REPORT

Recurrent design patterns in the feedback regulation of the mammalian signalling network

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Biochemical networks are characterized by recurrent patterns and motifs, but the design principles underlying the dynamics of the mammalian intracellular signalling network remain unclear. We systematically analysed decay rates of 134 signalling proteins and investigated their gene expression profiles in response to stimulation to get insights into transcriptional feedback regulation. We found a clear separation of the signalling pathways into flexible and static parts: for each pathway a subgroup of unstable signal inhibitors is transcriptionally induced upon stimulation, while the other constitutively expressed signalling proteins are long-lived. Kinetic modelling suggests that this design principle allows for swift feedback regulation and establishes latency phases after signalling, and that it might be an optimal design due to a trade-off between energy efficiency and flexibility.
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Introduction

Evolution gave rise to recurring patterns and motifs in biological networks, e.g. in signal-processing networks in bacteria, in metabolic networks, in neuronal networks and in ecological food webs (Jeong *et al*, 2000; Kollmann *et al*, 2005; Alon, 2007; Bluthgen *et al*, 2007). The recognition of these patterns helps to understand network optimization principles, and to interpret the network structure. Despite its importance, no such general evolutionary footprints have been identified in mammalian intracellular signalling networks.

Many extracellular stimuli elicit cellular responses by engaging intracellular signalling pathways, which activate nuclear transcription factors within minutes. These nuclear transcription factors induce changes in target gene expression on a timescale beyond 30 min. Some of these target genes are again involved in signalling, and thus slowly feed back into the signalling network. Cellular decision-making frequently requires ongoing signalling activity over several hours before the cell decides about commitment to a certain fate (cf. Supplementary information). Experimental evidence suggests that the slow transcriptional feedbacks efficiently modulate such late-phase signalling (Amit *et al*, 2007; see also Supplementary information), and thus phenotypic responses to extracellular stimulation. Depending on the cellular context and stimulus, some signalling pathways deactivate within minutes, by fast post-translational feedback regulation. In these cases, transcriptional feedback does not affect the signalling dynamics, but instead seems to prepare the system for future stimulation events (Mettetal *et al*, 2008) and to mediate adaptation. In this study, we identify design principles in early transcriptional feedback regulation of mammalian signalling pathways.

Results and discussion

We analyse published gene expression profiles from rat, mouse, and human cell lines in response to stimulation of any of five major mammalian signalling pathways. The pathways are growth factor signalling via MAPK cascades,

growth factor signalling via PI3K, β-adrenergic signalling via cAMP, TGFβ signalling via Smads, and cytokine signalling via JAKs and STATs (see Supplementary Figures 1–5, and Materials and methods). We focus on early transcriptional feedback, and thus restrict our analysis to gene expression changes within less than 4 h after extracellular stimulation. Key regulators in transcription factor networks are characterized by unstable mRNAs (Wang and Purisima, 2005). We expected that the same might be true for feedback regulators of signalling pathways, and therefore included mRNA and protein half-life measurements in our analysis. Protein half-lives were manually collected from the published literature (see Supplementary information) and mRNA data were taken from genome-wide microarray measurements (Raghavan *et al*, 2002; Yang *et al*, 2003).

Figure 1 relates transcriptional induction in response to extracellular stimulation with half-life data, and thus summarizes the results of our analysis. We found that the expression of at least one of the mRNAs changes in each of the five pathways analysed, confirming that transcriptional feedback is a general design principle in biochemical signalling networks. In total, 15 out of 134 signal proteins were significantly upregulated throughout all analysed pathways, with repression (negative values at the horizontal axis in Figure 1) being rare. Our half-life analysis revealed that the mRNAs and proteins of transcriptionally induced signalling species were all unstable (vertical axis in Figure 1). On the other hand, mRNAs and proteins of uninduced signalling species tended to be stable.
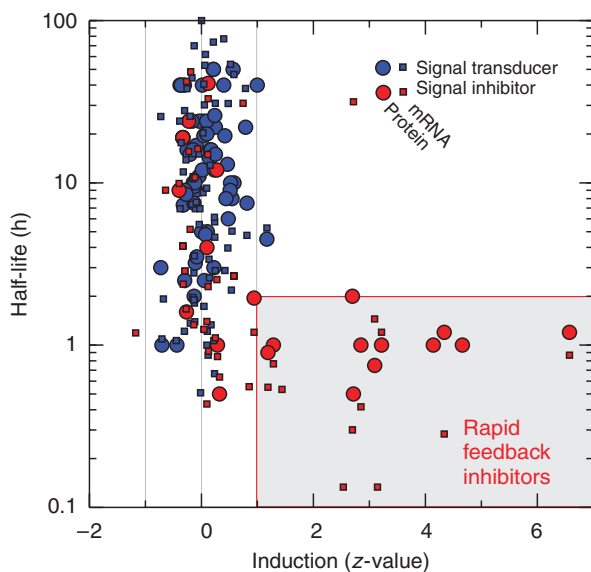

Figure 1 Correlation between half-life and degree of induction after signal activation for signal transducers and signal inhibitors of five important signal transduction networks. Shown are stimulus-induced expression changes of 134 signalling proteins divided by the standard deviation of all log2-fold change values in the corresponding array data set (z-value; horizontal dimension) in relation to the mRNA and protein half-lives, respectively (vertical dimension). Rapid feedback inhibitors (RFIs), defined as signal inhibitors whose mRNAs are induced ($z > 1$) within 4 h after signal administration (grey box), are generally characterized by mRNAs (squares) and proteins (circles) with very short half-life (< 2 h). By contrast, signal transducers (blue) and most long-lived signal inhibitors (red) are not induced within 4 h and have significantly longer half-lives than the RFIs.

We classified signalling proteins into two functional categories ('signal transducers' and 'signal inhibitors') to get further insights into the design principles of transcriptional feedback. The term signal transducer refers to proteins that are required to sense and to transmit the signal (e.g. kinases), while signal inhibitors attenuate information transfer, e.g. by catalysing kinase dephosphorylation. We found that 14 out of 51 signal inhibitors were induced in response to extracellular stimulation of their signal transduction chain (red symbols in Figure 1), while only 1 out of 83 signal transducers was marginally upregulated (blue symbols). This revealed two design principles at once: (i) transcriptional *negative* feedback is the dominant general design principle in intracellular signalling in mammals, whereas *positive* feedback seems to play no major role and (ii) the negative feedback regulation is asymmetric in these biochemical signalling networks, i.e. negative feedback does not occur by downregulation of signal transducers, but relies on the induction of a subset of signal inhibitors ($P < 0.0001$, Fisher's exact test). We call these induced proteins 'rapid feedback inhibitors' (RFIs).

The vertical dimension of Figure 1 shows that the induced RFIs have very short average mRNA (with one exception) and protein half-lives (median 0.5 and 1 h, respectively). The RFI mRNAs and proteins are significantly less stable than all other molecules analysed (both $P < 0.0005$, two-sided Mann–Whitney test). The average RFI half-lives are an order of magnitude shorter than those of signal transducers (median mRNA and protein half-life of 6 and 12 h, respectively; both $P < 0.0005$, two-sided Mann–Whitney test). The constitutively expressed, non-inducible signal inhibitors have a median mRNA and protein half-life of 2.6 and 9 h, respectively, and are thus more stable than the inducible RFIs (both $P < 0.01$, two-sided Mann–Whitney test).

We next asked whether RFIs differ from constitutively expressed signal inhibitors in terms of position or function in the mammalian signal transduction network. Figure 2 depicts the signalling pathways considered in this study, with functional groups containing RFIs are in boxes with dark grey background. MAPK phosphatases (DUSPs) are induced in response to MAPK signalling (cf. Amit *et al*, 2007). Similarly, in all other analysed pathways RFIs were induced, such as protein phosphatase 1 (PPR15A), phosphodiesterases (PDE4B/D), Smad7, SnoN, and SOCS proteins. In all pathways, RFIs attenuate signal transduction, often at multiple levels, ranging from cell surface receptors to transcription factors. Moreover, there is no common mode of inhibition, as some RFIs inhibit catalytically (PDEs, DUSPs, and PPR15A), others act by binding to their targets (Sprouty, SnoN, BAMBI, and TGIF) and yet others may combine these mechanisms (SOCS and Smad7). In terms of their mode of action, RFIs do not differ appreciably from that of constitutively expressed signal inhibitors.

Taken together, we found that early transcriptional feedback regulation in the mammalian signalling network is mediated exclusively by induction of a subgroup of signal inhibitors (RFIs). These RFIs are highly unstable in terms of mRNA and protein. This is consistent with the idea that rapid transcriptional regulation of steady-state protein expression requires short mRNA and protein half-lives (Box 1). It was surprising to see that negative feedback exclusively relies on rapid RFI induction, downregulation of signal transducers playing no role. Neither did we expect that the mRNAs and proteins of
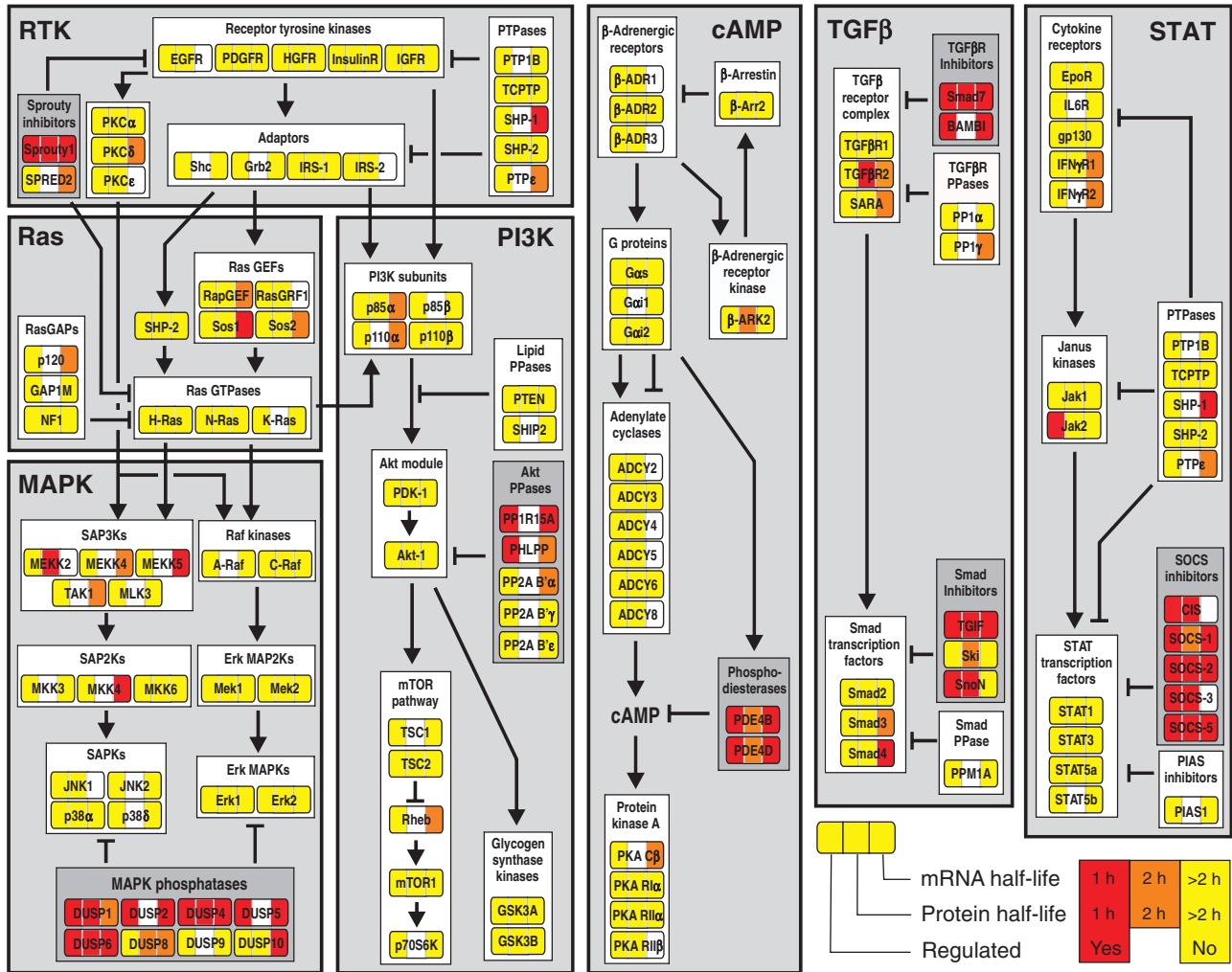
Figure 2 Induction and lifetimes of signal transduction proteins (and their mRNAs) at various positions in signal transduction networks. Signal transducers and signal inhibitors were classified into groups of functionally similar proteins (e.g. receptor tyrosine kinases). Post-translational regulatory interactions among these groups are indicated by arrows. Individual proteins are indicated by small boxes, which are divided into three parts. The left part indicates whether the factor is transcriptionally regulated upon external activation of the pathway (red), while the centre and the right parts depict the protein and mRNA half-lives, respectively (see legend). Empty parts indicate missing data. Functional groups containing rapid feedback inhibitors (RFIs) are highlighted with a dark grey background. RFIs possess short protein and mRNA half-lives and they regulate all levels of the signalling network, from receptors to terminal kinases.

constitutively expressed signalling proteins are generally stable. Thus, our analysis reveals a clear subdivision of the mammalian signalling network into two parts: (i) a constitutively expressed *static* part comprising stable signalling proteins required to receive and transmit the signal and (ii) a *flexible* part that is transcriptionally induced upon stimulation, and mediates negative feedback regulation.

The separation of the signalling network into flexible and static parts suggests that, as compared to signal transducer downregulation, RFI induction could be more effective in achieving signal attenuation. Accordingly, it has previously been shown for the MAPK cascade that phosphatases exert stronger control on signal duration than kinases (Hornberg *et al*, 2005). We therefore analysed the time courses of signal attenuation for both scenarios in more detail (Box 1), and indeed found a difference for catalytic inhibitors: the induction of a short-lived phosphatase allows for faster inhibition of the pathway when compared to the downregulation of a short-lived

kinase. Moreover, upregulation of phosphatases will result in a longer latency period, i.e. in a more prolonged attenuated state after the external activation has stopped. Such quick attenuation combined with long latency might be advantageous for the cell since it prevents repetitive activation, and allows for more efficient feedback regulation. Yet, RFI action can, in principle, be rapidly reversed (e.g. by inhibitory phosphorylation of RFIs), while recovery from transducer downregulation can only occur by slow resynthesis. Several RFIs do not inhibit their targets by a catalytic mode of action, but merely act as stoichiometric inhibitors (see above). We found that the dynamics of attenuation by upregulation of stoichiometric inhibitors does not appreciably differ from downregulation of the corresponding target. Taken together, these simulations suggest that the design pattern of negative feedback regulation by a small group of RFIs might have been evolved to speed up the adaptation upon activation, and to introduce a lag phase upon deactivation in some (but not all) signalling pathways.

An alternative but not mutually exclusive explanation for the separation of the signalling network into flexible and static parts might be improved energy efficiency. Unstable proteins and unstable mRNAs are energetically disadvantageous because they require a high production rate and therefore high ATP consumption to achieve a particular protein

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Box 1   Kinetic analysis of RFI action.

A

$$\frac{d(mRNA)}{dt} = k_1 - d_1 \cdot mRNA$$

$$\frac{d(Protein)}{dt} = k_2 \cdot mRNA - d_2 \cdot Protein$$

B
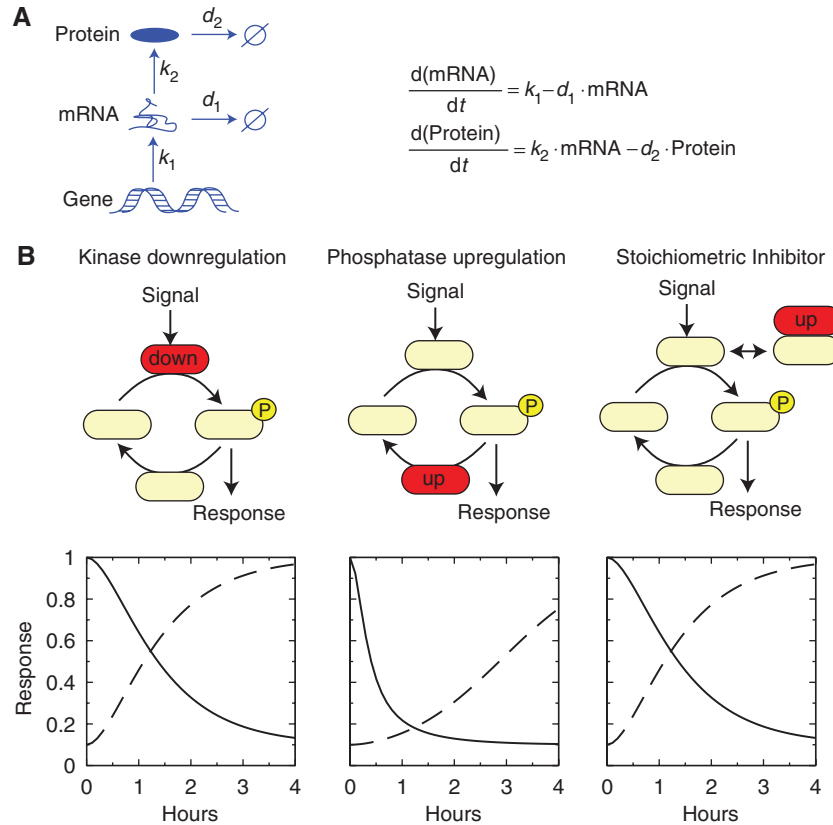
Box 1   *Dynamics of gene expression*: The expression of a gene into its protein is determined by four processes: transcription, translation, mRNA degradation, and protein degradation (schematically depicted above). As illustrated in panel **A**, the dynamics of gene expression may be described by two differential equations incorporating these four reactions. The steady-state protein concentration, P_{SS}, of a gene product is given by:

$$P_{ss} = \frac{k_1 \cdot k_2}{d_1 \cdot d_2} \tag{1}$$

If a gene is regulated at the transcriptional level (i.e. if the transcription rate is changed to k_1 at $t=0$) the time course of protein expression $P(t)$ is given by:

$$P(t) = P_{ss} \cdot \left(1 - \frac{P_{ss} - P(0)}{P_{ss}} \cdot \frac{d_1 \cdot e^{-d_2 t} - d_2 \cdot e^{-d_1 t}}{d_1 - d_2} \right) \tag{2}$$

Thus, the response time, defined as the time required to reach the new steady state, is solely determined by the decay rates. The response time depends on both, d_1 and d_2, if the protein and the mRNA half-lives are of similar magnitude, while it is mainly set by the slowest decay in case mRNA and protein stability differ significantly from each other. This implies that for transcriptional regulation, both the mRNA and the protein have to be unstable to attain a new steady state rapidly.

Unstable proteins and mRNAs need higher translation or transcription rates, respectively, to reach the same steady-state protein concentration (equation (1)). Therefore, their production consumes more free energy, as the energy expenditure is proportional to the transcription and translation rates (k_1 and k_2[mRNA]). Thus, a trade-off exists between making the protein network flexible (by increasing d_1 and d_2, and simultaneously increasing k_1 and k_2 to maintain the expression level), and making it energy efficient (by decreasing k_1 or k_2 and, to compensate, simultaneously decreasing d_1 or d_2).

Transcriptional regulation and the dynamics of signal transduction: The activation of signalling networks can be modulated by transcriptional regulation of the concentrations of their components. The time required to attain a new signalling steady state defined by transcriptional regulation of a signal inhibitor is determined by the stability of the signal inhibitor mRNA and protein (see above). The behaviour is slightly more complex if feedback is involved: a negative feedback system subjected to activation reaches a steady state faster than expected from the decay rates of the feedback regulator, while no such acceleration is observed upon deactivation (Alon, 2007). Rapid transcriptional feedback regulation of the signalling network requires that both the mRNA and the protein of the transcriptional feedback regulator need to be unstable, since otherwise: (i) feedback induction upon stimulus addition implies continuously increasing feedback strength over many hours and (ii) long latency will be observed upon stimulus removal.

Transcriptional negative regulation of the signalling network can, in principle, occur by upregulation of signal inhibitors or by downregulation of signal transducers. In the following, we compare the dynamic behaviour of a generic protein kinase cascade for three different transcriptional regulatory designs to get insights into kinetic implications of RFI action: (i) repression of a kinase acting as a signal transducer (panel **B**, left); (ii) induction of a phosphatase acting as a catalytic RFI (panel B, middle); and (iii) induction of a stoichiometric inhibitor acting as a non-catalytic RFI (panel **B**, right).

Box 1 Continued.

In a weakly activated phosphorylation/dephosphorylation cycle (modelled with linear kinetics), the amount of active phosphorylated protein at steady state is proportional to the ratio of kinase to phosphatase concentrations (Heinrich *et al*, 2002). Thus, the signal can be reduced to 10% of its original value, either by reducing kinase expression to 10% or by a 10-fold phosphatase upregulation. The figure above shows how the signal cascade activation level (i.e. the ratio of kinase and phosphatase activities) follows a slow change in kinase or phosphatase expression, if modelled according to equation (2) (with $d_1=2/h$ and $d_2=1/h$). A 10-fold phosphatase upregulation allows to switch off the signal much more quickly (middle graph, solid line) when compared to 10-fold kinase downregulation (panel **B**, left graph, solid line). We also analysed the recovery time after removal of the external activation if the kinase and phosphatase expression are regulated in the opposite direction. In this case, kinase upregulation (left graph, dashed line) allows for faster disappearance of the signalling than phosphatase downregulation (middle graph, dashed line). Thus, the signalling activity immediately follows transcriptional regulation of kinase expression (due to direct proportionality), while phosphatases regulate signalling pathways asymmetrically, with a long latency for recovery (this is due to the inverse proportionality). Similar conclusions also hold for strongly activated kinase cascades, although the difference between phosphatase and kinase regulation becomes less pronounced (not shown).

Several RFIs act as stoichiometric inhibitors, that is, they inhibit signal transduction non-catalytically by binding reversibly to their targets (as depicted schematically in the figure, right). We analysed a limiting case of stoichiometric inhibition, where the inhibitor binds to a kinase with very high affinity. Then, all available inhibitor I will be bound, unless the inhibitor is in present in excess over its target. Thus, the free, active concentration K of the targeted kinase with the total concentration K_T is given by:

$$K = \max(0, [K_T] - [I]) \tag{3}$$

The cascade activity was assumed to be proportional to the free kinase concentration K (see above), and was analysed for slow inhibitor up- and downregulation according to equation (2) (panel B, right; $d_1=2/h$ and $d_2=1/h$). The change in the signal level (again 10-fold ultimately) immediately follows alterations in inhibitor protein expression. This statement holds true for as long as the inhibitor is not induced too strongly. Otherwise, the concentration I exceeds K_T, so that the system shows some latency before it recovers. In any case, the signalling dynamics in response to inhibitor regulation do not differ from those observed upon kinase regulation (compare left and right graphs on panel B).

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expression level (Box 1). Thus, cells face a trade-off between flexibility and energy efficiency in the evolution of their regulatory networks: the nodes in the network can either be designed in a flexible, rapidly responding manner (unstable mRNAs and proteins) or such that free energy expenditure is minimal (stable mRNAs/proteins), but not both. The mammalian signalling network seems to circumvent this trade-off in an especially elegant manner; the network consumes only low amounts of free energy in the unstimulated state because constitutively expressed signal transducers required to receive the signal have stable mRNAs and proteins (static part). In contrast, the mRNAs and proteins of stimulus-induced RFIs are highly unstable, and thus allow for rapid stimulus-dependent negative feedback. This part of the network is flexible, and consumes energy only when the pathway is stimulated.

Experimental evidence supports the physiological relevance of the proposed energy minimization principle: depending on the cellular context, protein turnover requires between 30 and 70% of the total cellular energy budget (Wieser and Krumschnabel, 2001). From quantitative experimental measurements, we estimate that signalling proteins make up $\sim 5\%$ of the total cellular protein mass (Supplementary information). The most abundant cellular proteins, i.e. housekeeping and structural proteins, are very stable with half-lives of up to 60 days (Nissen *et al*, 1978) and thus do not contribute strongly to the cellular energy budget. We therefore expect that signalling protein turnover consumes much more than 5% of the total energy spent for protein synthesis, and thus constitutes one of the dominant energy sinks in mammalian cells. Our analysis showed that signalling pathways are transcriptionally regulated at multiple points by the induction of different and possibly cell-type-specific inhibitors (see Figure 2 and Supplementary information). If these flexible parts of the signalling pathway would be highly turned over constitutively, they would likely represent a strong energy burden. However, we show that the constitutively expressed

signalling proteins are generally stable. Thus, our analysis suggests that an energy minimization principle might have contributed to an evolutionary selection pressure favouring this strategy of regulation. Kinetic modelling therefore reveals that the criteria of rapid feedback regulation and energy efficiency favour the same wiring of the regulatory network. Consequently, we have two possible explanations for the observed separation of the signalling network into flexible and static parts, but we cannot presently select between them.

A circuitry involving RFIs could also be beneficial for simpler eukaryotic organisms like yeast. Accordingly, it is known that all three yeast MAPK signalling cascades induce their phosphatases PTP2, PTP3, and MSG5 (reviewed by Martin *et al*, 2005). We analysed transcriptional feedback in response to cAMP signalling and found specific upregulation of the signal inhibitor RGS2 (see Supplementary information). If yeast harbours RFIs, we expect these feedback regulators to be unstable as well. Therefore, we analysed a genome-wide data set of yeast protein half-lives (Belle *et al*, 2006). For the cell integrity, high osmolarity and cAMP pathways, we found that all 20 analysed signal transducers had a protein half-life of more than 15 min, while 4 out of 17 inhibitors were short-lived proteins with half-lives of 15 min or less. Therefore, unstable proteins in these pathways are inhibitors ($P<0.05$, two-sided Fisher's exact test). Moreover, transcriptional feedback occurs by induction of these unstable signal inhibitors, particularly via PTP2 with a half-life of only 3 min. This suggests that the yeast signalling network shows a similar design pattern as mammalian cells.

In pheromone signalling, we found an exception to our finding that transcriptional regulation of signalling is through *negative* feedback: the transducers FUS3, STE12, and STE2 in the MAPK pathway required to receive the pheromone signal are transcriptionally upregulated in response to pheromone stimulation, with rapid kinetics (<15 min) well below the cell cycle time (Roberts *et al*, 2000). Therefore, this is positive feedback. Interestingly, for positive feedback, the two possible

selection criteria we discussed (energy efficiency and quick feedback regulation) predicted that induction of the transducer should be a better strategy than repression of the inhibitor (not shown). This is indeed observed with pheromone signalling. And, some of these signal transducers in this pathway are short-lived proteins, much like the signal inhibitors involved in transcriptional negative feedback, which ensures that the pathway can be upregulated quickly. The pheromone pathway of yeast is different when compared to many other signalling pathways, as it is only required in certain phases of the cellular life cycle and therefore might require a positive feedback. However, the limited amount of available data does not allow us to substantiate this explanation.

Rapid transcriptional feedback inhibition as a general regulatory principle might allow signal transduction cascades to tone down signalling (to 'adapt'), as suggested by experimental studies where signalling persisted if transcriptional feedback was blocked by protein biosynthesis inhibitors (Amit *et al*, 2007; see also Supplementary information). Efficient signal adaptation might enable signalling cascades to induce controlled pulses of gene expression in a robust manner, independent of environmental variations and transcriptional noise (Rao *et al*, 2002; Sauro and Kholodenko, 2004; Dublanche *et al*, 2006; Alon, 2007). Consistent with a role in signal termination, many of the RFIs given in Figure 2 are identified as tumour suppressors or, in the case of cytostatic TGFβ signalling, as oncogenes. Cellular decision-making frequently requires ongoing signalling activity over several hours (see also Supplementary information). This suggests that RFIs are key regulators of the cell fate, while rapidly acting post-translational feedbacks might often be more important for initial signal processing and specificity (Altan-Bonnet and Germain, 2005; Santos *et al*, 2007).

Materials and methods

Microarray data

Microarray data were collected from the Gene Expression Omnibus database (Barrett *et al*, 2005) using R and bioconductor. Data sets with the following accession numbers were used: GDS896, GSE6783, GSE6462 (MAPK signalling); GDS854, GDS855, GSE5232 (TGFβ signalling); GSE3737, GSE6783, GSE6462 (PI3K/AKT signalling); GDS323, GDS1036, GDS1365, GDS1489 (JAK/STAT signalling); GDS1038 (cAMP signalling).

The expression values were log2-transformed where they were not already stored as log2-transformed values. For each gene and each stimulus duration in each data set, the median value of replicas was calculated and the value for unstimulated cells was subtracted. The result corresponds to log2-fold changes displayed in the heatmaps in the Supplementary information. For the main figures, the values were further normalized to account for different fold changes in the different experiments. This was done by dividing by the standard deviation of all log2-fold change values in the array data set, resulting in 'z-values'. A gene was marked as induced if the median z-value over all data sets, conditions and time points was larger than 1. Expression data where the majority of replicas were reported as not expressed (absent call) were left out.

Only proteins for which mRNA or protein half-life *and* microarray measurements were available were taken into account in the analysis. For four induced inhibitors (SOCS5, TGIF, BAMBI, and SRTY1), microarray data showed no induction but literature data were available that showed rapid induction. They were marked as induced in the pathway map in the main text (Figure 2) and marked with asterisks

with the reference in the expression heatmaps in the supplement, but were considered unchanged in the statistical analysis.

mRNA half-lives

mRNA half-lives were taken from the genome-wide data set reported in Yang *et al* (2003) and Raghavan *et al* (2002). We considered all three different treatment conditions in Raghavan *et al* (2002). The mRNA half-lives used in the display and calculations are the median over all half-life measurements for each gene (including all conditions in Raghavan *et al*, 2002).

Protein half-lives

Protein half-lives (listed in Supplementary information) were collected from literature studies, which measured protein decay after administration of the translation inhibitor cycloheximide or by pulse-chase assays. In some cases, the amount of protein decreased by less than 50% within the measurement time, T. If the protein decayed to almost 50% within the measurement time, the half-life was classified as $t_{1/2} > T$, and the value $t_{1/2} = T + 2$ h was used for further analyses. If the protein hardly decayed within the measurement time, the half-life was classified as $t_{1/2} \gg T$, and the value $t_{1/2} = T + 4$ h was used for further analyses. For those proteins whose half-lives were measured in multiple literature studies, we used the median of all measured half-lives to reduce the influence of outliers.

Some protein half-lives had been measured both under stimulated and unstimulated conditions with different results. In this case, we used the half-life under unstimulated conditions for uninduced proteins, while the half-life upon stimulation was taken for transcriptionally induced proteins. Our rationale follows: we assumed the unstimulated state to be the default situation for the cell, and thus basal protein turnover rate should be relevant in our context. We also hypothesized that rapid induction of feedback mediators requires short mRNA/protein half-lives. Therefore, we took the protein half-life upon stimulation for rapidly induced genes. In the light of our energy hypothesis, these rapidly induced factors can contribute to free-energy dissipation minimization if they are much more stable in the basal state when compared to stimulation conditions. Qualitatively similar results were obtained if the half-lives for stimulated cells were taken for transcriptionally uninduced proteins.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

Acknowledgements

References

Alon U (2007) Network motifs: theory and experimental approaches. *Nat Rev Genet* **8**: 450–461

Altan-Bonnet G, Germain RN (2005) Modeling T cell antigen discrimination based on feedback control of digital ERK responses. *PLoS Biol* **3**: e356

Amit I, Citri A, Shay T, Lu Y, Katz M, Zhang F, Tarcic G, Siwak D, Lahad J, Jacob-Hirsch J, Amariglio N, Vaisman N, Segal E, Rechavi G, Alon U, Mills GB, Domany E, Yarden Y (2007) A module of negative feedback regulators defines growth factor signaling. *Nat Genet* **39:** 503–512

Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau WC, Ledoux P, Rudnev D, Lash AE, Fujibuchi W, Edgar R (2005) NCBI GEO: mining millions of expression profiles–database and tools. *Nucleic Acids Res* **33** (Database issue)**:** D562–D566

Belle A, Tanay A, Bitincka L, Shamir R, O'Shea EK (2006) Quantification of protein half-lives in the budding yeast proteome. *Proc Natl Acad Sci USA* **103:** 13004–13009

Bluthgen N, Menzel F, Hovestadt T, Fiala B, Bluthgen N (2007) Specialization, constraints, and conflicting interests in mutualistic networks. *Curr Biol* **17:** 341–346

Dublanche Y, Michalodimitrakis K, Kummerer N, Foglierini M, Serrano L (2006) Noise in transcription negative feedback loops: simulation and experimental analysis. *Mol Syst Biol* **2:** 41

Heinrich R, Neel BG, Rapoport TA (2002) Mathematical models of protein kinase signal transduction. *Mol Cell* **9:** 957–970

Hornberg JJ, Bruggeman FJ, Binder B, Geest CR, de Vaate AJ, Lankelma J, Heinrich R, Westerhoff HV (2005) Principles behind the multifarious control of signal transduction. ERK phosphorylation and kinase/phosphatase control. *FEBS J* **272:** 244–258

Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL (2000) The large-scale organization of metabolic networks. *Nature* **407:** 651–654

Kollmann M, Lovdok L, Bartholome K, Timmer J, Sourjik V (2005) Design principles of a bacterial signalling network. *Nature* **438:** 504–507

Martin H, Flandez M, Nombela C, Molina M (2005) Protein phosphatases in MAPK signalling: we keep learning from yeast. *Mol Microbiol* **58:** 6–16

Mettetal JT, Muzzey D, Gomez-Uribe C, van Oudenaarden A (2008) The frequency dependence of osmo-adaptation in *Saccharomyces cerevisiae*. *Science* **319:** 482–484

Nissen R, Cardinale GJ, Udenfriend S (1978) Increased turnover of arterial collagen in hypertensive rats. *Proc Natl Acad Sci USA* **75:** 451–453

Raghavan A, Ogilvie RL, Reilly C, Abelson ML, Raghavan S, Vasdewani J, Krathwohl M, Bohjanen PR (2002) Genome-wide analysis of mRNA decay in resting and activated primary human T lymphocytes. *Nucleic Acids Res* **30:** 5529–5538

Rao CV, Wolf DM, Arkin AP (2002) Control, exploitation and tolerance of intracellular noise. *Nature* **420:** 231–237

Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, Bennett HA, He YD, Dai H, Walker WL, Hughes TR, Tyers M, Boone C, Friend SH (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. *Science* **287:** 873–880

Santos SD, Verveer PJ, Bastiaens PI (2007) Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. *Nat Cell Biol* **9:** 324–330

Sauro HM, Kholodenko BN (2004) Quantitative analysis of signaling networks. *Prog Biophys Mol Biol* **86:** 5–43

Wang E, Purisima E (2005) Network motifs are enriched with transcription factors whose transcripts have short half-lives. *Trends Genet* **21:** 492–495

Wieser W, Krumschnabel G (2001) Hierarchies of ATP-consuming processes: direct compared with indirect measurements, and comparative aspects. *Biochem J* **355:** 389–395

Yang E, van Nimwegen E, Zavolan M, Rajewsky N, Schroeder M, Magnasco M, Darnell Jr JE (2003) Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. *Genome Res* **13:** 1863–1872