Transcriptional feedbacks in mammalian signal transduction pathways facilitate rapid and reliable protein induction

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Signal transduction pathways translate the cellular context into context-dependent expression of genes. In response to extracellular stimuli, proteins have to be up-regulated quickly and reliably. However, rapid and reliable control of target genes by a signalling pathway faces two major challenges. Firstly, swift changes in protein levels require short-lived proteins, which is not resource-optimal. Secondly, gene expression is an intrinsically noisy process, and fluctuations in the protein numbers are likely to reduce the functionality of the proteins. Mammalian signalling pathways frequently induce the transcription of their own inhibitors, resulting in negative feedback regulation. However, the functional role of these transcriptional feedbacks in mammalian signal transduction is unclear. Here, we analyse a mathematical model of a prototypical signalling pathway, the MAPK cascade, in order to investigate how transcriptional negative feedbacks may help to overcome the challenge of fast and reliable gene induction. It is shown that a transcriptional negative feedback helps to decouple protein stability and response times, thus allowing for swift up-regulation even of long-lived proteins. Furthermore, transcriptional negative feedbacks filter out the extrinsic component of gene expression noise, which dominates the uncertainty in gene expression in mammalian cells, thus making gene expression more reliable. Model analysis predicts that both goals can be achieved if (i) proteins and mRNAs of the feedback regulators and (ii) mRNAs of all targets are short-lived. These predictions are confirmed by large-scale measurements of mRNA and protein half-lives. Therefore, the design of the mammalian signal transduction network with its rapid feedback inhibition allows for swift and reliable target gene expression.

Introduction

The mammalian intracellular signalling network controls the expression of genes depending on the extra- or intra-cellular presence of signals such as hormones. The products of these target genes execute the appropriate genetic program in response to the signals and shift the cell from one state to another. In response to many cellular stimuli, it is essential that the cell shifts quickly from one state to the next. For example, it is important that cells in close proximity to a wound proliferate and migrate quickly after wounding, thus proteins involved in growth and migration have to be up-regulated quickly upon stimulation.

Additionally, it is important that up-regulation works reliably, *i.e.* that the protein levels reach a specific concentration. If expression levels of some targets are too low, the processes will not be carried out, and the cell is left in some intermediate state. At the same time proteins should not be over-expressed, since protein function is mainly controlled through specific binding to other proteins and, when over-expressed, specificity of binding is reduced and proteins often start to malfunction.[1] Thus it is essential that signalling pathways up-regulate the target proteins quickly *and* reliably.

However, quick and reliable induction is complicated by two reasons: Firstly, it has been demonstrated that changes in the transcription rate are transmitted to the steady-state protein level on a timescale that is determined by the decay rates of the target's mRNA and protein.[2] In particular, the slowest of these two decay rates, typically the protein decay rate, dominates the timescale on which gene expression changes are transmitted to the protein level.[3] It requires therefore more than 2 protein half-lives after induction until a protein reaches 80% of its steady-state expression level. Thus, mRNAs and proteins need to be short-lived in order to respond quickly. However, it may often be beneficial if target proteins are long lived. Protein expression is one of the central energy sinks in mammalian cells, requiring about 30–60% of cellular energy resources,[4] with short-lived proteins contributing a major part to the turn-over.[5] Significantly expressed proteins are therefore under evolutionary pressure to be long-lived as the expression of long-lived proteins requires less resources at the same steady-state expression level. Therefore, it seems beneficial that cells evolve regulatory systems that decouple decay-rates from the regulation time. Secondly, a major challenge for reliable target gene induction is noise in the expression of target genes. It has been estimated that the standard deviation of protein expression in clonal

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mammalian cells is about 30–40% of the mean expression level.[6] It is useful to conceptually divide the origin of the noise as follows:[7] Firstly, noise in protein levels can originate from the stochastic nature of processes like transcription initiation, transcription and translation. Such noise has been termed intrinsic noise. Secondly, so-called extrinsic noise can arise from global fluctuations in levels of polymerases, ribosomes, transcription factors, signalling molecules, or other determinants of the cellular state. It is characteristic of extrinsic noise that it influences genes regulated by the same pathway in a similar way, giving rise to correlated fluctuations.[8] Unlike in bacteria, extrinsic noise seems to account for the major part of protein expression noise in mammalian cells,[6] despite much uncorrelated fluctuations on the mRNA level.[9] Consequently, mammalian cells need to overcome extrinsic, correlated fluctuations for precise and robust regulation of target genes. While some insights have been gained into how bacterial signalling networks cope with noise,[10] little is known about which strategies the mammalian signalling network employs to overcome noise.

For bacterial gene regulatory networks, it has been demonstrated that a gene can reach its target expression value more quickly if it is controlled by negative auto-regulation.[11] The mRNA is first induced when stimulated and causes a quick rise in protein levels. Subsequently, the mRNA level is lowered through negative auto-regulation to a level where it can maintain the steady-state. In bacterial cells such negative auto-regulation is realised through the expression of inhibitory transcription factors from the same operon.[12] Mammalian cells do not have this possibility as they do not organise their genes in operons.

Recently it has been discovered that the MAPK signalling cascade induces a phalanx of negative feedback regulators among its target genes.[13] We reviewed many high-throughput data sets and found that this behavior is not limited to MAPK signalling but is a re-occurring pattern in all major signalling pathways:[3] All major signalling pathways, including PI3K-mediated, TGF-β-induced, JAK/STAT signalling,[3] as well as NFκB,[14] WNT[15] and p53 signalling[16] induce inhibitors that down-regulate the pathway's activity such as specific phosphatases and stoichiometric inhibitors. These pathways also have in common that the induced inhibitors are mediated by short-lived mRNA and are short-lived proteins.[3] However, the functional role of these feedbacks remains unclear. Here we explore by means of mathematical modelling whether the re-occurring design of transcriptional negative feedback regulation helps to overcome the problem of swift, reliable regulation of target genes. For illustration we will use models of MAPK signalling and feedback regulation *via* a dual-specificity phosphatase (DUSP). The results presented are however generic.

Results

The speed of target gene induction

If a gene is regulated at the transcriptional level, *i.e.* if its transcription rate is increased or decreased, the protein level $P(t)$ follows the increase or decrease with a delay. Starting from the previous expression level P_0, it approaches the new steady-state expression level P_1 on a time-scale that is fully determined by the decay rates of the mRNA and protein (denoted by d_1 and d_2, respectively):

$$P(t) = P_1\left(1 - \frac{P_1 - P_0}{P_1}\,\frac{d_1 e^{-d_2 t} - d_2 e^{-d_1 t}}{d_1 - d_2}\right) \qquad (1)$$

This equation shows that the decay rate of the more stable component determines the time-scale on which a protein can be regulated by changing the expression rate.[2] mRNA and Protein half-lives ($t_{1/2}$) are inversely related to the decay rates ($t_{1/2} = \log(2)/d$). In most cases, the mRNAs are less stable than their final protein product. In that case, the half-live of the protein dominates the time-scale on which a protein is upregulated, and it takes *e.g.* more than 2 protein half-lives after induction until the protein reaches 80% of its final steady-state expression level. Therefore, swift regulation of long-lived proteins requires other mechanisms than simply changing the rate of the production of the gene.

Negative feedback can decouple protein life-time and regulatory time-scale

Major mammalian signalling pathways are controlled by negative transcriptional feedback,[3] similarly to bacterial systems where many transcription factors are negatively auto-regulated. Previous reports show that in bacterial systems negative auto-regulation of transcription factors reduce the time on which the transcription factors approach a new steady-state.[11] Inspired by these results, we reasoned that negative feedback regulation of signalling could potentially help to solve the problem of swift regulation. Therefore, we set up a mathematical model of transcriptional negative feedback regulation (depicted in Fig. 1A, equations see Experimental). Since exact parameter values for the system are not known, we use a Monte-Carlo approach to explore the model's behaviour exhaustively for different parameter settings. The decay rate of the target protein (d_4) was set to a fixed value of 0.2 h^{-1} to represent a typical human protein (with medium live-time of approx. 3.5 h). Thus, without any feedback, the target protein level would reach 80% of the steady-state after stimulation within about 7 h (or longer, if the mRNAs are stable, see Eqn. (1)). All other rate constants within the model were then drawn randomly from a log-normal distribution with a standard-deviation of two orders of magnitude. The distributions were centred at 0.2 h^{-1} for the decay rates, at 1 for the half-maximal inhibition constant (K_M), and at 5 h^{-1} for the transcription and translation rates, such that the steady-state of the protein in a model with average parameter set (without feedback) would be 1, and therefore in the rage of the half-maximal inhibition. One million such random parameter sets were drawn, and the model behaviour was simulated after stimulation. A surprising observation was that the typical behaviour of the model was not swift response, but rather a response time similar to feedback-less systems: For more than 80% of these random parameter sets, the model showed no speed up of the time it requires to up-regulate the target protein (for representative time courses, see Fig. 1B).

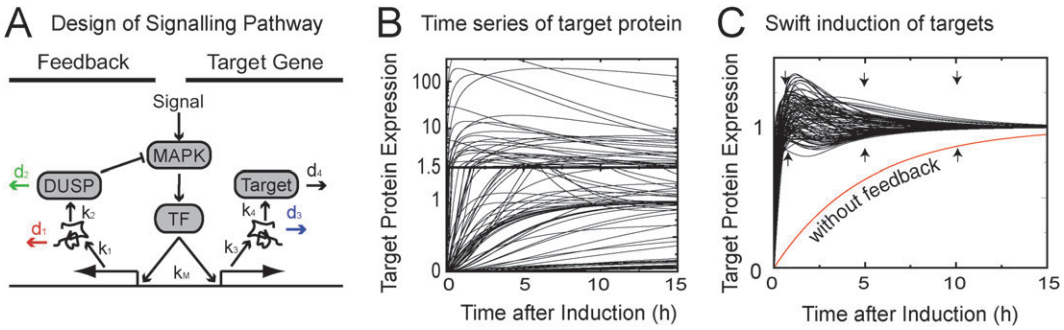

Fig. 1 Swift response due to transcriptional negative feedback. (A) The scheme shows the structure of the analysed model. A signal activates the MAPK signalling pathway, which in turn activates a transcription factor (TF). The transcription factor regulates the expression of the mRNA of DUSP and another target gene. Production rate constants are denoted by k's, degradation rate constants by d's. In the mathematical description, the post-translational modifications are assumed to be fast compared to transcription and translation. Therefore, they are assumed to be in a quasi steady-state, and are lumped together in one formula describing the activity of the transcription factor. (B) Typical, normalised time courses of target protein expression in 100 simulations of the model with random parameter sets. Most parameter sets yield a response time which is similar to a feedback-less system. Note that the *y*-axis changes from linear to logarithmic at $y = 1.5$. (C) Time courses of 100 simulations of the model with parameter sets yielding swift regulation (arrows mark the regions for selection). For comparison, upregulation without feedback is shown.

We therefore inspected the behaviour of the model more systematically, and classified the behaviour of the model for each parameter set by whether they respond much more quickly to a stimulus than the life-time of the target protein. We decided to classify a model as swift responder whenever the expression of the target protein approaches its steady-state (±20%) within 1 h, *e.g.* is up-regulated more than 7 times faster than a gene without feedback (see Eqn. (1)). For about 2% of the parameter sets the model showed such quick up-regulation of the target protein. For another 16% of the parameters, the target protein overshoots the new steady-state by more than 20% at 1 h after stimulation. Since overshooting of protein-levels, *i.e.* temporary over-expression of a target protein, can cause malfunctioning,[1] we disregarded these model parameter sets.

We inspected the time series of the model for the 2% of parameter sets that were swift responders, and saw that also in this set in most cases the target protein overshoots the steady state drastically after 1 h and only slowly approaches the steady-state afterwards. In order to filter out the parameter sets where the target protein overshoots drastically, we additionally set the requirement that target protein expression stays within the range of ±20% around the steady state at 5 h and 10 h post stimulation. Only a very small fraction (0.17%) of the models were finally selected that showed this desired swift response, showing that increased speed of target gene regulation is not a generic property of transcriptional negative feedback regulation but requires tuned rate constants — a clear difference to auto-regulatory loops in bacteria, where swift regulation was shown not to depend strongly on the parameter choice.[11] Typical time series of protein expression in such swiftly responding models are shown in Fig. 1C, together with a time-series of proteins expressed without feedback. The protein levels rise sharply, and even overshoot the desired steady-state levels slightly.

Since many signal transduction pathways as well as promoters react non-linearly towards the stimulation and inhibition, we thought that including a Hill-function at the level of transcription regulation increases the fraction of swiftly regulating networks. However, for a Hill-function with coefficient of 2, 3 and 4, only a slightly increased fraction responds swiftly (0.22% for all three settings).

Design requirements for fast induction

To understand what requirements have to be fulfilled in the model to generate the desired swift response, we inspected the parameter sets for which the regulation of the target was fast (displayed in Fig. 2A). We saw that the feedback typically follows a specific design. Firstly, the feedback needs to be strong which manifests itself in strong transcription and translation rates (both higher than random, *i.e.* $k_1 > 5\ h^{-1}$ and $k_2 > 5\ h^{-1}$, respectively) as well as high sensitivity of the feedback ($k_M < 1$). In contrast, the transcription and translation rate constants of the target (k_3 and k_4) are not significantly different from random in the swift responders (see Fig. 1A left panel). Secondly, the mRNA and protein involved in the feedback are required to have short half-lives, *i.e.* their decay rates are faster than that of the target protein ($d_1 > 0.2\ h^{-1}$ and $d_2 > 0.2\ h^{-1}$, see Fig. 1A right panel). The strongest constraint seemed to be that the target mRNA needs to be rapidly degraded ($d_3 \gg 0.2\ h^{-1}$, Fig. 1A right panel).

It is currently too difficult to investigate experimentally whether the feedbacks are very strong as suggested by the model. However, the decay rates of mRNAs and proteins have been measured on larger scales[17–19] and it can be directly compared with the model simulations, which predicted that the decay rates are biased towards faster decay. Thus, in order to determine whether the decay rates in mammalian signalling network do indeed follow the proposed design pattern we tested whether (i) the decay rates of feedback mRNA and proteins are unusually high, and (ii) whether the target mRNAs have a high decay rate. Data on half-lives of mRNA and protein decay rates of signalling proteins have been collected previously.[3] Additionally we collected mRNA-decay rates for target genes of MAPK signalling.[20] These data is summarised in Fig. 2B. It shows that indeed mRNAs and proteins involved in feedback regulation

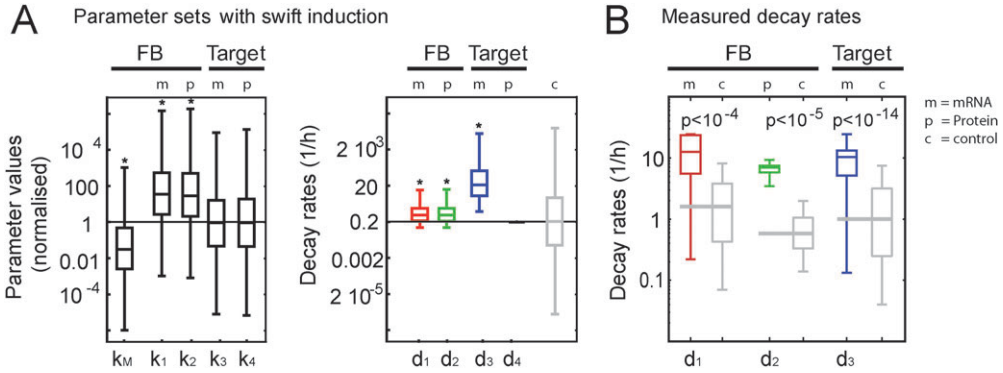

Fig. 2 Parameters of swift responders compared to experimental data sets. (A) Distribution of parameter sets for which the model exhibits swift target induction. Distributions of inhibition constant K_M and transcription and translation rates are shown on the left hand side, normalised by the average of random parameters. FB indicates feedback, * indicates significant deviation from random ($p < 0.01$, Wilcox rank sum test). Decay rates are shown on the right hand side. Average random parameters are shown as horizontal bar. Within the feedback, all parameters deviate significantly from random parameters. (B) Measured decay rates of feedback mRNAs and proteins, and decay rates of MAPK target mRNAs, in comparison to mRNA and protein decay rates for signalling molecules and mRNA decay rates of all non MAPK-targets (denoted by c).

(marked with m and p, respectively) have decay rates about one order of magnitude larger than other unregulated signalling molecules (marked with c). Additionally, the target genes of MAPK signalling are characterised by mRNAs with significantly larger decay rates than unregulated genes. Thus, the decay rates of the mammalian signalling pathways and their target genes follow the design required for swift target gene induction.

Functioning of the negative feedback

How does the negative feedback decouple the half-live of the target protein from the response time? Inspecting the time-series of the simulations shows that the negative feedback acts the following way (representative time-series shown in Fig. 3). After stimulation, the transcription factors become active and expression of the feedback (DUSP) and target are induced. Subsequently, the feedback deactivates the signalling pathway and thereby the transcription factor. Overall, the transcription factor shows a pulse-like activity after the onset of the stimulation, which results in a brief production of the target mRNA, which in turn leads to a strong production of the target protein in the beginning. Once the pulse is over, the mRNA level of the target drops strongly, resulting in a lower production of the protein — just enough to maintain the steady-state. From this interpretation one can also understand why the feedback players have to decay quickly: They have to rise and approach the new steady-state rapidly, in order to lower the transcription factor activity to the new steady state swiftly. Also, the target mRNA has to be short lived since otherwise the mRNA level would not fall quickly enough once the transcription factor activity is reduced, but will remain high and the protein level will increase over a longer period.

Transcriptional feedback rather than post-translational feedback can filter out the extrinsic component of gene expression noise

One could argue that pulse-like activation of the transcription factor could be also achieved by negative feedback regulation on the post-translational level.[21] For example, receptors could

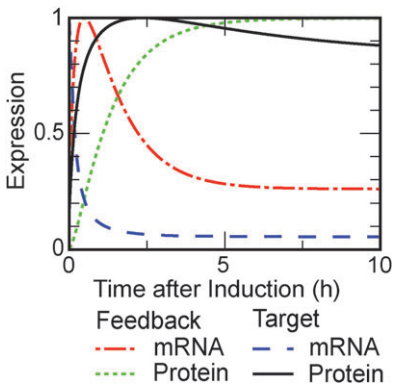

Fig. 3 Representative time course of a swift responder. Time courses of feedback mRNA, protein and target mRNA and protein for a representative parameter set which was selected for swift response.

be internalised or deactivated by phosphorylation of the terminal kinase, which in turn might deactivate the transcription factor. And indeed many post-translational feedbacks — most of them negative — exist in mammalian signalling, such as feedbacks from ERK to Raf, Src and SOS in MAPK signalling.[17]

However, fluctuations in gene expression rates are one of the major challenges for reliable control of target gene expression.[22] We reasoned that the transcriptional negative feedback might help to reduce the noise in target gene expression. In order to analyse whether the observed transcriptional feedback design investigated within the first part of this paper indeed reduces noise, we set out to analyse how the model performs when gene-expression noise is considered. We therefore reformulated the deterministic model used in the previous section to allow for stochastic simulations. To realistically model gene expression noise, we had to include both intrinsic and extrinsic noise in the model. Intrinsic noise was added to the model by formulating the model in terms of the chemical master equation and simulating it using the Gillespie algorithm. Extrinsic noise (*e.g.* upstream noise and noise coming from polymerase fluctuations) was

added by multiplying the translation rates with a random variable from an Ornstein-Uhlenbeck process and drawing the transcription factor concentration from a log-normal distribution. In our analysis, noise is measured as noise strength η defined as standard-deviation of the target protein level σ_{TP} normalised by the mean protein expression value T_P ($\eta = \sigma_{TP}/\langle T_P \rangle$). The parameters for these stochastic models were chosen such that they on average resemble the noise distributions recently measured for mammalian cells (total noise of $\eta_{tot} = 0.31$, with a dominating extrinsic component ($\eta_{ext} = 0.28$) and a smaller intrinsic component $\eta_{int} = 0.14$, see ref. 6). Parameters for the Ornstein-Uhlenbeck process were derived from Rausenberger and colleagues.[23]

For 100 randomly selected parameter sets that showed swift target induction in the deterministic model we then simulated the induction for four hours. Apart from the full model of transcriptional feedback regulation (Fig. 4A) we simulated two further scenarios: Firstly, post-translational feedback was mimicked by removing the extrinsic component of noise from the feedback gene. In this system, the feedback is only subject to relatively modest molecular noise and experiences no "extrinsic" fluctuations correlated with the expression of the target (Fig. 4B). Secondly, we investigated how the system would behave at another extreme, if extrinsic noise would be uncorrelated, for example if the feedback would be controlled by a different pathway or if all noise would be due to intrinsic fluctuations. (Fig. 4C). We observed that transcriptional feedback does indeed reduce noise strongly when compared to post-translational feedback (Fig. 4A and B). However, if noise was uncorrelated, the transcriptional feedback increases the noise level (Fig. 4C).

Since the noise levels differed significantly within one model class, we investigated how the total noise level depends on the intrinsic component. Therefore, we simulated the model with intrinsic noise only, and sorted the simulation according to the level of noise due to intrinsic noise (Fig. 4D). As long as the intrinsic noise is low ($\eta_{int} < 0.2$), the total noise level is relatively constant and a transcriptional feedback always reduces noise. In contrast, when intrinsic noise dominates, the noise-reduction capacity of the transcriptional feedback disappears. Taken together, the design of mammalian signal transduction pathway allows only to reduce the correlated extrinsic component of noise, while the intrinsic component is unchanged or might even be amplified.

Experimental

Deterministic model

Since the model is designed to investigate transcriptional regulation, the relevant timescale will be that of transcription and translation. Therefore, in the model it is assumed that the dynamics of signalling events such as phosphorylation and dephosphorylation are much faster than timescales of transcription and translation. Thus signalling and activation of transcription factors can be treated using a quasi steady-state assumption. The feedback which is investigated is a transcriptional feedback that targets the terminal kinase of the MAPK signalling pathway, MAPK. In the model it is assumed that once the pathway is stimulated the signal persists, and it is only deactivated through the modelled transcriptional feedback. Then, all signal processing upstream of that can be lumped into one number (called the signal S), which then corresponds to the steady-state activity of the MAPK activating kinase MEK.

Furthermore, the activation/deactivation of MAPK by MEK and DUSP, and the transcription factor activation have been lumped together in a quasi-steady state approximation. Then, the activity of the transcription factor as function of the Signal S and the feedback protein $DUSP_p$ is expressed as:

$$TF = \frac{S}{(DUSP_p/K_M)^h + 1}. \qquad (2)$$

The Hill coefficient h was chosen to be one in all simulations except where noted. Stimulation was modelled through an increase of S from 0 to 1 at time $t = 0$. For DUSP and the target T, the subscript m and p indicate mRNA and protein

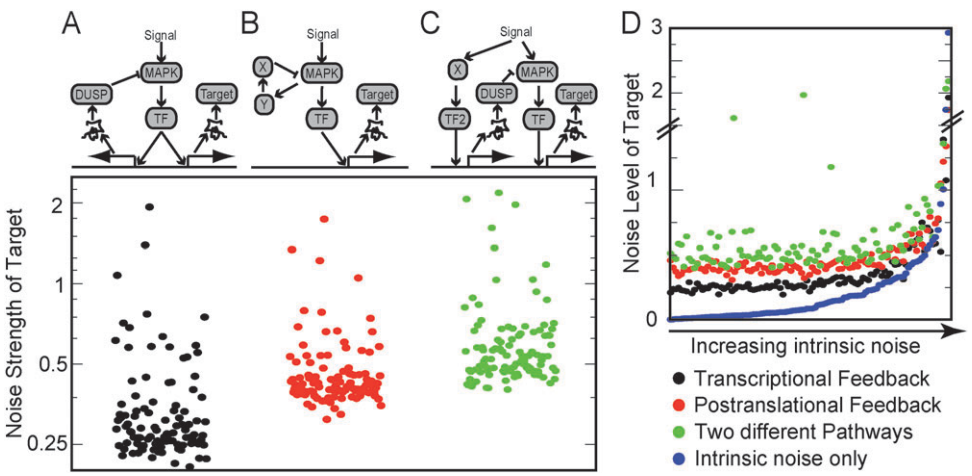

Fig. 4 Transcriptional feedback filters out extrinsic noise. The noise strength of different feedback designs are shown for 100 representative parameter sets of the stochastic model. (A) Transcriptional feedback. (B) Post-translational feedback. (C) Feedback through different pathways (uncorrelated extrinsic noise). (D) Noise strength for the 100 parameter sets sorted by the noise strength for the situation that no extrinsic noise was present.

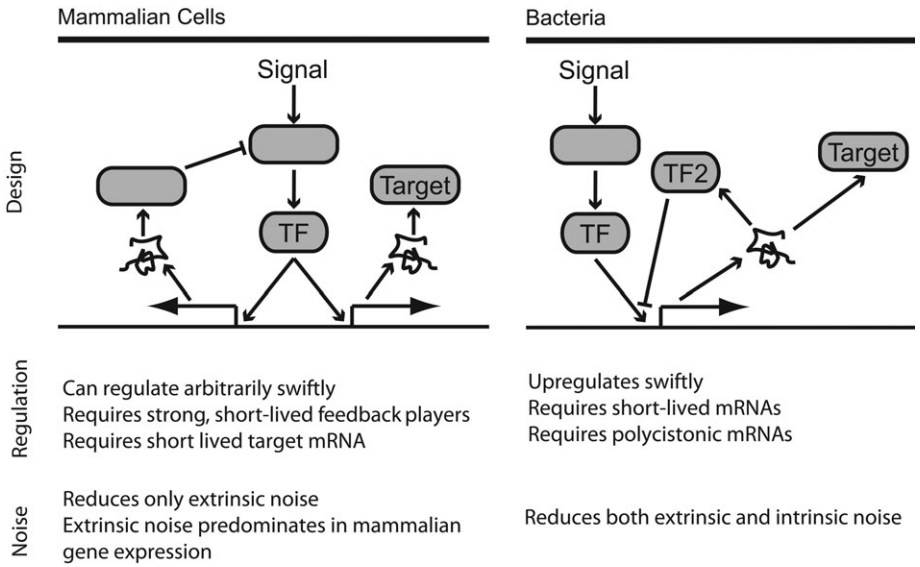

Fig. 5 Differences in the design of target gene expression in mammals and bacteria. While mammalian signalling pathways induce proteins that interfere with the signalling pathway, bacterial operons encode a repressing transcription factor. Both designs have implications for the speed of regulation and noise filtering.

species. Expression of the feedback regulator DUSP and the target gene T is modelled as follows:

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{DUSP}_m &= k_1\,\mathrm{TF} - d_1\,\mathrm{DUSP}_m\\ \frac{\mathrm{d}}{\mathrm{d}t}\mathrm{DUSP}_p &= k_2\,\mathrm{DUSP}_m - d_2\,\mathrm{DUSP}_p\\ \frac{d}{\mathrm{d}t}T_m &= k_3\,\mathrm{TF} - d_3\,T_m\\ \frac{\mathrm{d}}{\mathrm{d}t}T_p &= k_4\,T_m - d_4\,T_p p\end{aligned} \tag{3}$$

Stochastic model

Intrinsic noise was modelled by converting the differential equations above into chemical master equations. To generate reasonable numbers of mRNAs and protein, it was assumed that one unit of concentration in the deterministic model equates to 10,000 molecules. The resulting model was simulated with the Gillespie algorithm, where the propensities were drawn according to the reaction-rates.[24]

Additionally, extrinsic noise was added at two points in the model. First, noise in the transcription factor pathway was added by multiplying TF with a log-normal distribution with unity mean and 0.3 standard-deviation. Second, other sources of extrinsic noise were added by multiplying the translation rate constants k_2 and k_4 with an extrinsic factor $E(t)$, which varied time-dependent using an Ornstein-Uhlenbeck process. $E(0)$ is drawn from a Gaussian distribution with mean $\mu_E = 1$ and variance σ_E. For each time-step dt in the Gillespie algorithm, $E(t)$ is calculated by:[25]

$$\begin{aligned}E(t) &= E(t-\mathrm{d}t)e^{-\gamma \mathrm{d}t} + \mu_E(1-e^{-\gamma \mathrm{d}t})\\ &+ \sqrt{D\frac{(1-e^{-\gamma \mathrm{d}t})}{2\gamma}}\eta\end{aligned} \tag{4}$$

with γ being the inverse of the auto-correlation time and D being a diffusion constant given by $D = 2\gamma\sigma_E$, and η being drawn from a Gaussian distribution with zero mean and unity standard deviation. Given these constants, $E(t)$ represents the extrinsic noise with standard deviation σ_E around $\mu_E = 1$ with an auto-correlation time of γ^{-1}. Coupling the Ornstein-Uhlenbeck process and the Gillespie algorithm this way is not exact, as the value of the extrinsic variable changes the propensities in the Gillespie algorithm within a time-step. However, the time steps in the Gillespie algorithm are much smaller than the auto-correlation time γ^{-1}, thus the $E(t)$ is practically constant during a time step of the Gillespie algorithm. Therefore, the error made is negligible. The parameters were chosen as $\gamma_E = 1.6\,\mathrm{d}^{-1}$, $D = 0.28\,\gamma_E$, which can be derived from Rausenberger and Kollmann assuming a cell-cycle time of 24 h.[23]

mRNA decay rates

Data for mRNA half-lives were taken from the genome-wide data-set reported by Raghavan *et al.* and by Yang *et al.*[18,19] We considered all three different treatment conditions in Raghavan *et al.* The mRNA half-lives used in the display and calculations are the median over all half-life measurements for each gene.

MAPK target genes

The MAPK target genes were taken from the table in ref. 20.

Model implementation

All models were implemented in standard C++, integration was performed using the integrator lsode written in Fortran. Matlab software was used to analyse and visualise the model results, and the statistical package R was used to analyse the micro-array and decay rate data. All computations were performed on a standard desktop Apple MacPro.

Discussion

Our simulations show that the re-occurring design of transcriptional feedback regulation allows for fast and reliable target gene induction. Negative feedback helps to decouple the life-time of the target protein and the time it requires for its up-regulation. After the signalling pathway is stimulated the transcription factor is activated rapidly, but due to the negative feedback, this activation is rather pulse-like, and also the expression of the target gene mRNA shows a pulse-like expression. Within this pulse, the target protein is translated at high rate due to the high mRNA concentration, and thereby the protein accumulates quickly. Afterwards, the feedback reduces the transcription rate and the mRNA of the target drops to a level which is required to maintain the protein steady-state.

Such pulse-like activation in MAPK signalling has been observed many times. For example, in EGF or TGF-α stimulated cells, MAPK shows transient activation, which is however likely to be caused by other, posttranslational feedbacks.[26] For these kind of adaptation-like dynamics, thorough network analyses have been performed and negative feedback was shown to be a major motif facilitating such pulse-like dynamics.[27]

By using a Monte-Carlo approach, we have shown that the transcriptional feedback has to fulfil specific requirements to cause rapid up-regulation of target proteins: The feedback needs to be strong, and it needs to be mediated by short-lived mRNAs and proteins. It has been previously observed that feedbacks in mammalian signal transduction are designed this way, however, it was unclear why.[3] Moreover we found that target genes should be encoded on short-lived mRNAs. We confirmed that indeed typical target mRNAs of the MAPK signalling pathway have remarkable short half-lives. It can be therefore concluded that mammalian signalling pathways follow the design required for swift target regulation.

The present study underlines the usefulness of Monte-Carlo methods to systematically explore the behaviour of mathematical models. Similar approaches have been used *e.g.* to calculate the control distribution in the translation pathway,[28] and to explore the stability of metabolic pathways.[29] Monte-Carlo approaches can also be extended to explore different model structures, for example to investigate the topology in signalling pathways.[30] The present analysis shows that such strategies seem particularly suited if the model shows only for a subspace of the parameter space an interesting behaviour, which can then be compared to large scale data sets, such as genome-wide measurements of mRNA decay rates.

Signalling pathways are often controlled by a combination of post-translational and transcriptional feedbacks. We reasoned that only transcriptional feedbacks can be involved in noise reduction of target gene expression. In order to investigate this, we added intrinsic and extrinsic noise at a physiological level to the feedback and target gene, and could show that only the transcriptional feedback can filter out noise. It is very interesting to see that the transcriptional feedback design in mammals cannot filter out intrinsic noise in gene expression. Thus one may interpret the transcriptional negative feedback as a noise sensor for the extrinsic component of the noise: If, for example, the translation rate is higher by chance (*e.g.* due to larger amounts of ribosomes), then the negative feedback will switch off the signal earlier, thus reducing the impact of the accelerated translation.

The presence of noise in the expression of proteins in mammalian cells and its consequences for the cellular phenotype have been recently investigated in depth for several mammalian systems, for example in Apoptosis[31] or in the control of the cytokine interleukin-4.[32] Additionally, theoretical analyses have shown other means of apparent noise reduction in gene expression, for example time-scale separation and multiple processes that have to occur in series.[33,34] Furthermore, it has been shown that positive feedbacks might be optimal for noise reduction if the signal processing pathway needs to be ultra-sensitive.[34,35] While in the present analysis it is assumed that noise in target gene expression might be deleterious, there may be many cases where noise in protein expression might be utilised to generate heterogeneous cell populations[36,37]

The results presented here show that the negative feedback regulation of gene expression in mammalian cells may account for swift, noise-resistant regulation similarly to the characteristics of bacterial negative auto-regulation of transcription factors. Such auto-regulatory systems in bacteria have been shown to reduce transcriptional noise both *in silico* and *in vivo*.[38–40] Moreover, these auto-regulatory systems show a 5-fold decrease in the time to up-regulate the transcription factor.[11]

Nevertheless, the motifs by which negative feedbacks are realised in signal processing systems in bacteria and mammals are different (Fig. 5). In bacteria, transcription factors auto-regulate the operons on which they are encoded. In mammals, a subset of the induced genes encode for proteins that interfere with the signalling pathways. While both designs do increase the speed of regulation and reduce the gene expression noise, they show strongly different characteristics. Firstly, the mammalian design allows for quicker up-regulation of target genes than the bacterial design. This is due to decoupling of mRNA production of target and feedback genes. Secondly, the mammalian design reduces only the correlated extrinsic noise in gene expression, whereas the bacterial design can reduce both sources of noise. Therefore, the different design patterns of mammalian feedback regulation and bacterial auto-regulation might have evolved to cope with similar challenges. But since genomic organisation and gene expression noise are different in these systems (*i.e.* extrinsic noise dominates in the expression of most mammalian genes), bacteria and mammals found different solutions to the problem.

It should be noticed that the design of transcriptional feedback does not allow for rapid downregulation of the target protein level. Here, other mechanisms have to be employed to quickly stop a biological process, such as marking the proteins for rapid degradation.

The design of transcriptional feedback regulation in mammalian cells might have strong consequences for the interpretation of experimental data. First, due to the negative feedback and differences in the decay rates of the target

mRNA and protein the levels of mRNAs and proteins are decoupled in time: During the first phase of the stimulation, the mRNA level reaches its maximum, while the protein level will be still low but rises. Subsequently, when then the protein is fully expressed, the mRNA level is lowered. Thus, for snapshots in time, the mRNA and protein levels will not correlate. Such lack of correlation between mRNA and protein level has been observed experimentally, but until now attributed to post-translational regulation.[41] Our results from simulating the translational feedback, however, may suggest that the lack of correlation in proteomics and transcriptomics time series may still be in agreement with transcriptional regulation. Second, the stochastic simulations of the models show that the heterogeneity in the activity of signalling pathways in clonal populations of cells might not be the cause of the noisy expression of proteins, but a consequence of the strategy to reduce the noise in target gene expression.

Conclusions

Taken together, the analysis of the transcriptional feedback design of mammalian signalling pathways shows that this design facilitates rapid and reliable changes of protein levels in response to stimuli. Thus, transcriptional feedbacks allow cells to shift from one state to another after stimulation without ending up in spurious intermediate states due to the noise in gene expression.

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