

Structurally conserved robustness and fragility in oscillatory genetic circuits

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1. INTRODUCTION

Robustness can be understood as a system's ability to perform its specific function even when subjected to external (i.e. via adaptation) or internal (e.g. changes in kinetic parameters) perturbations. With robustness being a key property of biological systems, its analysis has become an important issue in systems biology [5]. Regulation, especially in complex systems, however, always implies a trade-off between robustness and fragility [2]. Understanding the design principles of cellular regulatory networks, which evolved to (optimally) cope with this constraint, therefore represents a major, but largely unresolved challenge.

Genetic oscillators, in particular those generating circadian rhythms, have emerged as model systems for studying these questions experimentally and theoretically. The evolutionarily conserved period generator displays a complex architecture. In *Drosophila*, the regulatory network consists of two negative autoregulatory feedback loops controlling the expression of timeless (*tim*) and period (*per*) interlocked with a positive feedback loop established via the *dClock* gene. Complex formation, regulated translocation and degradation of several of these gene products, which is additionally controlled (and delayed) by protein phosphorylation, add further levels of complexity to the system [8].

Model-based analyses of this network pointed out its remarkable robustness in the presence of molecular noise [1, 11, 4] and with respect to parametric perturbances [9, 7]. Different models display model-specific robustness and fragility properties [12]. A systematic investigation of the relationship between parameter sensitivity, model structure and behaviour, however, is lacking. Employing tools from systems engineering, we performed a comparative analysis of global robustness and fragility properties of two published mathematical models for circadian clocks, which shows a high degree of conservation of robustness and fragility. Biological implications of these findings will be discussed.

2. SYSTEMS ANALYSIS

We analyzed two models for genetic control networks underlying circadian rhythms in *Drosophila*. Both models rely upon negative autoregulatory feedback for generating the oscillations. A less complex 5-state model with only one branch [3] and a 10-state model including two distinct

branches of the control system for *per* and *tim* [7] were considered. To gain insight into the structure-function relationship, we studied the deterministic models' robustness towards internal perturbations (as opposed to assessing the effect of stochastic noise) by numerical computation of the parameter sensitivities $\frac{\partial \mathbf{x}}{\partial \mathbf{p}}$. Subsequently, we determined the Fisher information matrix $\mathbf{F}(\mathbf{p}^*)$

$$\mathbf{F}(\mathbf{p}^*) = \sum_{i=1}^N \left[\left. \frac{\partial \mathbf{x}}{\partial \mathbf{p}} \right|_{\mathbf{p}^*, t_i}^T \mathbf{C}^{-1}(t_i) \left. \frac{\partial \mathbf{x}}{\partial \mathbf{p}} \right|_{\mathbf{p}^*, t_i} \right] \quad (1)$$

with the covariance matrix $\mathbf{C}(t_i)$ derived from the trajectories by assuming fixed absolute and relative errors. Through eigenvalue analysis of $\mathbf{F}(\mathbf{p}^*)$, a lower bound σ_j for the accuracy of parameter j was obtained as an indicator of its sensitivity towards perturbations [10].

For the 10-state model, Fig. 1 (upper panel) shows the accuracy of the first model parameter (v_{sP}) specifying *per* gene expression in a two-dimensional plane of the 38-dimensional parameter space. Here, $\sigma_{v_{sP}}$ is plotted as a function of v_{mT} and v_{dT} , which affect *tim* mRNA and protein degradation, respectively. In this parameter plane, different behavioural modes occur, including stable steady state, simple periodic oscillations, birhythmicity and chaos [7]. As would be expected for such a situation, the sensitivity of the system towards changes in v_{sP} varies over several orders of magnitude. Although to a lesser extent, variability in absolute sensitivity is found for all model parameters (Fig. 1, lower panel). However, when the parameters are rank-ordered with respect to σ , the relative sensitivities for the fragile parts of the model remain largely unaffected by the system's operating regime. We performed two-dimensional sensitivity analyses for all possible parameter combinations in the 5-state model. The rank-ordering of the parameters was consistent with conservation of robust and fragile model parts, irrespective of the operating regime and the parameter combinations considered. In particular, highly sensitive parameters described globally controlled processes like general transcription, translation or proteolysis, whereas local processes (such as *per* and *tim* phosphorylation) showed higher robustness. Interpreted in biological terms, these results are suggestive of a design principle of cellular regulation, in which robustness of specific (local) functions is achieved by delegation of fragilities to global control circuits.

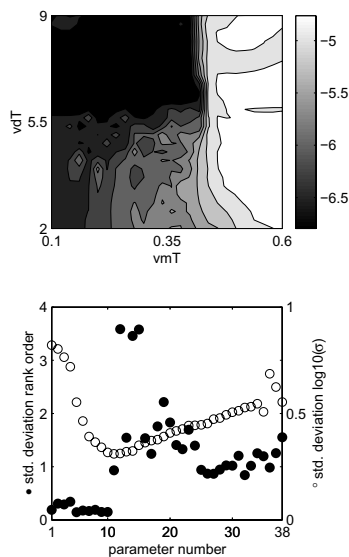


Figure 1: Two-dimensional sensitivity analysis. Log_{10} of σ_1 (top) and the standard deviation of accuracy (\circ) and rank order (\bullet) for all model parameters rank-ordered according to their accuracy (bottom).

Next, we employed singular value decomposition (SVD) of the (scaled) sensitivity matrix to assess the effects of multi-parametric perturbances. In general, the lead singular value dominates the next largest by one to two orders of magnitude, such that the lead vector direction can be used to identify the weakest directions for systematic perturbances. For the 10-state model, analysis of this vector indicated, again, the importance of the aforementioned 'global' parameters for the fragility of the local gene regulatory network underlying the circadian clock. The most sensitive direction of parametric disturbances implied breaking the symmetry between the two feedback loops described by the model, as already suggested by [4, 11]. More specifically, the most efficient perturbation could be achieved by simultaneously acting on local control of phosphorylation, and global control. However, as divergence in the rate of global processes such as general transcription is unlikely to occur, the hierarchical architecture of the regulatory network could contribute to enhance robustness of circadian rhythms.

We have begun a third analysis that directly addresses the sensitivity of the oscillation period to parametric disturbances. Using the method of Larter *et al.* [6], we observed that the elements of the v_1 vector from the SVD of the sensitivity matrix for the 5-state model are approximately scalar multiples of the period sensitivities. Thus the SVD of the sensitivity matrix for periodic systems may be a convenient way to estimate the relative ordering of period sensitivities. We observed that this ordering can depend on the operating regime of this system. For example, the period was highly sensitive to the maximum transcription rate for one parameter set [4], while it was robust to local variation in this parameter for another [3], even though the period for both parameter sets was about equal. The biological implications of this, and whether similar observations will be made for more complex models, are currently being investigated.

3. CONCLUSIONS

Systematic analysis of parametric sensitivity of two models for the circadian clock in *Drosophila* revealed that robustness and fragility of the network are largely determined by its structure, not by the actual operational regime. In particular, the ordered sensitivity of model parameters reveals that 'global' parameters are the most important, remarkably conserved points of fragility. Singular value decomposition additionally identifies the most influential direction of multi-parametric perturbances, which results in symmetry-breaking. Based on these results, we suggest a design principle of cellular regulation, namely to 'export' a specialized control circuit's points of fragility to global, well-controlled regulatory systems like general transcription. In future studies, it will be intriguing to test the method presented herein using more complex models of oscillatory genetic circuits, for instance involving (interlocked) positive and negative feedback loops [11, 9]. Including other regulatory networks could also serve to gain deeper insight in the relations between (hierarchical) control structures of cellular regulation and the key properties of robustness and fragility.

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