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Sensitivity analysis of programmed cell death and implications for crosstalk phenomena during Tumor Necrosis Factor stimulation

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Abstract—**Different methods for analyzing the sensitivity of the direct signal transduction pathway of receptor-induced apoptosis to parameter changes are presented. Apoptosis is a form of programmed cell death, removing unwanted cells within multicellular organisms to maintain a proper balance between cell reproduction and death. The results indicate the importance of controlling activated caspases by direct inhibition to prevent apoptosis. A misregulation of IAP molecules, one of the main inhibitors, appears to be especially critical. The results indicate how an increased production of this molecule promotes survival and might promote cancer progression, while a reduced degradation might not, thereby providing insight of potential pharmaceutical relevance and also stimulating experimental verification. The different engineering methods applied, nicely complement each other to provide valuable insight into this important process. Because IAPs, among others, are also an important connection to other signaling pathways, the results will enable a more efficient extension of the current model. This is outlined at the example of Tumor Necrosis Factor induced signaling pathways.**

I. Introduction

MATHEMATICAL modeling of biological processes has recently received renewed attention and is a fast growing area [1-3]. Whereas metabolic processes are often studied using steady state approaches, the modeling and analysis of signal transduction needs a special focus on dynamical properties [4]. Biological processes are usually very complex and non-linear [5]. While systems and control theory already offer a wide range of tools for modeling and analysis of technical systems, these need to be specifically tailored and enhanced to suit the needs of biological systems. Then, the interplay of engineering and biology is promising to not only enable a deeper understanding of the molecular basis of life, but to inspire new ideas that can also be applied to more generic technical systems.

In this paper, we describe approaches to model the intracellular signal transduction pathways elicited by the cytokine (i.e. hormone like molecule) Tumor Necrosis Factor (TNF) focusing on the pathway leading to apoptosis (programmed cell death) [6-9]. TNF activates several signal transduction pathways, which interact in a dynamic network. These pathways are also relevant in other signal transduction processes and for TNF itself, are of high pharmaceutical interest. Misregulation is implicated in severe pathological alterations including developmental defects, autoimmune diseases, neurodegeneration and cancer [10].

In the adult human TNF plays a key role in the activation and coordination of responses of the immune system. Focusing on the molecular level, TNF induces its cellular functions by binding to specific membrane standing receptors of the target cell (Fig. 1). These receptors propagate the signal through the plasma membrane. Via additional adaptor proteins mainly three pathways are activated, i.e. the NFκB (nuclear factor kappa B) pathway, the apoptotic pathway and the Jun N-terminal kinase (JNK) pathway. NFκB is a transcription factor important in inflammatory responses. Interestingly, it also up-regulates proteins which antagonize the apoptotic pathway elicited in parallel. Apoptosis is a form of programmed cell death and involves a suicide program resulting in the controlled degradation of the cell without evoking inflammation. Apoptosis therefore can be regarded as an altruistic cell death for the sake of the whole organism [11]. It can be triggered intrinsically by cellular malfunction or extrinsically by death receptors of the TNF superfamily. The extrinsic pathway either proceeds directly (in so-called Type I cells) or utilizes a feed forward loop involving the mitochondria (in so-called Type II cells) [12, 13]. The function of the JNK pathway remains contradictory and is not further investigated in this study [8].

Although many details about TNF and the pathways it activates are known, evidenced by about 100.000 relevant entries in literature databases, a deep understanding of the underlying signaling system is missing. As many positive and negative feedback loops are involved in the signaling process, mathematical modeling is promising to contribute to a detailed elucidation. However, mathematical modeling

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has only recently been applied to the involved pathways, either focusing on a reproduction of the overall behavior [14-17], or working out certain characteristics in more detail [18-21].

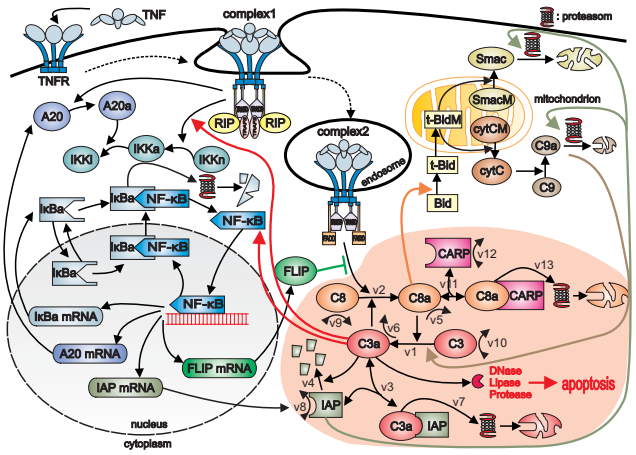

Fig. 1. Overview on TNF signaling pathways. The interplay of the different pathways is currently under investigation. This study focuses on the direct apoptotic pathway, highlighted in red. The state variable abbreviations of the corresponding model are explained in the text and can be combined into a vector $\boldsymbol{x}$. Then, the ODEs are $d\boldsymbol{x}/dt=\boldsymbol{N}\cdot\boldsymbol{v}(\boldsymbol{x})$, where the stoichiometric matrix $\boldsymbol{N}$ follows by simple balancing with the rates $\mathbf{v}(\mathbf{x})$: $v1=k_1\cdot[C8a]\cdot[C3]$, $v2=k_2\cdot[C3a]\cdot[C8]$, $v3=k_3\cdot[C3a]\cdot[IAP]-k_{-3}\cdot[iC3aIAP]$, $v4=k_4\cdot[C3a]\cdot[IAP]$, $v5=k_5\cdot[C8a]$, $v6=k_6\cdot[C3a]$, $v7=k_7\cdot[iC3aIAP]$, $v8=k_8\cdot[IAP]-k_{-8}$, $v9=k_9\cdot[C8]-k_{-9}$, $v10=k_{10}\cdot[C3]-k_{-10}$, $v11=k_{11}\cdot[C8a]\cdot[CARP]-k_{-11}\cdot[iC8aCARP]$, $v12=k_{12}\cdot[CARP]-k_{-12}$, and $v13=k_{13}\cdot[iC8aCARP]$. The experimentally estimated initial conditions in the unit "molecules/cell" are $[C8]_{t=0}=130{,}000$, $[C3]_{t=0}=21{,}000$, $[IAP]_{t=0}=[CARP]_{t=0}=40{,}000$. The other initial concentrations are zero. The standard parameter values are explained in [18] or can be obtained from www.sysbio.de/projects/tnf/jbc/.

We have here employed different kinds of sensitivity analyses to gain a deeper understanding of a previously published model for the direct pathway of receptor induced apoptosis [19]. Sensitivity analysis can also indicate biological effects of mutations or drugs, both altering system parameters. Using simulation studies with changed parameter values we focus on the above mentioned connections to the NFκB pathway, demonstrating that the connecting molecules have a strong influence in the cellular decision regarding life or death. We complement these studies by evaluating the effect of modifying each system parameter over four decades on characteristic system responses. These analyses show that the connections to the NFκB pathway are among the most influential parameters of the system. While the latter two studies have been limited to analyze the sensitivity of the output, we further use local sensitivity analyses of a characteristic steady state behavior for all states and parameters to provide on overview for the whole system. Although limited by the linear approximations employed, these analyses supplement and extend our results to provide a detailed insight into the mathematical model and the biological process it characterizes.

II. Modeling and Simulation Results of the Apoptotic Core

A. Biological Model Description

As described above, TNF binds to its corresponding receptors on the cellular surface (Fig. 1). The first signaling complex formed activates the NFκB pathway whose main steps are the activation of the inhibitor of κB kinase (IKK), which then phosphorylates the inhibitor of κB (IκB) marking it for proteasomal degradation, thereby liberating NFκB which rapidly translocates into the nucleus to activate transcription of many genes. The nucleo-cytoplasmic shuttling has been shown to be important and is, together with the negative feedback imposed by the up-regulation of its own inhibitor, responsible for oscillations in the nuclear pool of NFκB [20, 22].

After the internalization of the receptor a second signaling complex is formed, which is able to activate receptor associated upstream pro-caspases (summarized here by C8 in the pro-form and C8a in the activated form). This molecule belongs to the family of caspases, which are at the heart of apoptosis. All caspases are produced as inactive zymogens (pro-proteases) being activated by proteolytic cleavage to yield the active enzyme which itself can cleave further proteins. The substrates of C8a are limited, an important one being the molecule Bid, which subsequently activates the mitochondrial pathway of apoptosis leading to cytochrome c release from the mitochondria and finally to the activation of executioner caspases via the apoptosome, as recently modeled in [18]. As mentioned before, in Type I cells C8a directly activates the executioner caspases (summarized here by C3 in the pro-form and C3a in the active form).

The mathematical model for the direct activation of the executioner caspases consists of the reactions numbered in Fig. 1 [19]. In short, C8a, in general derived from activated death receptor complexes, serves as an input. The mutual activation of caspase 8 and 3 constitutes a positive feedback loop (v1 and v2). C3a serves as an apoptosis marker (output) as this molecule cleaves many cellular targets critical for function, and a high level of irreversible activation commits a cell to die. This process is inhibited by different inhibitor of apoptosis proteins (summarized here by IAP, v3), whereby C3a in parallel can also cleave and inactivate its own inhibitors (v4). In addition, an inhibition of C8a (v11) is considered. Although the exact nature and kinetics of this reaction remain to be established, there are both theoretical and experimental evidence arguing for its presence [19, 23, 24]. Finally, a turnover is introduced for all molecular species (v5-10, v12-13).

B. Mathematical Model Description

The model was formulated as a set of ordinary differential equations (ODEs) using mass action kinetics and the mass balance approach. The stochastic nature can to a wide extent be neglected as was shown previously [24]. Overall, the

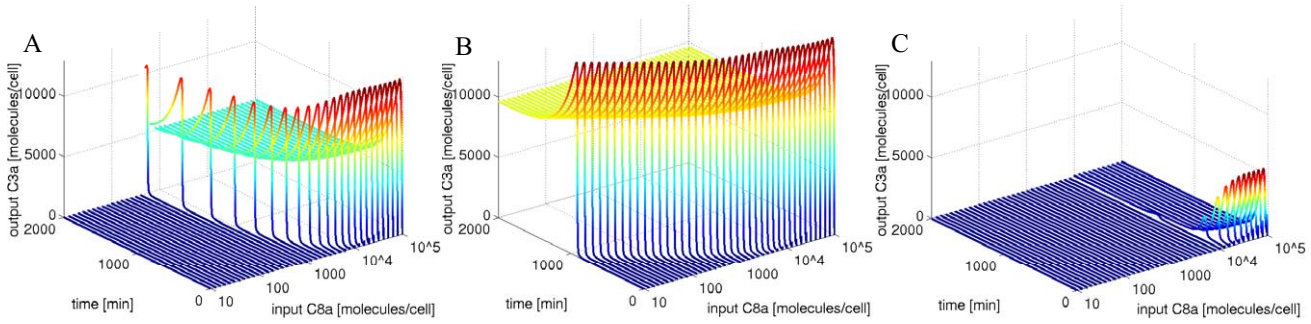

Fig. 2. Simulation experiments with varying inputs in A) the nominal case, B) with the IAP concentration two fold down-regulated, or C) with the IAP concentration two fold up-regulated.

model describes the dynamic behavior of 8 components and includes 13 reactions. This yields 13 independent parameters, for which experimental data are generally available. The parameters corresponding to protein production were chosen to provide a steady state for given degradation rates and initial conditions with zero input. The input was realized by assigning corresponding initial conditions to C8a mimicking a pulse stimulus. See Fig. 1 legend for more details.

C. *Simulation Results*

Fig. 2.A shows a simulation result obtained for the time courses of activated caspase 3 applying different initial conditions of activated caspase 8. Main characteristics of the biological system are covered by the mathematical model [19]. The first is that for some minor levels of initial caspase activation no significant activation of C3 takes place. This threshold behavior is very important in the real system to avoid accidental apoptosis by random activation of some molecules of C8. For a C8a input strength above this threshold the system undergoes a lag phase. The length of this decision phase is inversely proportional to the input strength. Then, an activation phase follows, where the majorities of caspase 3 (and 8, not shown) are activated within minutes, nicely matching experimental data obtained from single cell measurements [25]. After the peaking of the caspase activities, they finally settle to a high steady state value. Here, the model obviously looses its validity, as there follow many additional processes not considered.

The continuously increasing input is converted into an all-or-none discrete output signal. From the system sciences point of view, a bistable switching behavior between two stable steady states is obtained. The "life steady" state corresponds to no activated caspases. In the "death steady state" the almost complete activation of caspases leads to cell death. The areas of attraction of the two stable steady states are separated by the stable manifold of the unstable steady state [26].

III. Analysis using *in silico* Experiments

Having established a suitable model for the apoptotic core reactions that describes the bistable behavior, we evaluated how this behavior depends on the initial conditions and kinetic parameter values.

A. *NFκB crosstalk and Inhibitor* in silico *mutation*

To reveal the relevance of the cross talking inhibitors IAP and FLIP (FLice Inhibitory Protein) simulation studies were performed with modified protein concentrations. In the biological system, this modification of protein concentrations is controlled by the transcription factor NFκB, which is also activated by the TNF receptor complex as described above. Therefore, the IAP and FLIP levels are potential key signals linking pro- and anti-apoptotic pathways (crosstalk).

Changed FLIP levels directly affect the input, and the input dependent threshold behavior has already been discussed above in part. Altered IAP concentrations were simulated by changing the initial condition and the respective production rate constant to give a steady state with zero input to account for altered gene expression.

As can be seen in Fig. 2 the effects of a two fold up- or down-regulation (Fig. 2.C or B respectively) of the effector caspase inhibitor IAP are strong. A down-regulation leads to an earlier onset of significant effector caspase activation for comparable inputs. Both the peak and the death steady state level of activated caspases are increased. Further, the threshold below which no caspase activation occurs virtually vanishes (below one molecule C8a input). This is in contrast to XIAP knock out mice, which hardly show a phenotype. However, this can be explained by the redundancy of IAP molecules *in vivo*, represented as a single pool *in silico* [27]. The up-regulation almost completely abolishes effector caspase activation even for high inputs, only allowing a temporal activation of a much lower magnitude. Not only the peak but especially the death steady state level of activated caspases is decreased yielding a signal that is almost only transient (almost returning to zero C3a). This is interesting as it not only shows how cells can be protected against weaker apoptotic signals, but it also indicates that for large inputs temporarily restricted caspase activation can occur. It is known that in certain cancers IAP proteins are up-regulated [28]. This might not only prevent the efficient removal of cancerous cells, but when encountering strong

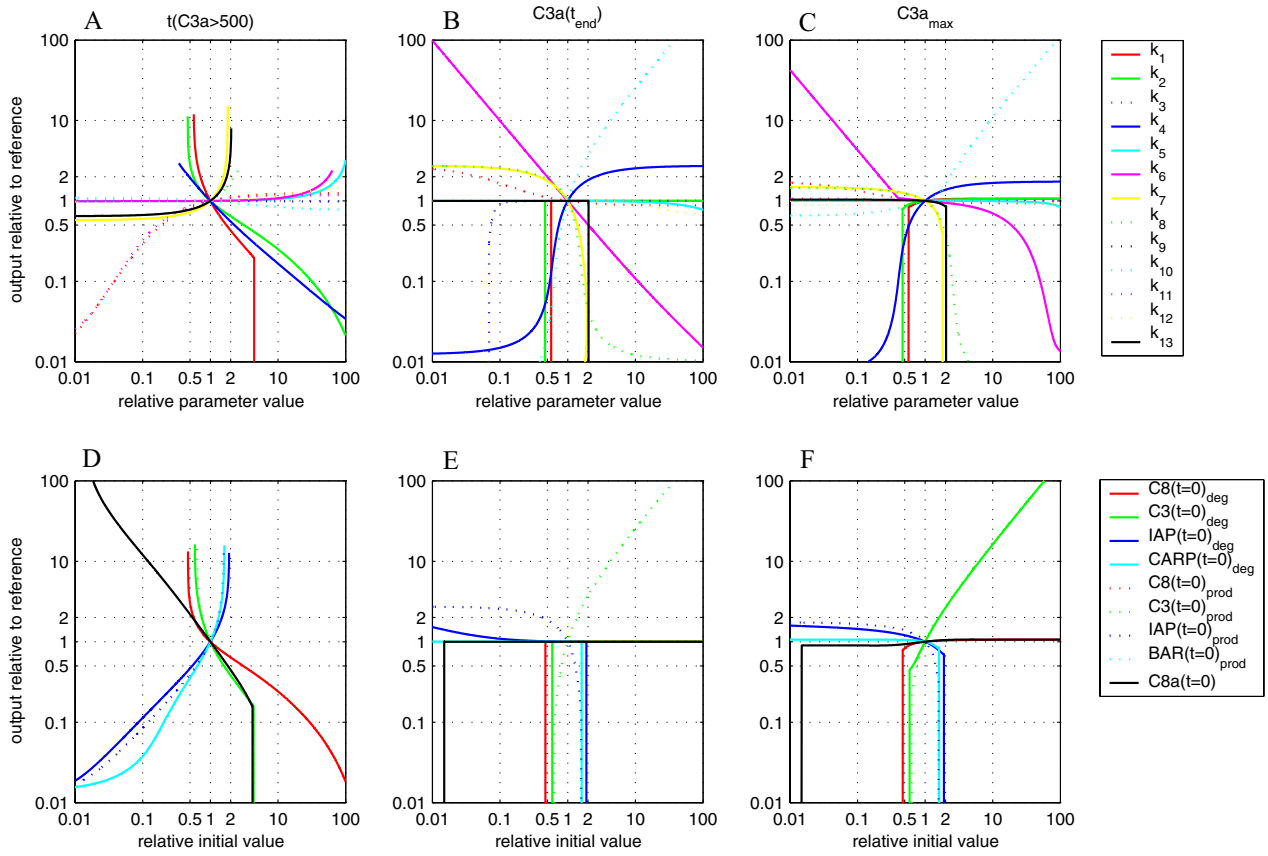

Fig. 3. Sensitivity plots. The dependence of the three characteristic outputs t(C3a>500) (A, D), C3a(t_{end}) (B, E) and C3a$_{max}$ (C, F) on the kinetic parameter values (A-C) and the initial conditions (D-F) is shown. Both axes are in logarithmic scale and the values normalized to the nominal case.

apoptotic triggers these cells might be damaged by partial caspase activation. Partial executioner caspase activation might lead to partial DNA cleavage favoring further mutations or to damages in the cytoskeleton potentially making the tumor cell more invasive, both being hallmarks of cancer progression [29].

Modifying the level of the third inhibitor CARP, which might as well be controlled by NFκB, qualitatively yields the same results (not shown). The inhibitors strongly shift the input dependent threshold up or down, depending on whether they are up- or down-regulated, respectively. Therefore, NFκB can efficiently influence the apoptotic outcome.

The simulations clearly indicate an important role for IAP molecules in controlling the apoptotic pathway. In addition, they indicate how these molecules might contribute to cancer progression. Further, the simulation studies can not only hint to the effects of NFκB but also indicate what effects mutations or pharmaceutical effectors, i.e. drugs might have. Therefore, we would like to extend these studies to all possible reactions and components in the apoptotic pathway.

B. Wide range parameter sensitivity analysis

While the previous studies were able to provide a dynamic and clear insight into the system behavior when certain system properties are altered, the approach is cumbersome to provide a survey for the effects of all different rates and components. Therefore, we employed a systematic analysis for all systems parameters.

Fig. 3 shows how certain output characteristics change when varying a kinetic parameter or an initial condition. When varying an initial condition, in addition either the degradation rate constants or the production rate constants had been varied accordingly (denoted by the subscript “deg” and “prod” respectively) to achieve a steady state without input. For parameter changes the ratio was kept constant (i.e. only the forward rates had been varied and if the reactions were reversible, the back rates had been changed in the respective way to preserve the ratio). Compared to the previous studies, we kept the input signal constant at 5000 molecules/cell. As output characteristics we did not evaluate the whole time trajectories but chose a) the time until the number of activated effector caspases exceeds 500 molecules/cell (t(C3a>500)) corresponding to the length of the lag phase, b) the number of caspase molecules at the end of the simulation corresponding to either the life or death steady state depending on whether the input signal is above or below the threshold for the given conditions (C3a(t_{end}=10^5 min)) and c) the maximal effector caspase activation achieved (C3a$_{max}$). Both, the fold parameter or initial condition change as well as the output characteristic, were normalized to the nominal system (i.e. all curves

intersect in the middle of the plot and e.g. a relative initial value of two indicates a two fold up-regulation). The choice of a double logarithmic scale was also motivated by the fact that wet lab experiments often proceed in fold changes. A 100 fold down-regulation nearly corresponds to a knock out, whereas a more than 10 fold up-regulation (although applied in certain experiments) might be considered as physiologically irrelevant.

The length of the lag phase (Fig. 3, left column) is mainly determined by the kinetics of the mutual caspase activation (k_1 and k_2), the degradation of the activated caspase-inhibitor complexes (k_7 and k_{13}) and the turnover of IAP molecules (k_4 and k_8). Unexpectedly, the reactions involving the IAP binding to C3a itself (k_3), the reactions involving CARP, but also the caspase turnover (Fig. 3.A) only have a minor impact. The effects are clearly nonlinear and also asymmetric, i.e. a two fold up-regulation has not the quantitatively inverse effect of a two fold down-regulation. All initial conditions exert a strong effect on the length of the lag phase (Fig. 3.D). The effect of CARP up-regulation is slightly more pronounced when compared to the effect of IAP up-regulation. This can be explained by the fact that the same inhibition kinetics were assumed, but the CARPs are inhibiting the more potent caspase. However, as pointed out in the model description, more experimental details regarding the CARP kinetics are needed to confirm this point. Interestingly, both CARP and IAP have a stronger impact on the caspase activation than the input. This points to the relevance of FLIP, and therefore its role might not be as dominant as that of the inhibitors IAP or CARP. The discontinuities at about one fifth of the nominal time for certain parameters or initial concentrations can be explained by the appearance of a second, earlier peak which is already visible in Fig. 2 for high inputs (the vertical connecting lines are plotting artifacts and not removed because they are helpful in the interpretation). This early peak is derived from the initial activation before C8a molecules become bound to the CARPs. Whether a change in the initial concentration was accompanied by a corresponding change in the production or degradation rate hardly influences the system behavior.

Regarding the final values of activated caspase 3 molecules (Fig. 3, middle column) the parameters and initial concentrations can be divided into two groups: those that have no impact on the location of the steady states, but influence whether the life or death steady state is achieved (i.e. giving a 1 or 0 output as can be seen in the discontinuous output; again the vertical connections were not removed) and those parameters which also influence the location of the steady state. The latter group contains the rates that affect the turnover of (activated) caspase 3 molecules as well as the corresponding initial concentration (k_6 and k_{10} in Fig. 3.B, $C3_{prod}$ in Fig. 3.E). Interestingly, an up-regulation through increased expression strongly increases the final C3a value, while an up-regulation

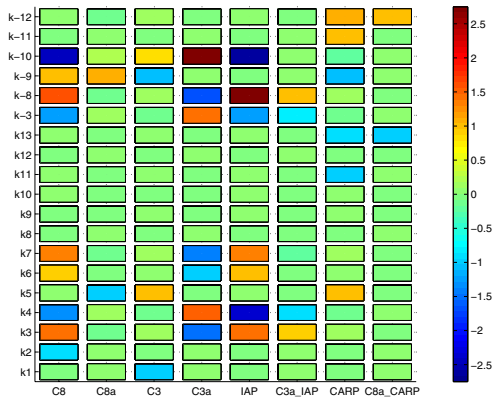

Fig. 4. Normalized local sensitivities.

through decreased degradation reveals no such effects. This can be explained by the fact that in the apoptotic case, i.e. after a significant caspase activation was achieved, almost all caspase molecules will become activated, and while the degradation of pro-caspases is proportional to their concentration, the production is independent of this. The same effect can be seen for IAP molecules (Fig. 3.E). An up-regulation through production lowers the final value of C3a, while an up-regulation through a reduced degradation has no impact on the final value, although at high IAP concentrations activation is completely abolished for the given input signal. This can be explained by the fact that in the apoptotic case the majority of IAP molecules are degraded by the cleavage reaction, independent of the degradation rate. This feature can also be observed in plots similar to Fig. 2. As described the up-regulation through increased *in silico* expression yields an almost transient signal for high inputs. The same fold up-regulation through decreased degradation only shifts the threshold but not the principle form of the original signal (data not shown). Thus, while apoptosis prevention through increasing IAP expression might lead to pathological cell states as described above, this might not be the case if the up-regulation is achieved through a decreased degradation (e.g. through interference in the proteasomal degradation pathway) pointing to an interesting feature, that awaits experimental validation.

The maximal effector caspase activation (Fig. 3, right column) is similarly influenced. The information in these plots can also be used to fine tune the parameters when more quantitative experimental data becomes available.

Taken together, analyzing the dependence of the systems behavior to wide range parameter variations indicates that many parameters can significantly influence the systems behavior. In that respect, the direct inhibitors of active caspases, IAP and CARP, appear especially powerful. Regarding their potential pharmaceutical role, these analyses indicate that a decreased degradation should be more desirable than an increased expression.

C. Local parameter sensitivity analysis

So far the analyses have focused on the influence of parameters on a single output. However, molecules such as IAP members are also important in other pathways. It is therefore interesting to investigate the effects of parameter changes on other state variables. To do this, we applied a local sensitivity analysis. We evaluated the dependence of the stable apoptotic steady state (Fig. 4) to 1 % parameter changes. The sensitivities were calculated using the Matlab Systems Biology Toolbox [30]. The results provide a normalized output relative to the nominal steady state and parameter values, i.e. the sensitivity of state x_i with respect to parameter p_j is defined as $S_{ij}=p_j/x_{SSi}\cdot(x_{SSi}(p_j+\Delta p_j)-x_{SSi})/\Delta p_j$.

For the C3a-column in Fig. 4 the local sensitivities of the death steady state correspond to the tangents in Fig. 3.B (point 1|1), although for the local analysis the parameter ratios have not been kept constant and thus a direct comparison is only possible for unidirectional reactions. Indeed, $k_{7,}$ dictating the degradation speed of the C3a-IAP complex, is strongly negative in Fig. 4 and also has a steep negative slope in Fig. 3.B. For bidirectional reactions the sum of the two local values should roughly (locally) provide results comparable to the analysis shown in Fig. 3.B. For example, strong but opposing effects are exerted by the parameters k_{-8} and k_{-10} describing the IAP and C3 production respectively. For these, the local analysis shows that the steep slopes in Fig. 3.B almost completely derive from the back reaction rate constant and not from the forward degradation rate constants. Opposing effects for the rate constants within one reaction can be seen for reaction 3. Although the slope in Fig. 3.B indicates a minor impact, the local analysis reveals a strong but opposing effect of the association and dissociation constant. While unexpected before, this can now be easily understood as this reaction corresponds to the reversible inhibition of C3a by IAP molecules. A stronger binding acts anti-apoptotic lowering the concentration of C3a.

Apart from activated caspase 3, the most sensitive states are IAP and caspase 8. Regarding IAP, the most sensitive parameter is k_{-8} corresponding to its expression. Interestingly, C8 most strongly depends on rate constants which do not directly influence the state (k_3, k_4, k_{-8}, k_{-10}). From a dynamic systems point of view, this behavior can easily be envisioned for a non-linear system containing feedback loops; however, for biologists this can provide valuable unexpected insight. Another important point to notice is that these local analyses are obviously not able to predict non-local behaviors. For example, both the local and the wide range sensitivity analysis predict that varying k_1 or k_{13} will have no impact on the final C3a concentration, but only the wide range analysis shows that this is only true for a restricted parameter interval. Outside of this interval, the system completely changes its behavior.

In summary, the local analyses can nicely complement the previous studies not only providing an overview, but additional insight. The IAP molecules not only have strong impact on the output, but appear themselves to be sensitive to several parameters.

IV. Outlook

Based on the mathematical model of the apoptotic core further biological and theoretical questions can be tackled.

As discussed in the introduction two apparently conflicting signaling pathways are induced by the TNF receptor complex. The main focus of our current work is directed to modeling and analysis of the whole signaling network as depicted in Fig. 1. Therefore, a detailed mathematical model of the receptor dynamics after TNF stimulation, of the NFκB signaling pathway, and of the interaction between the two pathways will be necessary. Regarding the NFκB pathway some models have been published within the last years [20, 22, 31, 32]. It was shown that the form of the induced NFκB signal plays an important role. Based on published models and using own experimental work, the model parameters have to be identified to fit to our experimental system. When building a large model including NFκB signaling one has to pay special attention to the accurate description and experimental validation of the expression of IAP, but also of FLIP as this work further reveals the strong dependence of the system behavior on these parameters.

Based on an overall model the interplay of both pathways can be studied. One main goal is again the identification of key components and sensitive parameters. This will allow to proposal of meaningful experiments to reveal a maximum of information.

On a long term time scale, a vision of this project is to gain enough understanding about the cross talking pathways, so that it allows influencing the balance of their interplay into a desired direction. This might give some important insight for the design of specific drugs to restore the healthy balance of pro- and anti-apoptotic signaling in diseased cells. From a control engineering point of view it will also be interesting to investigate the complex regulation networks in order to detect new efficient strategies adaptable to manmade systems.

V. Conclusion

Our results demonstrate that local sensitivity analyses can reveal a useful overview, but also have to be complemented because they cannot capture the non-linear and asymmetric effects of parameter changes as observed. These can be captured by wide range parameter changes and in more detail by simulation studies. However, latter methods become overwhelming when used to study all state and parameter dimensions. Taken together the different methods for analyzing the sensitivity of system characteristics to parameter variations not only provide an interesting case study for the important process of apoptosis, but also illustrate how classical system theoretic methods can be

applied to cellular signaling.

The model of apoptotic signals analyzed here was shown to be highly sensitive to several parameter changes. This is somewhat in contrast to observations for other biological processes [33, 34]. However, one has to keep in mind that additional processes increasing the robustness of the model have been neglected in order to enable a better insight into the principal behavior.

The results of our analysis outline the importance of controlling activated caspases for prevention of apoptosis. Here, it appears to be more efficient to inhibit activated caspases (i.e. IAPs and CARPs) than to inhibit the activation (i.e. FLIP). Especially the role of the NFκB induced IAP molecules appears to be critical in the regulation of apoptosis. The analyses also indicate how tumors might become more aggressive, suggesting strategies to prevent tumor progression. Further, the results will enable a more efficient modeling of additional pathways, as the main interaction points have been thoroughly studied here.

APPENDIX

A Matlab simulation file of the described model is available at www.sysbio.de/projects/tnf/jbc/.

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REFERENCES

[1] H. Kitano, "Systems biology: a brief overview," Science, vol. 295, pp. 1662-4, 2002.
[2] T. Ideker, "Systems biology 101--what you need to know," Nat. Biotechnol., vol. 22, pp. 473-5, 2004.
[3] U. Alon, "Biological networks: the tinkerer as an engineer," Science, vol. 301, pp. 1866-7, 2003.
[4] O. Wolkenhauer, M. Ullah, P. Wellstead, and K. H. Cho, "The dynamic systems approach to control and regulation of intracellular networks," FEBS Lett., vol. 579, pp. 1846-53, 2005.
[5] J. J. Tyson and M. C. Mackey, "Molecular, metabolic, and genetic control: An introduction," Chaos, vol. 11, pp. 81-83, 2001.
[6] M. O. Hengartner, "The biochemistry of apoptosis," Nature, vol. 407, pp. 770-6, 2000.
[7] M. Leist and M. Jaattela, "Four deaths and a funeral: from caspases to alternative mechanisms," Nat. Rev. Mol. Cell Biol., vol. 2, pp. 589-98, 2001.
[8] H. Wajant, K. Pfizenmaier, and P. Scheurich, "Tumor necrosis factor signaling," Cell Death Differ., vol. 10, pp. 45-65, 2003.
[9] G. Chen and D. V. Goeddel, "TNF-R1 signaling: a beautiful pathway," Science, vol. 296, pp. 1634-5., 2002.
[10] R. M. Locksley, N. Killeen, and M. J. Lenardo, "The TNF and TNF receptor superfamilies: integrating mammalian biology," Cell, vol. 104, pp. 487-501., 2001.
[11] J. Savill and V. Fadok, "Corpse clearance defines the meaning of cell death," Nature, vol. 407, pp. 784-8, 2000.
[12] C. Scaffidi, I. Schmitz, J. Zha, S. J. Korsmeyer, P. H. Krammer, and M. E. Peter, "Differential modulation of apoptosis sensitivity in CD95 type I and type II cells," J. Biol. Chem., vol. 274, pp. 22532-8., 1999.
[13] C. Scaffidi, S. Fulda, et al., "Two CD95 (APO-1/Fas) signaling pathways," EMBO J., vol. 17, pp. 1675-87, 1998.
[14] M. Bentele, I. Lavrik, et al., "Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis," J. Cell Biol., vol. 166, pp. 839-51, 2004.
[15] B. Schoeberl, E. D. Gilles, and P. Scheurich, "A mathematical vision of TNF receptor interaction," Proceedings of the International Conference on Systems Biology, Pasadena, CA, pp. 158-167, 2001.
[16] M. Fussenegger, J. E. Bailey, and J. Varner, "A mathematical model of caspase function in apoptosis," Nat. Biotechnol., vol. 18, pp. 768-74, 2000.
[17] K. A. Janes, J. G. Albeck, S. Gaudet, P. K. Sorger, D. A. Lauffenburger, and M. B. Yaffe, "A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis," Science, vol. 310, pp. 1646-53, 2005.
[18] E. Z. Bagci, Y. Vodovotz, T. R. Billiar, G. B. Ermentrout, and I. Bahar, "Bistability in Apoptosis: Roles of Bax, Bcl-2 and Mitochondrial Permeability Transition Pores," Biophys. J., vol. 90, pp. 1546-59, 2006.
[19] T. Eißing, H. Conzelmann, E. D. Gilles, F. Allgöwer, E. Bullinger, and P. Scheurich, "Bistability analyses of a caspase activation model for receptor-induced apoptosis," J. Biol. Chem., vol. 279, pp. 36892-7, 2004.
[20] A. Hoffmann, A. Levchenko, M. L. Scott, and D. Baltimore, "The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation," Science, vol. 298, pp. 1241-5, 2002.
[21] D. E. Nelson, A. E. Ihekwaba, et al., "Oscillations in NF-kappaB signaling control the dynamics of gene expression," Science, vol. 306, pp. 704-8, 2004.
[22] T. Lipniacki, P. Paszek, A. R. Brasier, B. Luxon, and M. Kimmel, "Mathematical model of NF-kappaB regulatory module," J. Theor. Biol., vol. 228, pp. 195-215, 2004.
[23] E. R. McDonald, 3rd and W. S. El-Deiry, "Suppression of caspase-8- and -10-associated RING proteins results in sensitization to death ligands and inhibition of tumor cell growth," Proc. Natl. Acad. Sci. USA, vol. 101, pp. 6170-5, 2004.
[24] T. Eißing, F. Allgöwer, and E. Bullinger, "Robustness properties of apoptosis models with respect to parameter variations and stochastic influences," IEE Proc. Syst. Biol., vol. 152, pp. 221-228, 2005.
[25] M. Rehm, H. Dussmann, R. U. Janicke, J. M. Tavare, D. Kogel, and J. H. Prehn, "Single-cell fluorescence resonance energy transfer analysis demonstrates that caspase activation during apoptosis is a rapid process.," J. Biol. Chem., vol. 277, pp. 24506-14, 2002.
[26] E. Bullinger, "System analysis of a programmed cell death model," Proceedings of the IEEE Conference on Decison and Control, Seville, Spain, pp. 7994-9, 2005.
[27] H. Harlin, S. B. Reffey, C. S. Duckett, T. Lindsten, and C. B. Thompson, "Characterization of XIAP-deficient mice," Mol. Cell. Biol., vol. 21, pp. 3604-8, 2001.
[28] B. Nachmias, Y. Ashhab, and D. Ben-Yehuda, "The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer," Semin. Cancer Biol., vol. 14, pp. 231-43, 2004.
[29] D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," Cell, vol. 100, pp. 57-70, 2000.
[30] H. Schmidt and M. Jirstrand, "Systems Biology Toolbox for MATLAB: a computational platform for research in Systems Biology," Bioinformatics, vol. 22, pp. 514-5, 2005.
[31] S. L. Werner, D. Barken, and A. Hoffmann, "Stimulus specificity of gene expression programs determined by temporal control of IKK activity," Science, vol. 309, pp. 1857-61, 2005.
[32] M. W. Covert, T. H. Leung, J. E. Gaston, and D. Baltimore, "Achieving stability of lipopolysaccharide-induced NF-kappaB activation," Science, vol. 309, pp. 1854-7, 2005.
[33] N. Barkai and S. Leibler, "Robustness in simple biochemical networks," Nature, vol. 387, pp. 913-7, 1997.
[34] J. Stelling, U. Sauer, Z. Szallasi, F. J. Doyle, 3rd, and J. Doyle, "Robustness of cellular functions," Cell, vol. 118, pp. 675-85, 2004.