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Noise in eukaryotic gene expression

William J. Blake*, **Mads Kærn***, **Charles R. Cantor & J. J. Collins**

Center for BioDynamics, Center for Advanced Biotechnology, Bioinformatics Program, and Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA
* *These authors contributed equally to this work*

Transcription in eukaryotic cells has been described as quantal[1], with pulses of messenger RNA produced in a probabilistic manner[2,3]. This description reflects the inherently stochastic nature[4–9] of gene expression, known to be a major factor in the heterogeneous response of individual cells within a clonal population to an inducing stimulus[10–16]. Here we show in *Saccharomyces cerevisiae* that stochasticity (noise) arising from transcription contributes significantly to the level of heterogeneity within a eukaryotic clonal population, in contrast to observations in prokaryotes[15], and that such noise can be modulated at the translational level. We use a stochastic model of transcription initiation specific to eukaryotes to show that pulsatile mRNA production, through reinitiation, is crucial for the dependence of noise on transcriptional efficiency, highlighting a key difference between eukaryotic and prokaryotic sources of noise. Furthermore, we explore the propagation of noise in a gene cascade network and demonstrate experimentally that increased noise in the transcription of a regulatory protein leads to increased cell–cell variability in the target gene output, resulting in prolonged bistable expression states. This result has implications for the role of noise in phenotypic variation and cellular differentiation.

To explore the effects of transcriptional variation and control on the level of noise in eukaryotic gene expression, we used both native and artificial modes of transcriptional regulation in the yeast *GAL1* promoter (Fig. 1a). In its natural context, the *GAL1* promoter is activated in response to galactose (in the absence of preferentially metabolized glucose) through an upstream activation sequence (UAS$_G$) composed of multiple binding sites for the transcriptional activator Gal4p. Like many eukaryotic activators[17], Gal4p acts by recruiting protein complexes involved in chromatin remodelling and the ordered assembly of a transcription preinitiation complex[18,19]. Because Gal4p is a galactose-dependent transcriptional activator, activation-based expression from the *GAL1* promoter is effectively modulated with galactose. As a second mode of transcriptional control, distinct from the native complexity of the yeast galactose-utilization pathway, we constructed an artificial, Tet-responsive *GAL1* promoter (P$_{GAL1*}$) by inserting tandem *tet* operators ($2 \times tetO_2$) downstream of the *GAL1* TATA box. In contrast to Gal4p-mediated activation, bound Tet repressor (TetR) might act by sterically hindering the assembly of the transcriptional machinery, effectively repressing expression from P$_{GAL1*}$. TetR-mediated repression can be relieved by the addition of the chemical inducer anhydrotetracycline (ATc), which binds directly to TetR. Constitutive expression of TetR therefore allows rheostat-like control of P$_{GAL1*}$ transcriptional efficiency through the use of varying levels of ATc. The gene encoding the yeast-enhanced green fluorescent protein (*yEGFP*) was expressed from P$_{GAL1*}$ as a quantifiable reporter, and fluorescence histograms were obtained from flow cytometric measurement of similarly sized cells containing chromosomally integrated, single genetic copies of each construct.

Transcription from P$_{GAL1*}$ is modulated over a broad dynamic range by both native and artificial modes of regulation (Fig. 1b), allowing a direct comparison between galactose- and ATc-mediated
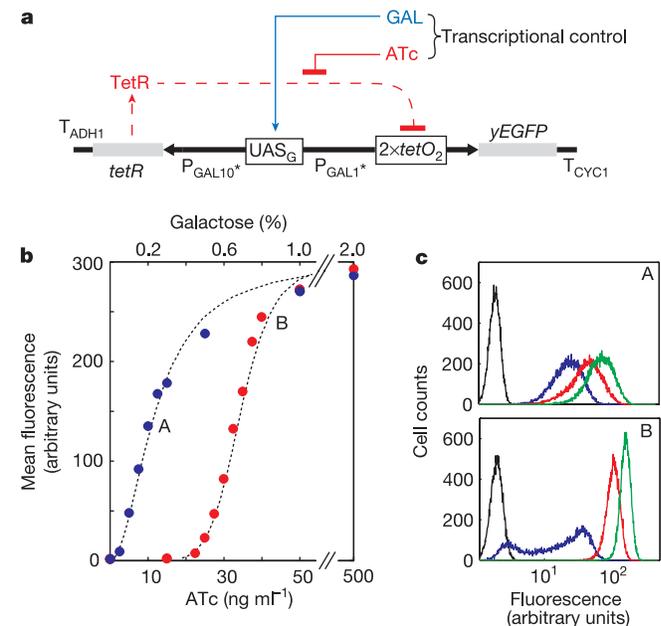

Figure 1 Transcriptional control of P$_{GAL1*}$. **a**, TetR, expressed from P$_{GAL10*}$, represses expression of *yEGFP*. Anhydrotetracycline (ATc) and galactose (GAL) are required to induce yEGFP expression. Transcription terminators (T$_{ADH1}$, T$_{CYC1}$) are indicated. **b**, Dose–response curve of P$_{GAL1*}$ expressing yEGFP to ATc at full galactose induction (2%; red points), and to galactose at full ATc induction (500 ng ml^{-1}; blue points). Broken lines were obtained from stochastic simulations (Box 1). **c**, Transient response of cells marked A and B in **b**, induced with 0.2% galactose and 40 ng ml^{-1} ATc, respectively. Histograms correspond to preinduction (black), and 150 min (blue), 290 min (red) and 440 min (green) after induction.

induction of yEGFP expression from a single promoter. Each point of the dose–response curves shown in Fig. 1b represents the averaged response of an isogenic population to a particular level of induction with either ATc or galactose. Population averages were obtained from fluorescence histograms of individual cell measurements after more than 45 h of growth at a particular inducer level. Distinct modes of transcriptional control of P_{GAL1*} yield strikingly different transient responses, with native galactose-mediated induction resulting in a graded shift in the fluorescence distribution and ATc-mediated induction causing a bimodal response (Fig. 1c). It is likely that these responses reflect the differential effects of activator and repressor proteins on the promoter itself[20,21]. Steady-state fluorescence histograms for populations maintained at similar mean fluorescence values with either ATc- or galactose-mediated

induction show considerable variation, with ATc-mediated induction causing a more heterogeneous response (histograms not shown; see Fig. 2a). We quantify this cell–cell variability in yEGFP in terms of population variance divided by population mean and refer to this value as the noise strength[15].

Noise strength, measured over a wide range of P_{GAL1*} transcriptional efficiencies, shows a striking response marked by low noise at low transcriptional efficiency, an increase at transcriptional efficiencies of 20–40%, and a gradual decrease to a low-noise state at full induction (Fig. 2a). To control for any TetR effects during galactose-mediated induction, noise strength was also measured from P_{GAL1*} in the absence of TetR (under similar conditions of varying galactose), and was found to exhibit an identical behaviour to that of P_{GAL1*} with full TetR induction (not shown).

Box 1
Transcriptional noise in eukaryotic gene expression

We developed a stochastic model of gene expression that incorporates several features specific to eukaryotic transcription, including sequential assembly of the core transcription apparatus, slow chromatin remodelling, rate-limiting binding of the TATA-box-binding protein (TBP) and pulsatile mRNA production due to reinitiation. The model assumes random transitions between different promoter states in transcription initiation as represented by the scheme:
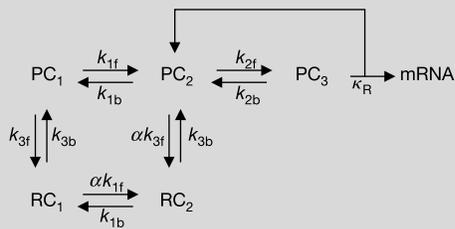
The states PC_1, PC_2 and PC_3 represent, respectively, the inactive (or silenced) promoter, an intermediate complex with TBP and various transcription factors bound, and the preinitiation complex where all components required for transcription are assembled on the promoter. Reinitiation is modelled as a return to the intermediate complex PC_2 (and subsequent transition back to PC_3) after transcription initiation has occurred from PC_3. The states RC_1 and RC_2 represent different repressed promoter configurations. The factor α characterizes the masking of unoccupied DNA-binding sites in the repressed complex RC_1 and the intermediate complex PC_2. Transcript elongation and translation are modelled as single-step processes with stochastic decay of mRNA and protein (see Supplementary Information).

Figure 3a shows that the model can reproduce the correlation between noise strength and transcriptional efficiency for both ATc- and galactose-mediated induction using the same parameter set (see Supplementary Information) that gives quantitative agreement with the dose–response curves (Fig. 1b). With these parameter values, the model predicts linear correlations between noise strength and translational efficiency (Fig. 3b) that are in excellent agreement with the experimental observations (Fig. 2b). The model cannot reproduce the experimental results if the probability of producing a transcript is constant (Fig. 3a, b).

Our experiments involve a chromosomally carried repressor gene, to minimize repressor fluctuations. It was previously shown in bacteria[16] that substantially increased fluctuations in a controlling repressor, introduced by carrying the repressor gene on a plasmid, can result in increased levels of noise at intermediate levels of expression. As shown in Fig. 4b, the level of noise in GFPmut3b expression from P_{GAL10*} ranges between about 1 and 3 arbitrary units (AU) and is about 1.3 AU at full galactose induction. Given that GFPmut3b and tetR have similar

codon adaptation indices (0.183 and 0.115, respectively), the level of noise in GFPmut3b expression can, when assuming similar mRNA decay rates, be used as an indicator of tetR gene expression noise. By using a value of 4 AU for tetR gene expression noise in the simulations, we overestimate repressor fluctuations and show that, in the absence of transcriptional reinitiation, these fluctuations alone (broken lines in Fig. 3a) cannot account for the noise levels observed in our experiments (Fig. 2a).

Eukaryotic activators are known to act on various steps in the transcription process[25], including (1) promoter activation, through remodelling of chromatin structure and/or recruitment of TBP; (2) stabilization of an intermediate, TBP-containing complex; and (3) recruitment of RNA polymerase II (RNAP). In the model, these steps correspond to an increase in k_{1f} (step 1), a decrease in k_{1b} (step 2) and an increase in k_{2f} (step 3), respectively. For example, in Fig. 3a the effect of increasing galactose (mediated through the Gal4p activator) is assumed to increase k_{1f} and decrease k_{1b} (see Supplementary Information). In Fig. 3c we explore the potential role of activators in modulating gene expression noise by varying the rates k_{1f}, k_{1b} or k_{2f} in the model.

In the model, the transcription level (the average promoter occupancy by RNAP) and the magnitude of gene expression noise are both sensitive to the extent of reinitiation, ξ, defined as the average number of mRNA transcripts produced before promoter deactivation (transition from PC_2 to PC_1):

$$\xi = \kappa_R k_{2f}/[k_{1b}(k_{2b} + \kappa_R)].$$

A promoter that has an unstable intermediate complex (high k_{1b}) that is ineffective in recruiting RNAP (low k_{2f}) lacks the ability to promote high reinitiation, causing relatively low levels of transcriptional noise. Such a promoter is also limited to low levels of transcription, even if k_{1f} could become infinitely large in the presence of an activator (Fig. 3c). Promoter strength can be substantially increased by increasing the extent of reinitiation through, for example, more efficient recruitment of RNAP (high k_{2f}). However, this causes a marked increase in noise strength at low levels of expression (Fig. 3c) owing to an average of ξ transcripts being produced in pulses at irregular intervals rather than at a low steady rate. If an activator is directly involved in the stabilization of the intermediate complex (decreasing k_{1b}) and/or the rate of RNAP recruitment (increasing k_{2f}), promoter strength can be increased markedly without affecting noise at low levels of transcription. As shown in Fig. 3c, by decreasing k_{1b} or increasing k_{2f}, an activator can cause transcriptional noise to pass through a maximum when the promoter is about 50% activated with low transcriptional noise at both low and high levels of transcription. By acting on multiple steps in the transcription initiation process, transcriptional activators can be used to modulate gene expression noise and ensure tight basal expression, without compromising promoter strength or dynamic range.

The observed difference between noise signatures for ATc- and galactose-mediated induction might therefore reflect the different molecular mechanisms by which galactose and ATc induce promoter activation, as shown by transient graded and binary responses, respectively (Fig. 1c). To determine whether the qualitative characteristics of the observed noise signatures are specific to P_{GAL1*}, we engineered a second Tet-responsive promoter by inserting $2 \times tetO_2$ into the yeast ADH1 promoter in the same location, relative to the TATA box, as in P_{GAL1*}. The engineered ADH1 promoter (P_{ADH1*}) is 10-fold weaker than P_{GAL1*} at full ATc induction, but it shows a non-monotonic transcriptional noise signature similar to those observed for P_{GAL1*} (Fig. 2a, inset). P_{ADH1*} also exhibits transient bimodality when induced with ATc (not shown), offering further evidence that the mode of transcriptional control can have a marked effect on the resulting population response.

Recent bacterial studies[15,16] have explored the contribution of transcription to the level of noise in prokaryotic gene expression. It was demonstrated experimentally, with a chromosomally integrated LacI-repressible promoter, that the level of transcription (modulated with isopropyl β-D-thiogalactoside inducer) had little effect on the level of noise[15], demonstrated by a weak positive correlation between transcriptional efficiency and noise strength. The non-monotonic responses to varying levels of transcription exhibited by eukaryotic cells in the present study (Fig. 2a) contrast sharply with what was observed in a prokaryote[15], in which a similar noise measure and single-copy constructs make comparison amenable. One of the many factors that differ between prokaryotic and eukaryotic gene expression is the rate-limiting step of transcriptional initiation, which, for eukaryotic organisms, involves the binding of the TATA-box-binding protein (TBP) to the promoter region[22,23]. Highly expressed genes are probably associated with promoters that employ the process of transcriptional reinitiation[24], whereby a stabilized complex (including TBP) remains on the promoter for multiple rounds of transcription. By obviating the need to reassemble the preinitiation complex de novo, transcriptional reinitiation allows the faster production of a large amount of transcript.

The stochastic model presented in Box 1, and described in detail in Supplementary Information, was used to explore specific mechanisms of eukaryotic transcription apparatus assembly, as well as the process of reinitiation, which may contribute to the shape and magnitude of the observed noise signatures. The model incorporates random transitions between various states of promoter occupancy by activators, repressors and general transcription factors in the process of formi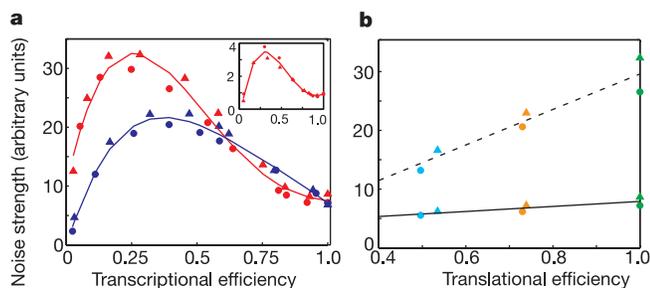ng a functional preinitiation complex before transcriptional initiation and reinitiation. Importantly, simulations of the model (Fig. 3a) demonstrate that pulsatile mRNA production (resulting from transcriptional reinitiation) is required to reproduce the experimental observations, providing further support for the quantal view of eukaryotic transcription[1] (see Box 1). An additional important factor in the control of eukaryotic gene expression is the role of transcriptional activators, which, through a variety of mechanisms[25], can affect the rates of transition between various states of promoter occupancy and accessibility. We use the model to explore these rates, as potential targets of activator action, and show how activators can modulate gene expression noise (see Box 1).

Translational efficiency, or the number of protein molecules produced from a single mRNA transcript, has been proposed[5,7], and recently shown[15], to be an important variable in determining the level of noise in prokaryotic gene expression. To determine whether translational efficiency contributes to the level of noise in eukaryotic gene expression, we modified the construct shown in Fig. 1a by replacing the yEGFP gene with two codon variants, GFPmut3b (the precursor to yEGFP) and yEGFPm1 (a hybrid gene containing sequences from yEGFP and GFPmut3b). Codon usage, as measured by the codon adaptation index (CAI)[26], is a reliable indicator of translational efficiency in yeast and is easily modified without disrupting the amino-acid sequence or functionality of the protein. Relative to yEGFP at full (100%) induction from P_{GAL1*}, GFPmut3b and yEGFPm1 showed low (~50%) and intermediate (~75%) expression, respectively, which correlate well with the calculated CAI values for each of these variants (Fig. 2b). As shown in Fig. 2b, an increase in the translational efficiency of the reporter causes a slight increase in the noise strength at full induction of P_{GAL1*}. However, increased translational efficiency
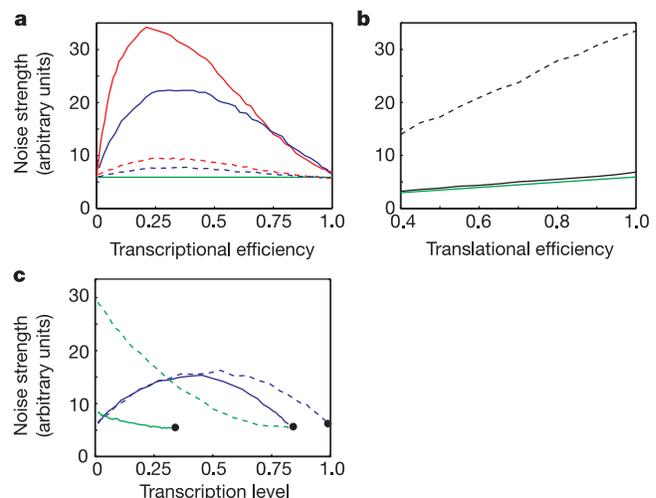
Figure 3 Simulations of eukaryotic gene expression noise. **a**, Correlation between noise strength and transcriptional efficiency varied with ATc (red curve), galactose (blue curve), and for constant probability of producing a transcript (green line). Simulations in the absence of transcriptional reinitiation are shown (broken curves). **b**, Correlation between noise strength and translational efficiency for full (solid line, slope = 5.7) and ~28% (broken line, slope = 33) transcriptional induction. The green line (slope = 4.9) is obtained when the probability of producing a transcript is constant. **c**, Correlation between level of transcription and noise at different model parameter values. The green curves show variation in the level of transcription through k_{1f} for low (solid curve, $k_{1b} = 1$, $k_{2f} = 1$, $\xi = 0.5$) and high (broken curve, $k_{1b} = 1$, $k_{2f} = 10$, $\xi = 5$) extents of reinitiation. The blue curves show variation in the level of transcription through k_{1b} for $k_{1f} = 0.1$, $k_{2f} = 10$ (solid curve); and through k_{2f} for $k_{1f} = 0.1$, $k_{1b} = 1$ (dotted curve). The level of transcription is reported relative to the highest level of mRNA theoretically possible. Repressor is absent ($k_{3f} = 0$) and RNA polymerase II binds tightly to the promoter ($k_{2b} = 1$).

Figure 2 Effect of transcriptional and translational efficiency on noise strength. Data points represent measurements taken at >30 h (circles) and >45 h (triangles) of growth. **a**, Transcriptional efficiency from P_{GAL1*} is varied with either ATc (red points) or galactose (blue points). The inset shows noise strength as a function of transcriptional efficiency (varied by ATc induction) for P_{ADH1*}. Fourth-order polynomial fits are included for clarity. **b**, Variants yEGFP (green points), yEGFPm1 (orange points) and GFPmut3b (light blue points) differ only in codon content (CAI = 0.596, 0.384 and 0.183, respectively). Linear fits to data obtained at full transcriptional induction (solid line, slope = 4.3), and at ~28% (30 ng ml^{-1} ATc) transcriptional induction (broken line, slope = 30.2) are shown.

has a substantial effect when coupled to a noisy transcriptional state (~28% induction), amplifying transcriptional noise considerably. This provides additional evidence that the level of gene expression noise in eukaryotic cells is strongly influenced by transcription, in contrast to observations in prokaryotic cells[15]. Strikingly similar results are produced with the stochastic model (Fig. 3b). The results in Fig. 2b show that translational efficiency (in particular, codon usage) can be used to regulate the level of noise in the expression of a eukaryotic gene; these findings indicate that noise strength is an important factor to be considered when studying the role of codon bias in gene expression.

There has been speculation about the effects of a noisy regulatory input on downstream gene expression[7,9,27], and it has been proposed that important regulatory genes have evolved to minimize noise[15]. To determine the extent to which fluctuations in the expression of a regulatory gene can affect cell–cell variability in the downstream target, we constructed a simple regulatory cascade (Fig. 4a) in which noise in the expression of the regulator (TetR) can be modulated independently of the regulated gene (yEGFP). Independent noise control is feasible because of the differential effect of galactose on the GAL10 and ADH1 promoters. Specifically, P_{ADH1*} expression is not regulated directly by galactose and the level of noise in transcription from P_{ADH1*} does not change significantly when galactose is varied (Fig. 4b). The level of noise in TetR (expressed

from P_{GAL10*}) can therefore be varied with galactose independently of noise in the expression from P_{ADH1*}. We set the tunable noise in TetR expression to either high (0.15% galactose) or low (2% galactose) (see Fig. 4b), and used ATc to control the regulatory effect of TetR on P_{ADH1*} transcriptional efficiency. Figure 4c shows that increased noise in TetR expression has a marked effect on the level of noise in ATc-regulated yEGFP expression, causing a substantial increase in target gene fluctuations over a broad range of transcriptional efficiencies.

As described above, ATc-induced expression of yEGFP from P_{ADH1*} results in a transient bimodal response (similar to that of P_{GAL1*} shown in Fig. 1c), which becomes unimodal when expression reaches a steady state. By directly tuning the level of noise in the TetR regulatory protein, independently of other factors involved in expression from P_{ADH1*}, we find that we can directly affect yEGFP expression state stability, as reflected in the maintenance of a bimodal response. Specifically, a low level of noise in TetR expression (noise strength ~1.3 arbitrary units (AU)) causes yEGFP expression to exhibit a single, unimodal fluorescence distribution (Fig. 4d), whereas when the TetR regulatory protein is tuned to a high noise level (noise strength ~2.7 AU), we observe that yEGFP exhibits prolonged bistable expression states that persist for the length of the experiment (Fig. 4e). These results show that the downstream effects of increased cell–cell variability in a regulatory protein can have profound phenotypic consequences, drastically affecting the stability of gene expression states. It was recently shown that increased noise in the expression of a tumour-suppressor gene can lead to altered cell phenotypes characterized by distinct morphological changes[28]. The cascading noise experiments described above demonstrate a plausible mechanism for such heritable changes in target gene expression, whereby increased levels of noise in the expression of a regulatory protein can cause a population of isogenic cells to exhibit prolonged bistable expression states.

We have shown how transcriptional efficiency and the mechanism of transcriptional control relate to noise strength in certain eukaryotic promoters, providing an important distinction between sources of noise in prokaryotic and eukaryotic cells. We have also demonstrated a clear link between codon usage and gene expression noise. Moreover, we have shown that increased noise in a regulatory protein can have profound effects on bimodal responses that can be critical for cellular differentiation and the maintenance of phenotypic variation. Together these findings contribute to a greater understanding of the origins of cell–cell variability and the consequences of such variability in the expression of a regulatory protein on cell phenotype. Our stochastic model of gene induction proposes specific mechanisms by which slow transitions between various states of transcription apparatus assembly and the process of reinitiation, and also the action of transcriptional activators, might modulate noise in gene expression. In addition to the proposed role of activators, the level of noise might involve other factors, such as the sequence of the promoter itself. The stability of the complex between transcription factor IIA, TBP and promoter is dependent on the sequence of the TATA box[29], and promoters that direct reinitiation have consensus TATA elements[30]. Variation in TATA box sequence and the use of TATA-less promoters might be additional mechanisms of noise control, as a high extent of reinitiation (caused by a stable intermediate promoter complex) can lead to high noise (see Box 1). All of these mechanisms are known to be involved in eukaryotic gene expression and might work synergistically to control the level of noise. □
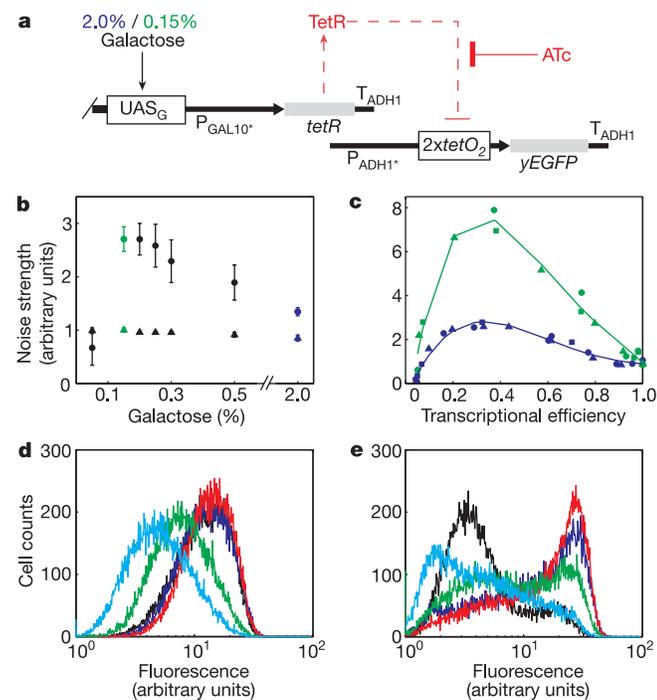

Figure 4 Cascading noise in a gene network and effect on cellular response. **a**, Diagram of simple cascading gene network with regulatory input (TetR) and regulated output (yEGFP). **b**, Noise strength as a function of galactose for P_{GAL10*} expressing *GFPmut3b* (circles) and P_{ADH1*} expressing *yEGFP* (triangles). Conditions of high (0.15% galactose; green points) and low (2% galactose; blue points) input noise are indicated. *GFPmut3b* (CAI = 0.183) is used to estimate the effect of galactose on noise in the expression of *tetR* (CAI = 0.115). Error bars are 95% confidence intervals for three independent measurements at >15 h, >30 h and >45 h induction with galactose. **c**, Noise in yEGFP expression as a function of transcriptional efficiency at high (blue points) and low (green points) levels of input noise. Data points represent day 4 (circles), day 5 (triangles) and day 7 (squares) measurements. Fourth-order polynomial fits are included for clarity. **d**, **e**, Histograms represent yEGFP fluorescence measurements taken after growth for 1 day (black), 2 days (dark blue), 3 days (red), 4 days (green) and 5 days (light blue). Low (**d**) and high (**e**) levels of TetR input noise cause different phenotypic responses.

Methods

Strains, media and growth

All yeast strains were created by targeted chromosomal integration of shuttle vector constructs at either the *GAL1-10* or the *ADH1* locus of *S. cerevisiae* strain YPH500 (α, ura3-52, lys2-801, ade2-101, trp1Δ63, his3Δ200, leu2Δ1) (Stratagene) or derivatives,

and confirmed by polymerase chain reaction (PCR) analysis. Cultures were grown in synthetic drop-out (SD) medium supplemented for selection of correct integrants and containing either 2% galactose with various amounts of ATc inducer, or 500 ng ml^{-1} ATc and 2% raffinose with various amounts of galactose as an inducer. For cascading noise experiments, all cultures contained 2% raffinose. Exponentially growing yeast cells were diluted 1:30 to 1:50 into SD medium containing the appropriate inducer concentrations and grown at 30 °C for 14–19 h to an OD$_{600}$ of 0.6 $\pm$ 0.3 for flow cytometric analysis. Inducer concentrations were maintained in subsequent dilutions, and cells were assayed every 14–19 h for 3–7 d. All cultures containing ATc were protected from light during growth. All cloning steps were performed in *Escherichia coli* XL10-Gold (Stratagene).

Artificial switch construction

All plasmid backbones were derived from pRS403 or pRS404 shuttle vectors (Stratagene). The *GAL1-10* promoter region was obtained from pESC-Leu (Stratagene) and the *ADH1* promoter region from pAD4Δ (a gift from T. Gilmore, Boston University). Promoters were modified by PCR-directed insertion of bacterial operator sites, 2×*tetO$_2$* or *lacO*, downstream of the *GAL1*, *ADH1* or *GAL10* TATA boxes to create P$_{GAL1^*}$, P$_{ADH1^*}$ and P$_{GAL10^*}$, respectively (only P$_{GAL10^*}$ contains *lacO*). Genes encoding TetR, yEGFP and GFPmut3b were amplified by PCR from pcDNA6/TR (Invitrogen), pEGFP3 (a gift from B. Cormack, Johns Hopkins University) and pGFP(LVA) (Clontech), respectively. The *yEGFPm1* gene was created by inserting the *Msc*I–*Hpa*I fragment from *GFPmut3b* into *yEGFP*. All *gfp* variants were sequenced with twofold coverage to ensure identical amino-acid sequences. CAI, a measure of codon bias, was calculated with CodonW (J. Peden, University of Nottingham, UK; http://bioweb.pasteur.fr/seqanal/interfaces/codonw.html).

Cytometry and data analysis

Expression data were obtained with a Becton-Dickinson FACSCalibur flow cytometer with a 488-nm argon excitation laser and a 515–545-nm emission filter (FL1). Cultures in mid-exponential-phase growth were pelleted and resuspended in filtered PBS before assay. Cell samples were assayed at a low flow rate until 30,000 cells had been collected within a small forward scatter and side scatter gate to minimize fluorescence variation due to cell size. Flow cytometry standard list-mode files were converted to ASCII format with MFI (E. Martz, University of Massachusetts, Amherst; http://www.umass.edu/microbio/mfi) and analysed by using Matlab (The MathWorks, Inc., Natick, Massachusetts). Transcriptional efficiency was defined relative to the fully induced (500 ng ml^{-1} ATc, 2% galactose) case for each measurement day, whereas translational efficiency was defined relative to yEGFP expression for each measurement day.

Simulations

Simulations of the reaction network described in Box 1 were performed with a stochastic algorithm as described in detail in the Supplementary Information. Mean values and variances were calculated from histograms obtained from 10^4 independent realizations of length 10^3 dimensionless time units (18-fold the protein half-life). For computational efficiency, fluorescence was assumed to equal the number of reporter protein molecules. With the exception of TetR and galactose, nuclear concentrations of transcription factors and cofactors were assumed to be constant and absorbed into the pseudo-first-order association (k_{if}) and dissociation (k_{ib}) rate constants. Cell–cell variability in rates of transcription elongation, translation and decay of mRNA and protein cause an increase in the noise strength at increased transcriptional efficiency (not shown), and are assumed to be low. The level of galactose in each individual cell was obtained by drawing a random value from a normal distribution with a standard deviation of 10% of the mean. Fluctuations in TetR protein were modelled using a two-step model of transcription and translation[15]. Broken lines in Fig. 3a were obtained with a model incorporating fast promoter state transitions with fluctuations in TetR (red) and cell–cell variability in galactose (blue), respectively (see Supplementary Information). The green lines in Fig. 3a, b were obtained from the same model in the absence of galactose and TetR cell–cell variability, and hence show a time-independent probability of producing a transcript. In Fig. 3b, translational efficiency was varied by changing the rate constant κ_P associated with translation (see Supplementary Information). The green line in Fig. 3b was obtained with 30 ng ml^{-1} ATc and 2% galactose. A coinciding curve is obtained with 500 ng ml^{-1} ATc and 2% galactose. In Fig. 3c, k_{1f}, k_{1b} and k_{2f} were varied between zero and infinity.

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Correspondence and requests for materials should be addressed to J.J.C. (e-mail: jcollins@bu.edu).