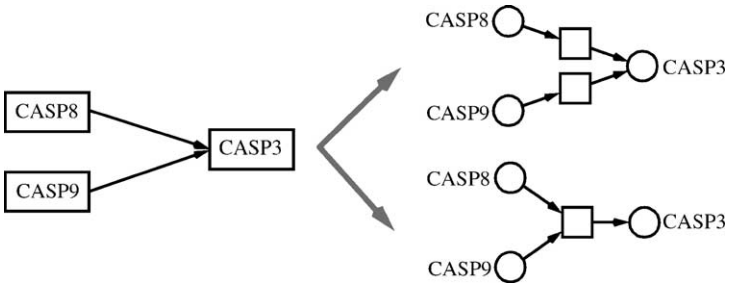

Fig. 7. Different interpretations of joining in a monochromatic graph representation. The given case has been taken from the KEGG representation of apoptosis, see Fig. 5. Possible interpretations of monochromatic joining: the activation of caspase-3 is triggered by caspase-8 and/or by caspase-9, one of them is sufficient (compare upper part); the activation of caspase-3 is only triggered if caspase-8 as well as caspase-9 are activated, both are necessary conditions (compare lower part).

meaning of node connections to several successors (branching) or vice versa from several predecessors (joining) is—in the given context of concurrent behaviour of independent atomic actions—open for interpretation, compare Figs. 6 and 7. The former shortcomings could be eliminated by a careful definition of the chosen notation, however the latter one is an inherent deficiency of any monochromatic notation.

That is one of the reasons why we favour Petri nets—a famous bichromatic graph model with a sound formal semantics—as the common intermediate representation language of unifying model representations. To model the different biopathway schemes into one Petri net, possibly consisting of different components, each biochemical compound or receptor is assigned to a place. For a future detailed analysis, like quantitative kinetic description at the biochemical elementary reaction level, all intermediate products (e.g. activated Fas receptor complex, death inducing signalling complex DISC, apoptosome) have to be included in the biopathway description, too.

The relations between any biochemical substances are represented basically by transitions with corresponding arcs, modelling a biochemical or signalling atomic event. However, while the modelling of the biochemical substances is quite straightforward, the elaboration of the transition structures tends to be rather time-consuming, requiring a lot of reading and interpretation of various verbal or graphical statements.

The absence of a widely accepted unambiguous arrow notation has to be considered as a severe lack in any graphical representations, in addition to the discussion above see also Schacherer (2002). To give an example, if a graphical scheme contains an arrow, touching another arrow, such a point of contact between arrows can be modelled by a transition, representing an interaction of the given biological entities, or by a place with corresponding transitions and arcs, representing the interaction product (e.g. receptor–ligand complexes or enzyme–substrate complexes).

We start the modelling with the apoptotic scheme represented in Fig. 3, which was also used by Matsuno et al. In Matsuno et al. (2003a) they present a corresponding continuous Petri net to model quantitative properties of apoptosis using an assumed initial concentration for each compound. Likewise, our Petri net model given in Fig. 8 [1] comprises the Fas-induced and mitochondrial DNA damage pathways as well as the Bid controlled cross-talk between them.

Usually, signal transduction does not involve the resetting of the triggering signal(s). Therefore, such events are modelled by test arcs. Similarly, enzyme reactions are catalytic reactions, i.e. there is no consumption of the biochemical compound, so they are modelled by test arcs, too.

In our model, apoptosis inhibitors are not taken into account. All inhibiting substances are only input substances for the considered system model. Input substances are not produced dynamically by the system behaviour, which means in the biological interpretation that they come from the system environment. That's why their presence would just exclude mod-

[1] The complete net examples are available on http://www.informatik.tu-cottbus.de/~wwwdssz. To visualise them you have to install the used Petri net EDitor PED, available via the same web page.

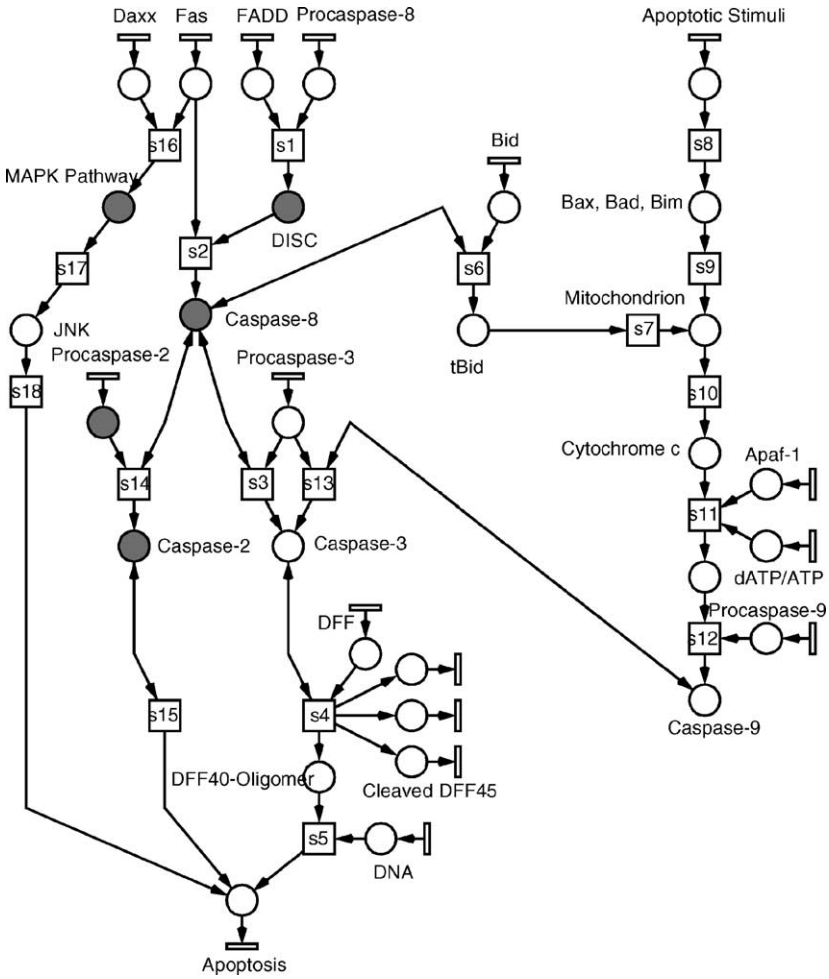

Fig. 8. Petri net of apoptosis induced by Fas receptor and intrinsic apoptotic stimuli. Please note, input and output transitions are drawn as flat hollow bars and have the same name as the place, they are related to. Grey nodes represent logical nodes and connect different net components (compare Fig. 9). Read arcs are used to reflect the signal transduction principle. They are replaced by normal arcs for the validation step. For a list of the abbreviations see Appendix.

elled system behaviour without adding new functionality.

The tokens for all input places are generated by input transitions (source nodes, having no predecessors), while the tokens of all output places are consumed by output transitions (sink nodes, having no successor nodes). To highlight the special meaning of these transitions for the whole model and in order to distinguish them from the ordinary ones, they are drawn as flat hollow bars.

In Fig. 9 we extend the basic Petri net model given in Fig. 8 by the pro-apoptotic TNFR-1 pathways. Both net components are glued together by the logical nodes, given in grey. The extension has been done intentionally by a separate net component to promote readability as well as to keep explicit traces concerning the development process and the composition principle of the total model. It should be obvious how to continue this line.

The transformation from an informal to a formal model involves the resolution of any ambiguities, which must not have been done necessarily in the right way. Therefore, the next step in a sound model-based

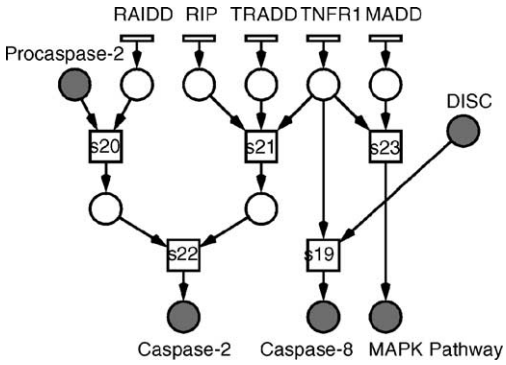

Fig. 9. Apoptosis induced by TNFR-1 receptor, as extension of Fig. 8.

technology for behaviour prediction is devoted to model validation.

4. Model validation

Model validation aims basically at increasing our confidence in the constructed model. There is no doubt that this should be a prerequisite before raising more sophisticated questions, where the answers should be found by help of the model and where we are usually ready to belief the answers we get. So, before thinking about model analysis, we are concerned with model validation.

To accomplish model validation, we need validation criteria, establishing consistency checks for the model. Looking for such criteria, we should take into account that our models are usually the outcome of a quite heuristic procedure assembling together separate pieces, perhaps with different possible interpretations, into a larger picture.Thus, a first and very evident question concerning a model of increasing size is the one, whether all the former basic behaviour of the smaller pieces, i.e. model components, are still maintained in the larger model, and that there are no unwanted additional ones. Due to the model's size and inherent complexity, such a property can hardly be decided without computational support.

For that purpose, we are going to exploit one of the basic behavioural properties, a Petri net may exhibit—the so-called T-invariants. T-Invariants are (multi-)sets of transitions, reproducing a given marking, i.e. in the context of metabolic Petri nets—sets of chemical reactions, reproducing a given distribution of chemical compounds, or more generally spoken in the context of arbitrary biological Petri nets—sets of actions, reproducing a given system state. Due to the fact of state reproduction, a behaviour, establishing a T-invariant, may happen infinitely often, resulting into cyclic system behaviour.

To describe all possible behaviour in a given cyclic system, it would be obviously of help to have all system's basic (cyclic) behaviour (in Schuster et al., 2000 called elementary mode), so that any system behaviour may be decomposed into a linear combination of basic behaviour. Then, model validation means to compare the calculated basic behaviour with the expected one.

To implement these considerations, we use—opposite to Schuster et al. (2000)—standard Petri net analysis techniques and tools. To make life easy, we take the empty Petri net (no tokens at all), where all input and output nodes are transitions. An input transition may fire forever, each time generating tokens on all its postplaces. Consequently, such a net structure represents an unbounded net (there is no finite upper bound for the total token number in the net), which are generally harder to handle then bounded ones. Contrary, an output transition consumes by each firing the tokens of its preplaces, therefore decreasing the total number of tokens.

So, if we now take the empty net, we are able to look for all T-invariants, i.e. for all multi-sets of transitions reproducing the empty marking. That seems to be—at least currently—the best way to handle inherently unbounded systems, without assuming anything about the system environment. But, to give the net a real chance to reproduce the empty marking, all read arcs in the model under discussion have to be transformed into unidirectional ones.

We get all minimal semi-positive T-invariants, i.e. all basic behaviour, by solving the following system of linear equations:

$$[C] \times \bar{x} = 0$$

whereby $[C]$: $(P \times T)$—incidence matrix; $\bar{x}$: transition vector.

Please note, the calculation of T-invariants requires only structural reasoning, the state space need not to be generated. Therefore, the danger of the famous state space explosion problem does not apply here.

When computing all minimal semi-positive T-invariants by help of the Integrated Net Analyser tool INA, see Starke (1998), we get the following results.

There are two receptors (Fas, TNFR-1) and three basic apoptotic pathways per receptor (caspase-8, JNK, caspase-2) as well as an apoptotic stimuli-induced pathway in our model. Altogether, there are ten T-invariants. (Please note, in the transition vectors given below the generating input and the consuming output transitions have been skipped for sake of simplicity.)

- Fas-induced:
  - s1, s2, s3, s4, s5: Fas/caspase-8/caspase-3—Fas-induced direct caspase-8/caspase-3 pathway
  - s1, s2, s6, s7, s10, s11, s12, s13, s4, s5: Fas/caspase-8w/mitochondrion/cytochrome c/caspase-9/caspase-3—Fas-induced Bid-controlled cross-talk
  - s16, s17, s18: Fas/MAPK-pathway/JNK—Fas-induced JNK pathway
  - s1, s2, s14, s15: Fas/caspase-8/caspase-2—Fas-induced caspase-8/caspase-2 path-way
- Apoptotic stimuli-induced:
  - s8, s9, s10, s11, s12, s13, s4, s5: apoptotic stimuli/Bax, Bad, Bim/mitochondrion/cyto-chrome c/Apaf-1/caspase-9/caspase-3—apoptotic stimuli-induced mitochondrial pathway
- TNFR-1-induced:
  - s1, s19, s3, s4, s5: TNFR-1/caspase-8/caspase-3—TNFR-1-induced direct caspase-8/caspase-3 pathway
  - s1, s19, s6, s7, s10, s11, s12, s13, s4, s5: TNFR-1/caspase-8/mitochondrion/cytochrome c/Apaf-1/caspase-9/caspase-3—TNFR-1-induced Bid-controlled cross-talk
  - s1, s19, s14, s15: TNFR-1/caspase-8/caspase-2—TNFR-1-induced caspase-8/caspase-2 pathway
  - s23, s17, s18: TNFR-1/MAPK-pathway/JNK—TNFR-1-induced JNK pathway
  - s20, s21, s22, s15: TNFR-1/caspase-2—TNFR-1-induced direct caspase-2 pathway

The minimal semi-positive T-invariants describe the basic system behaviour, because they represent the pairwise linearly independent solutions of the system of linear equations resulting from the incidence matrix C. All possible system behaviour can be described by linear combinations of these minimal semi-positive T-invariants. On the one side all known pathways in the modelled fragment of apoptosis are reflected in a corresponding T-invariant, and on the other side there is no computed T-invariant without an apoptosis-related interpretation.

5. Summary and outlook

Petri nets represent a concise formal representation, allowing a unifying view on knowledge stemming from wide-spread different sources, usually represented in various, sometimes even ambiguous, styles.

In this paper, we proposed a methodology how to develop and analyse larger biological models in a careful step-wise manner. The approach integrates different modelling objectives by using only one all-purpose model, which is extended step-wise according to the analysis questions in mind.

The proposed approach has been demonstrated using the up to now quite incomplete knowledge about the behaviour of apoptotic pathways. We have intentionally chosen that example to stress the fact that even incomplete and uncertain knowledge may be subject of our technology. Actually, we are convinced that step-wise incremental modelling accompanied by running repeated analyses are the only chance to get dependable larger models. This may help to put at the end the smaller pieces into a whole working picture.

Up to now, the qualitative analyses have just been used to check the model for self-consistency. The next steps are obvious: after being convinced of the model's integrity, we are ready to use the model for questions where the answers are not yet known. It has to be shown that available Petri net analyses techniques like model checking of temporal formulae will be still of help while turning to special biological and biotechnological questions.

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Appendix A. Abbreviations

Apaf-1	Apoplectic protease activating factor 1
Ask1	Apoptosis signal-regulating kinase-1
ATP	Adenosine triphosphate
Bad	Bcl-X_L/Bcl-2 associated death promoter
Bax	Bcl-2 associated X protein
Bcl-2	B-Cell lymphoma 2
Bid	Bcl-2 interacting domain
CASP	Caspase
Caspase	Cysteinyl aspartate-specific protease
CrmA	Cytokine response modifier A
Cyt c	Cytochrome c
dATP	Desoxyadenosine triphosphate
Daxx	Fas death domain associated protein xx
DFF	DNA fragmentation factor
DFF40	40 kDa unit of DFF
DFF45	45 kDa unit of DFF
DISC	Death inducing signaling complex
FADD	Fas associating protein with death domain
FAP-1	Fas associated phosphatase-1
Fas	Fas receptor
Fas-L, FasL	Fas ligand
FLIP	FLICE inhibitory protein
JNK	c-Jun amino-terminal kinase
MADD	Mitogen activated kinase activating death domain
MAPK	Mitogen activated protein kinase
RAIDD	RIP associated Ich-1/CED homologous protein with death domain
RIP	Receptor interacting protein
tBid	Truncated Bid
TNF, TNFα	Tumor necrosis factor
TNFα-R, TNFR-1	Tumor necrosis factor receptor
TRADD	TNF receptor 1 associated death domain

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