Influence of parameter values on the oscillation sensitivities of two p53–Mdm2 models

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Abstract Biomolecular networks that present oscillatory behavior are ubiquitous in nature. While some design principles for robust oscillations have been identified, it is not well understood how these oscillations are affected when the kinetic parameters are constantly changing or are not precisely known, as often occurs in cellular environments. Many models of diverse complexity level, for systems such as circadian rhythms, cell cycle or the p53 network, have been proposed. Here we assess the influence of hundreds of different parameter sets on the sensitivities of two configurations of a well-known oscillatory system, the p53 core network. We show that, for both models and all parameter sets, the parameter related to the p53 positive feedback, i.e. self-promotion, is the only one that presents sizeable sensitivities on extrema, periods and delay. Moreover, varying the parameter set values to change the dynamical characteristics of the response is more restricted in the simple model, whereas the complex model shows greater tunability. These results highlight the importance of the presence of specific network patterns, in addition to the role of parameter values, when we want to characterize oscillatory biochemical systems.

Keywords P53 network · Oscillatory systems · Global sensitivity

Introduction

Biomolecular networks are responsible for regulating many vital functions in living organisms. Among these networks, oscillatory systems represent a very important family because they help with the regulation of key cellular processes such as circadian rhythms, cell cycle, DNA damage response and others (Zhang and Kay 2010; Kaizu et al. 2010; Tiana et al. 2007; Sneppen et al. 2010). The time-keeping functionalities of oscillatory networks has led to efforts in the area of synthetic biology, with successful realization of several oscillatory systems (Purcell et al. 2010; Khalil and Collins 2010; Weitz et al. 2014; Prindle et al. 2014). Some researchers have even made advances in coupling artificial oscillators on to nanomechanical devices, bringing promise to the composition of larger functional artificial systems (Franco et al. 2011).

Oscillations often result from complex nonlinear interactions among tens of genes, proteins and metabolites, which makes very difficult to unveil their design principles. Despite that, some requirements for robust oscillation have been identified, e.g. negative feedback and time delay (Novak and Tyson 2008; Ananthasubramaniam and Herzog 2014). Importantly, the obtention of robust oscillatory system may necessitate the presence of additional specific traits. Tsai et al. (2008) demonstrated numerically that both increased robustness and tunability are achieved by adding a positive self-promotion feedback loop. Their results were later confirmed experimentally (Tigges et al. 2009; Stricker et al. 2008).

While there has been a large interest in determining the relationship between network architecture and functionality, it

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is still not very clear how the parameter values influence the latter. Some numerical studies have shown that the network parameter values can be tuned in order to obtain either integrative or oscillatory responses (Conrad et al. 2008). Moreover, there are also hints that by carefully choosing the parameter values it may be possible to obtain synchronized oscillations—previously thought to be dependent only on the network architecture (Potapov et al. 2011).

In order to characterize the response of dynamical systems, one can assess their sensitivities to parameters variations. These sensitivities can help with quantifying the level of influence of each parameter variation on specific aspects of the systems response. Most of the sensitivity related methods need a level of reference for each parameter, which is usually defined as the nominal value. The parameter variations can be small (local methods) or large (global methods). Many methods have been proposed for studying parametric sensitivities of biomolecular oscillators (Kramer et al. 1984; Leloup and Goldbeter 2004; Wilkins et al. 2007; Taylor et al. 2008), among them the metabolic control analysis (MCA) (Ingalls and Sauro 2003).

Quantitative models are playing a very important role in the quest to explaining the occurrence and functionality of biomolecular networks, including oscillatory ones (Tiana et al. 2007; Sneppen et al. 2010). However, two big issues remain. The first one is the insufficient information about the parameters that define the biochemical reactions, even for well studied systems. And the second one is the great variability that these parameters present in operating conditions (Geva-Zatorsky et al. 2006).

One approach to study the great variability of the system parameters is to use stochastic analysis, where the parameters are allowed to change over time. However, it may be difficult to identify in a straightforward way the effect of a parameter variation on the system response. In contrast, using a deterministic approach over a large range of variations of the model parameters may give some insights on the variability of the system response.

Here we study two models of the p53–Mdm2 core network. Both models correspond to a very important motif in regulatory networks where a protein upregulates a gene, which in turn promotes the protein degradation (Yeger-Lotem et al. 2004; Alon 2007). This configuration, also called amplified negative feedback oscillator, has successfully been implemented in some synthetic biochemical systems both in vivo and in vitro (Purcell et al. 2010; Atkinson et al. 2003; Kim and Winfree 2011), which reinforces the importance of performing a detailed analysis in order to properly characterize its response.

We begin by briefly describing the two p53–Mdm2 models, a global parametric sensitivity metric and the simulation setup (oscillatory response validation criteria

and techniques employed for plot generation). Next, we analyze the oscillations dependence on each parameter (parametric sensitivity) by considering the response characteristics. We also perform a clustering of sensitivities which readily permits to rank parametric sensitivities. By taking the sensitivity medians for a variety of parameter sets, it is possible to determine the global influence of each parameter on the response characteristic. Finally, we explore the effect of network architecture by employing the difference between ranking correlation medians for the two models.

Additional details about the simulation data and complementary plots can be found in the Electronic Supplementary Material (ESM) file.

Material and methods

Mathematical models

The transcription factor p53 has been identified as a major tumour-suppressor in multicellular organisms. The p53 pathway is highly complex, including several hundreds of genes and their products (Kohn and Pommier 2005; Levine et al. 2006). Building models of such complexity is a daunting task, not only many interactions are still not well characterized but also the kinetic constants that describe them are, in general, not known.

Here we study two simple models of the p53–Mdm2 network. The first model is proposed by Chickarmane et al. (2007) (from now on Chickarmane model), shown in Fig. 1a; and the second model proposed herein is a more detailed version of Chickarmane's, shown in Fig. 1b.

Chickarmane model includes only two species, p53 and Mdm2, with their time dependencies described by Eqs. 1a–b. Once DNA gets damaged, the phosphorylated *ATM* (ATM_p) increases and stays at a higher than normal constant level if damage persists. The p53 is then transcribed by this ATM_p . This description is a simplification of experimental findings (Batchelor et al. 2008). A very important feature in this model is the p53 positive feedback (self-promotion), represented by the Hill function term in Eq. 1a. Degradation of p53 can be dependent or independent of Mdm2. Considering the Mdm2, its transcription is due to both a basal rate and the p53, and its degradation is independent of any species in the model.

$$\frac{dp53}{dt} = a_0 ATM_p + a_1 \frac{p53^4}{k_1 + p53^4} - g_1 p53 Mdm2 - g_2 p53 \quad (1a)$$

$$\frac{dMdm2}{dt} = a_2 + a_3 \frac{p53^4}{k_2 + p53^4} - g_3 Mdm2 \quad (1b)$$

Fig. 1 The p53–Mdm2 core network models studied here: **a** Chickarmane model, **b** model proposed in this work

The second model herein proposed considers four species: inactivated p53 ($p53$), activated (phosphorylated) p53 ($p53_p$), cytoplasmic Mdm2 ($Mdm2_{cyt}$) and nuclear Mdm2 ($Mdm2_{nuc}$). Their corresponding relations are given by Eqs. 2a–d. Similar four species models have been proposed elsewhere (Zhang et al. 2007, 2009; Jolma et al. 2010). In this model ATM_p is also maintained at a constant level once DNA damage happens, but performs two tasks, promotion of p53 activation (Prives 1998) and $Mdm2_{nuc}$ degradation (Stommel and Wahl 2004). There is also a positive feedback loop that promotes the transcription of p53 due to $p53_p$. Yet, degradation of both versions of p53 are affected by Mdm2, $p53_p$ promotes the transcription of $Mdm2_{cyt}$ (Yin et al. 2002), and Mdm2 can be imported into (or expelled from) the nucleus.

$$\frac{dp53}{dt} = k_{1s53} + k_{2s53} \frac{p53_p^4}{J_{s53}^4 + p53_p^4} + k_{in} \frac{p53_p}{k_{m2} + p53_p} - a_f \frac{ATM_p p53}{k_{m1} + p53} - k_{1ac} p53 - (g_{in} + g_{in1} Mdm2_{nuc}) p53 \quad (2a)$$

$$\frac{dp53_p}{dt} = a_f \frac{ATM_p p53}{k_{m1} + p53} + k_{1ac} p53 - k_{in} \frac{p53_p}{k_{m2} + p53_p} - (g_{ac} + g_{ac1} Mdm2_{nuc}) p53_p \quad (2b)$$

$$\frac{dMdm2_{cyt}}{dt} = k_{1s2} + k_{2s2} \frac{p53_p^3}{J_{s2}^3 + p53_p^3} + k_o Mdm2_{nuc} - k_i Mdm2_{cyt} - k_{2d2} Mdm2_{cyt} \quad (2c)$$

$$\frac{dMdm2_{nuc}}{dt} = k_i Mdm2_{cyt} - k_o Mdm2_{nuc} - k_{1d2} Mdm2_{nuc} - k_{3d2} \frac{ATM_p Mdm2_{nuc}}{k_{md} + ATM_p} \quad (2d)$$

Global parametric sensitivity

In this study, oscillation gets defined in terms of its extremum and period. Furthermore, when two oscillations are considered, a delay between them can also be identified. In order to define a global parametric robustness metric, the following methodology is employed. First, the relative fold change of extremum (period or delay) value is plotted against a parameter relative fold change (shown in a base 10 logarithmic scale); the resulting curve is denoted as influence curve F . Relative fold change is considered to be the relative variation with respect to the nominal relative value 1. Figure 2b shows the case for the extremum and period when the parameter a_0 is changed. Second, the function $|F - 1|$ is defined (vertical bars represent the absolute value) and integrated along the parameter relative fold change, provided that oscillations occur. The result of this integration is then divided by the size of the domain of parameter relative fold changes associated with oscillations. The number obtained from this division is called global parametric sensitivity, or just sensitivity from now on. For all the calculations, the parameter relative fold change is considered in a base 10 logarithmic scale.

Simulations

For the two models considered herein, parameters are selected such that oscillations obtained resemble those of experimental results, which present periods of 5.5 ± 1.5 h and delays of 2 ± 0.5 h (Geva-Zatorsky et al. 2006). As a validation criteria a parameter set must render a period and delay in the range of 5.5 ± 0.3 h and 2 ± 0.3 h, respectively. Furthermore, for the inactivated state of the networks (corresponding to $ATM_p = 0$), both p53 and Mdm2 responses should not present oscillations. The differential equations were solved using the deterministic integrator RADAU, included in the software PyDSTool (Clewley et al. 2007), and for the integration of the mean relative fold change of extrema, periods and delay the adaptive Simpson quadrature numerical algorithm was employed.

Fig. 2 Sensitivity calculation procedure, corresponding to the Chickarmane model of the p53 core network. Results correspond to base parameter set “B0”: **a** p53 oscillations dependence on parameter a_0 relative fold change; the heat map values correspond to the oscillation amplitudes, **b** a_0 influence on mean values of extrema of p53 (Ep) and Mdm2 (Em), periods of p53 (Pp) and Mdm2 (Pm), and delay between p53 and Mdm2 (D), **c** oscillations range for each parameter in the model, **d** heat map for global parametric sensitivities as defined in this work

Base parameter sets

Starting from the base parameter set “B0” included in the ESM file (Tables S1 and S2), we generated randomly 5 additional base parameter sets, “B1–B5”, within the range of 0.001–1000 times each parameter value. Only in this case we relaxed the validation criteria to accept values for periods of 5.5 ± 3 h and delays of 2 ± 1 h.

Parameter sets

For each base parameter set, 100 additional parameter sets were randomly obtained within the range of 0.1–10 times each base parameter value. In order to obtain the 100 parameter sets that satisfied the period and delay validation criteria, it was necessary to evaluate responses from a few hundred thousands up to a few million trials, depending on both the model and the base parameter set.

Clustering

For the analysis of the data obtained, we used a hierarchical clustering method (Myat 2007) to group both parameter sets and model parameters that show similar sensitivities (see Fig. 3). Euclidean distance and average linkage were employed.

Fig. 3 Sensitivities for p53 period in Chickarmane model. All 100 parameter sets around the base parameter set “B0” are shown

Bootstrapping

For each parameter, and each one of the 6 base parameter sets “B0–B5”, we calculated the median of the corresponding sensitivities among the 100 parameter sets. For each base parameter set, we then arranged the parameters in a decreasing order of importance according to their sensitivity median; thus generating a sensitivity median ranking. The Spearman correlation between the resulting sensitivity median ranking and the ranking obtained considering sensitivities for each parameter set is calculated; 100 correlations are obtained for each base parameter set. By using the bootstrap method (Wilcox 2010) on the 100 ranking correlations, the difference between the ranking correlation medians of all base parameter sets, in combinations of two by two, is calculated using a 0.95 confidence interval (see Fig. 6).

Results

Equipped with an adequate metric (global parametric sensitivity) and a simulation validation criteria, a wide variety of simulations were performed for the Chickarmane model and the model herein proposed.

For the Chickarmane model (Chickarmane et al. 2007), 6 sets of base parameter values are shown in ESM, Table S1. Figure 2 is obtained by using the base parameter set “B0”. Figure 2a, b shows the influence of parameter a_0 on p53 and Mdm2 oscillations, as it is varied from 0.1 to 10

times its nominal value (only parameter a_0 changes); a_0 relative fold change is represented in logarithmic scale. In Fig. 2a, as parameter a_0 increases its value, the period of the p53 oscillations gets smaller. This dependence can also be observed more clearly in Fig. 2b, if we consider the monotonically decreasing curve labelled “Pp” (p53 period). Figure 2b also shows similar behavior for Mdm2 period “Pm”, as “Pm” overlaps “Pp”. Importantly, Fig. 2b presents the mean relative fold change, with respect to nominal values, of the extrema (maximum values), periods and delay for the p53 and Mdm2 oscillations. Note that, these curves are not defined in the whole range, since for small and large values of a_0 there are no oscillations. For both p53 and Mdm2 extrema, “Ep” and “Em” respectively, there is no monotonic dependency with the a_0 value. The delay between p53 and Mdm2, “D”, neither presents monotonic relationship to a_0 values. We present mean values of extrema because oscillations of the p53 and Mdm2 species concentrations are usually damped, i.e. have diminishing amplitudes and corresponding different local extrema. Likewise, we also show mean values of periods and delays as they can also present variations during oscillations.

In order to summarize the results presented in Fig. 2b and to include the influence of the remaining parameters, Fig. 2c, d is shown. The oscillating regions for all parameters considered in the Chickarmane model can be seen in Fig. 2c, where we observe that parameter g_2 is related to oscillations in the whole range, i.e. from 0.1 to 10, which corresponds to $\log(0.1) = -1$ to $\log(10) = 1$, giving a total logarithmic variation of 2. In Fig. 2d sensitivities for all parameters are shown. We observe, for example, that parameter g_2 has almost no influence on any of the five features considered (two extrema, two periods and one delay); which is not the case for a_1 . While Fig. 2d summarizes very well the sensitivities of all model parameters, those results are still dependent on the specific parameters set “B0” considered. This clearly presents the need for a more general approach for analyzing sensitivities.

Figure 3 shows the clustering of sensitivities corresponding to p53 period, for all 100 parameter sets found around the base parameter set “B0”. The parameter sets (horizontal axis) are divided into 10 clusters in order to find small groups of parameter sets whose sensitivities are similar. The model parameters (vertical axis) are divided into 3 clusters, corresponding to large, medium and small influence groups, respectively. Calculating a ranking of the parameters using the median values of their sensitivities, for all 100 parameter sets, it is found that g_3 (linked to Mdm2 degradation that is independent of any species in the model) presents the largest influence. However, even for this parameter g_3 , there are parameters sets (just about in the middle of the figure) that present small sensitivity. For

the parameter a_0 (related to p53 transcription due to ATM_p), there are specific parameter sets that present the largest sensitivities. The remaining parameters are grouped into the small influence cluster. All in all, this figure shows that sensitivities ranking can vary greatly depending on the specific parameter values.

For the model herein proposed, 6 base parameter sets are shown in the ESM, Table S2. Figure 4 shows the clustering of sensitivities for total p53 ($p53 + p53_p$) period, considering all 100 parameter sets found around the base parameter set “B0”. Parameters k_{1s53} (p53 basal production) and k_{3d2} (Mdm2_{nuc} degradation due to ATM_p) present, in general, the largest influence. Parameters k_{1d2} and k_{2d2} (related to both Mdm2_{nuc} and Mdm2_{cyt} degradation that is independent of any species in the model) present only marginal influence; this stands in contrast to the results obtained for Chickarmane model, where g_3 has the largest median value of the sensitivities. In addition, if we compare Figs. 3 and 4, it is possible to observe that the latter presents larger variability in the sensitivities from one parameter set to another.

Similarly to p53 period, we have calculated global parametric sensitivities for the remaining features, i.e. Mdm2 period, p53 and Mdm2 extrema, and delay, for all 6 base parameter sets “B0–B5”. These results are explored further below.

Figure 5 shows the sensitivity median of the p53 and Mdm2 extrema (Ep, Em), periods (Pp, Pm), and delays (D), for the two models under study. This figure takes into account the responses of all 100 parameter sets randomly generated from each of the six base parameter sets “B0–

Fig. 4 Sensitivities for p53 period in the model herein proposed. All 100 parameter sets around the base parameter set “B0” are shown

B5". Only parameters with the largest sensitivities within the parameter set and whose sensitivity values added correspond to 75 % of the total sensitivity amount are shown. We observe that, even in a very wide parameter space, the sensitivities of the Chickarmane model vary to a lesser extent than the ones corresponding to the model herein proposed. In fact, considering the model herein proposed, it is possible to find parameters that have the largest influence for a particular base parameter set, but have negligible influence for another. We can see that any change in the values of the kinetic parameters will have more impact in the model herein proposed—hence, making it more difficult to determine which parameters will be the most influential on any setting.

Taking a closer look at Fig. 5 the following can be observed. First, there are some “missing parameters”. For the Chickarmane model, Fig. 5a–d, we observe that, due to very low sensitivities, some parameters are missing, such as, p53 degradation independent of Mdm2 (g_2) and Mdm2 basal transcription (a_2). Alternatively, considering the model herein proposed, Fig. 5e–h, the parameters corresponding to the two processes mentioned above (g_{in} and g_{ac} , and k_{1s2} , respectively), when present, show sizeable sensitivities in only one base parameter set. Second, both models coincide in presenting large sensitivities for p53 degradation due to Mdm2 (g_1 , g_{in1} and g_{ac1}), on the extrema of Mdm2 and the delay; and also for the transcription of Mdm2 due to p53 (a_3 and k_{2s2}), on the delay. Third, most of the parameters present in the model herein

proposed, and not present on the Chickarmane model, do not have particularly noticeable sensitivities; with the notable exception of both the parameter related to the Mdm2 entrance to the nucleus (k_i) and the parameter corresponding to Mdm2 degradation due to ATMP (k_{3d2}), which have a high influence on the periods (k_i and k_{3d2}) and delay (k_i). Fourth, for both models, there is an evident level of sensitivity of extrema, periods and delay on variations of the parameter associated to p53 positive feedback (a_1 and k_{2s53}), for some of the base parameters considered. This result reinforces what was found previously by Tsai et al. (2008), who demonstrated that positive feedbacks were related to an increased robustness and tunability of biomolecular oscillators.

In order to complement the understanding of the effect of a wide range parameter variations, we explored the influence of the network architecture by comparing the ranking correlation medians of both Chickarmane model and the model herein proposed. If the difference between the correlation medians is always either positive, negative or zero, then the sensitivity rankings are more dependent on the network architecture. If, on the other hand, this difference can at times be positive, negative or zero, then the parameter values present noticeable influence on the sensitivity rankings.

Figure 6 shows any two comparisons of Chickarmane model and the model herein proposed obtained by considering only parameters accounting for the top 75 % of the sensitivities. In Fig. 6a, for the two base parameter sets

Fig. 5 Sensitivity medians for p53 and Mdm2 extrema (Ep, Em), periods (T), and delays (D), considering all 6 base parameter sets. For each base parameter set only parameters accounting for 75 % of the influence are shown: **a–d** Chickarmane model and **e, f** model herein proposed

Fig. 6 Differences between correlation medians of sensitivity rankings for the two models under study. Only parameters accounting for at least 75 % of the total influence are considered. **a** Difference between base parameter set “B0” of Chickarmane model and base parameter set “B1” of model herein proposed. **b** Difference between base parameter set “B2” of Chickarmane model and base parameter set “B5” of model herein proposed

considered, the differences between the ranking correlation medians are positive, for all extrema, periods and delay. In Fig. 6b, we observe that, for some properties, such as, periods and delay, these differences between the ranking correlation medians can be zero or negative. The whole two by two comparisons are shown in ESM, Figs. S2, S3, which correspond to parameters accounting for 75 and 100 % of the total influence, respectively. In general, it is observed that these differences are going to be positive with a few exceptions, specially for the cases where we consider all parameters in the calculations (100 % influence).

Discussion

Networks that show oscillatory behavior are present at all organizational levels in nature. Among them, biochemical oscillators are found performing vital tasks at the cellular level. In order to understand the occurrence and properties of these networks, a great deal of theoretical and experimental work has been performed, resulting in the discovery of some design principles that underline the importance of the network’s architecture. However, it is still little known how these oscillations are affected when the kinetic parameters are constantly changing or are not precisely known, as often occurs in cellular environments.

When performing modelling tasks we first need to decide on the level of detail we want. It is generally accepted that more complex models can provide thorough descriptions, but the effort needed to build them is larger. On the other side, simple models require fewer parameters, but if properly chosen, they can provide great insights, albeit at the expense of detail. Then, what to do when we deal with systems, such as biomolecular networks, where the model parameters are difficult to determine and/or

present great variability? Will simple models still present a reasonable description of the main features of the systems response?

We have studied two models of the p53 core network in order to characterize the influence of their parameters on their dynamical response. The simple model (Chickarmane model) has two species and 10 parameters, and the more complex one (model herein proposed), four species and 22 parameters. Global parametric sensitivities of extrema, periods and delay were calculated for both models; for each case, 600 parameter sets were identified in the range of 0.001–1000 times the nominal parameter set “B0”.

For all the parameter sets, and in both models, the parameter related to p53 self-promotion (a_1 in Chickarmane model and $k_{2,p53}$ in the model herein proposed) is the only one that presents sizeable sensitivities on extrema, periods and delay. These results add to previous findings describing the self-promotion motif as a fundamental component that increases the robustness of oscillatory systems. For other parameters, the dependency was less clear; for the model herein proposed, the parameters corresponding to the largest sensitivities presented greater variability, while for the Chickarmane model, the sensitivities in general did not present much variability.

Finally, the effect of the parameter values on the ranking of the most sensitive parameters is compared for the two models. In general, the medians of the ranking correlations for the Chickarmane model will be larger than that of the model herein proposed. However, for specific combinations of base parameter sets, the difference will tend to be zero or negative. In other words, the ranking of the most influential parameters was more dependent on the specific values of the parameters for the more complex model, or, varying the parameter set values to change the dynamical characteristics of the response is more restricted in the simple model.

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