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Post-transcriptional noise control

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SUMMARY

Recent evidence indicates that transcriptional bursts are intrinsically amplified by mRNA cytoplasmic processing to generate large stochastic fluctuations in protein levels. These fluctuations can be exploited by cells to enable probabilistic bet-hedging decisions. But, large fluctuations in gene expression can also destabilize cell-fate commitment. Thus, it was unclear if cells temporally switch from high to low noise and what mechanisms enable this switch. Here, we review the discovery of a post-transcriptional mechanism that attenuates noise in HIV. Early in its lifecycle, HIV amplifies transcriptional fluctuations to probabilistically select alternate fates, whereas, at late times, HIV utilizes a post-transcriptional feedback mechanism to commit to a specific fate. By re-analyzing reported post-transcriptional negative feedback architectures, we find that they attenuate noise more efficiently than classic transcriptional auto-repression and present an assay to detect post- transcriptional motifs. We hypothesize that coupling transcriptional and post-transcriptional autoregulation enables efficient temporal noise-control to benefit developmental bet-hedging decisions.

INTRODUCTION

A genetically identical population of cells will express different levels of mRNA and protein in each cell due to stochastic fluctuations in gene expression (i.e., noise). Both the sources of gene-expression noise, and the degree of variability (i.e., magnitude of the noise) can differ drastically depending on the organism or gene.[1–3] Intuitively, some sources of gene expression noise can be explained by, for instance, differences in cell size, cell-cycle state, cellular composition, or environmental stressors.[4] These global sources of variability are commonly termed extrinsic noise and will affect two identical genes within the same cell equally.[5] However, there is additional variability caused by the inherently probabilistic nature of molecular processes, termed intrinsic noise, that is the major driving force of gene-dependent fluctuations in expression levels noise.[5] Intrinsic noise is predominately caused by the low copy numbers of biomolecules involved in gene expression and the pulsatile or "bursty" nature of transcription.[6–10]

Since it is not exactly clear when either noise source dominates it is important to consider both extrinsic and intrinsic noise. Early work in bacteria showed that the variability arising

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from both bursty,[10] and constitutive (i.e., Poissonian) promoters[11] could be explained solely by taking into account intrinsic fluctuations. Yet, later studies found that only once cell-cycle or cell-size effects had been minimized does intrinsic noise dominate for bursty promotes or at low expression levels, while extrinsic noise dominates at high expression levels across bacteria,[3] yeast[2,3,12–14], and mammalian cells.[15] Conversely, a recent study in mammalian cells found that a host of extrinsic variables—including cell-size—could not explain the observed transcriptional variability for lower expressing genes,[16] implying that the variability for these genes is dominated by intrinsic noise. There is perhaps a deeper philosophical debate about when factors influencing variability are truly intrinsic rather than extrinsic, as previously discussed[7], and future results may require the field to revise its interpretations.

Fluctuations in protein expression levels are often harnessed by cells to drive alternate fate outcomes in bacterial cells, stem cells, during viral infection, or drug tolerance in cancer cells, facilitating bet-hedging strategies.[6,7,17–25] Yet, these fluctuations can be detrimental for phenotype stability,[2,12,26,27] requiring cells to implement specific mechanisms that can attenuate noise. For example, during *Drosophila* embryo development multiple nuclei share one cytoplasm, dampening noise that originates in the individual nuclei and allowing for precise embryonic development.[26,28] Yet, this dampening acts globally and is not genespecific. Examples of gene-specific noise-control include the promoter sequences, [29–32] or enhancers at the gene locus affecting promoter toggling frequencies,[33,34] as has been reviewed elsewhere.[35] These designs at the gene locus preclude the ability of a gene to switch from high expression variability to low variability, an attribute that is beneficial in cellular decision making or during bet-hedging strategies.

Therefore, a remaining question is how can cells temporally control variability in a gene-specific manner? The intuitive answer is feedback (or feedforward) loops, but it is unclear if these have evolved to control gene-expression noise and to what extent they can do so. Historically, most feedback loops are studied with respect to changes in mean expression levels, though positive noise-enhancing feedbacks have been shown to drive cell-fate decisions,[21,36–38] and classic negative autoregulation is thought to decrease noise in bacteria.[39,40] Post-transcriptional regulation by small RNAs in bacteria[41–43] and micro-RNAs in eukaryotes[44–46] have also been implicated in noise regulation, though herein we will mostly focus on autoregulatory motifs. More recent results, indicate that a post-transcriptional autoregulatory feedback is implemented to control noise in HIV protein expression and plays an important role in stabilizing viral fate- commitment to active-viral replication.[47] It is therefore likely that noise-control is a functional advantage of similar motifs in other systems. Below, we review how variability in gene expression can be temporally controlled by coupling transcriptional and post-transcriptional autoregulatory circuits. Using simulations, we examine a range of reported post-transcriptional feedback architectures and determine their respective abilities to attenuate mRNA and protein noise.

INTRINSIC GENE EXPRESSION VARIABILITY IS GENERALLY AMPLIFIED POST- TRAN SCRIPTIONALLY

The simplest models show that episodic (i.e. 'bursty') transcription occurs when a promoter toggles between a transcriptionally active and transcriptionally silent state.[1,9,10,48–52] These simple transcriptional bursting models, referred to as the two-state or random-telegraph models, assume the promoter toggles between an inactive OFF state and active ON state.[53] Though multi-state promoter models[15] can accurately capture experimental data, any model that has more than one promoter state will generate mRNA and protein fluctuations that are super-Poissonian.[9,54] On the other hand, models that have only one promoter state (i.e. for constitutively expressed genes) are simplified birth-death processes (i.e., "Poisson processes") and will generate Poissonian distributions of gene products. Poisson distributions accurately describe the variability for constitutive promoters,[9,55] however, non-Poissonian multi-state models (e.g., random-telegraph models) are required to fit measured cell-to-cell expression distributions that are super- Poissonian.[17,48,56]

In addition to promoter toggling, gene-expression variability can be modulated by the turnover of nuclear mRNA, cytoplasmic mRNA, and protein.[1,10,57,58] For example, slow nuclear export combined with a short cytoplasmic mRNA half-life, can decrease (attenuate) cytoplasmic mRNA noise.[59] Additionally, slow translation rates and long protein half-lives allow time-averaging of short-lived fluctuations caused by small, frequent transcriptional bursts.[19,30] Yet, these two forms of noise minimization are extremely costly for two reasons: first, they are inefficient, due to wasteful overproduction of mRNA;[40] and second, they cannot synergize because the long protein half-lives—required for protein time-averaging of short-lived fluctuations—counteract the noise reduction caused by slow export, making the two processes mutually exclusive.[60] Exploiting biochemical rates for noise amplification on the other hand, is significantly less costly for a cell, owing to the ability for a high degree of synergy between amplification steps. For example, high translation efficiency amplifies phenotypic variability.[11,40,58] This amplification occurs because several hundred to thousands of proteins can be translated from one mRNA, so any transcriptional bursts can be enhanced more than 100 fold, thereby augmenting a population's ability to inhabit two phenotypic states.[12,58,61]

Additionally, recent results show that the effects of cytoplasmic mRNA processing, and translation further amplify gene-expression noise, resulting in cytoplasmic mRNA and protein distributions that are far from minimal Poisson noise.[56] Therefore, the majority of physiologically relevant rates involved in mRNA expression will cause noise amplification (Fig. 1a, left), especially assuming widespread noise amplification due to cytoplasmic mRNA processing (Fig. 1a, right).[56] A substantial body of literature argues that transcriptional noise propagates and is amplifies at the protein level and can drive diversifying (positive) selection for bet-hedging phenotypes.[6,20,62,63] Therefore, efficient and accurately timed noise-control, for example in the form of positive or negative autoregulation (Fig. 1b), are required to switch from high to low gene expression variability (Fig. 1c).

EFFICIENT POST-TRANSCRITPIONAL NOISE CONTROL IN HIV

Until recently it remained unclear if transcriptional noise, when it is utilized by cells to drive fate decisions, is subsequently attenuated to allow cells to commit to a given fate, and if so, by what mechanisms. In the early 2000s however, it was predicted that efficient noise attenuating circuits would act post- transcriptionally.[64] This prediction remained theoretical until recently[47] when it was discovered that a post-transcriptional negative feedback in human immunodeficiency virus type 1 (HIV) efficiently minimizes fluctuations following a noise-driven fate decision.

Upon integration into the host-cell genome, HIV amplifies transcriptional fluctuations to enable a noise- driven binary decision[21,22,65] leading to either active viral replication or a long-lived transcriptionally silent state called proviral latency (Fig. 2a, green).[66,67] Though T-cell activation impacts latency establishment and reversal, the fate decision itself is inherently probabilistic and cell state is insufficient to deterministically regulate latency.[68,69] However, once this decision has been made, large expression fluctuations are detrimental, destabilizing the active state. The resulting question was: how does HIV attenuate noise following the fate decision to stabilize its active state? In essence, HIV solves this problem by incorporating a post-transcriptional negative feedback into its regulatory circuit to decrease the amplitude of transcriptional fluctuations (i.e., noise) and stabilizing fate-commitment (Fig. 2a, blue).[47]

HIV transcripts are spliced into three classes of transcripts: unspliced (US) mRNA; singly spliced (SS) mRNA; and multiply spliced (MS) mRNA. The MS transcript class produces, among others, two regulatory proteins: Tat and Rev (Fig. 2b). Tat is responsible for a positive feedback acting on the HIV long terminal repeat (LTR) promoter (Fig. 2b, green), which amplifies fluctuations in protein expression that drive the viral fate-decision (Fig. 2c, green).[22] Rev on the other hand, is a regulatory protein that binds to the Rev Response Element (RRE) present solely on the US and SS transcript classes and exports them out of the nucleus (Fig. 2b, blue) via cellular chromosome region maintenance 1 (CRM1).[70,71] Rev was thought to negatively autoregulate, but the mechanism by which it did so was unclear. Surprisingly, we found that US mRNA is post-transcriptionally spliced into SS and MS mRNAs and this post-transcriptional splicing is obligate for HIV negative autoregulation (Fig. 2b, blue).[47] Simulations predicted that the large Tat-induced fluctuations in protein expression (Fig. 2c, green)—which drive cell-fate decision by generating strong bimodality (Fig. 2d, green)—are attenuated by the Rev negative feedback (Fig. 2c, blue) stabilizing viral commitment to the active state (Fig. 2d, blue).

HIV post-transcriptional splicing was demonstrated using a pulse-chase experiment that relied on single molecule RNA FISH as a readout.[47] One benefit of using RNA FISH is that, as an imaging technique, nuclear and cytoplasmic mRNAs can be readily distinguished, which was essential for this analysis. The pulse phase of the experiment was comprised of a short 14-minute induction with tumor necrosis factor alpha (TNF) to induce nuclear factor kappa B (NFkB) sites on the HIV LTR. Then, transcription was shut off using the transcriptional poison Actinomycin D (ActD). Once transcription was halted, the nuclear US transcript class decreased as expected. However, the nuclear SS transcripts continued to

increase in abundance for 20–30 minutes despite transcription being halted, and the nuclear MS transcripts continued to steadily increase in abundance for the length of the experiment, ~1 hour (Fig. 3a). These data indicated that HIV splicing could not be co-transcriptional and that MS RNAs, which code for Rev and Tat, were products of US RNAs. Subsequent, imaging provided direct evidence that Rev export of US and SS mRNAs resulted in depletion of MS mRNAs, including the Rev mRNA, thereby constituting a negative feedback loop. This post-transcriptional feedback architecture was dubbed ‘auto-depletion’ to differentiate it from the classical transcriptional auto-repression loops.[72] Analytical theory and simulations had predicted that such auto-depletion architectures may provide superior noise attenuation compared to transcriptional auto-repression.[47,64]

To determine how efficiently Rev auto-depletion circuitry attenuated noise, HIV-mutants with increased splicing efficiency were generated since computational models predicted this would significantly reduce negative-feedback strength. Single-cell time-lapse imaging trajectories from a representative HIV mutant with reduced negative feedback are shown (Fig. 3b, left); note the large increase in mean expression level and noise compared to wild type (Fig. 3b, right) as well as the lack of overshoot kinetics—a classic hallmark of a negative feedback—present in the wild-type trajectories but absent in the feedback mutants. Strikingly, the HIV mutant with reduced negative feedback showed decreased stability in commitment to active replication (Fig. 3c), as was predicted by simulations (Fig. 2d). Together the data demonstrated that in the absence of negative feedback (i.e., the splicing mutants), HIV expression noise is not efficiently attenuated and the active viral state is substantially destabilized.[47]

From an evolutionary perspective, HIV’s coupling of noise-amplification circuitry (Tat positive feedback) with downstream noise-attenuation circuitry (Rev negative feedback), is perhaps an expensive regulatory architecture. However, this coupled circuitry allows for temporal regulation of noise and has the benefit of optimizing fitness for probabilistic bet-hedging strategies.[62] Therefore, a plethora of genes may have selected post-transcriptional auto-regulatory architectures for their ability to temporally control noise in addition to mean. This hypothesis is supported by the elements of the HV negative feedback appearing conserved in lentiviruses.[47]

EXAMPLES OF POST-TRANSCRIPTIONAL FEEDBACK

The noise-attenuation effects of HIV’s auto-regulatory feedback suggest a functional basis for similar motifs in other systems. For example, a central mechanism by which serine- and arginine-rich (SR) proteins regulate their homeostasis is by splicing their pre-mRNAs to untranslated variants.[73,74] Here, we thus review a set of reported post-transcriptional feedback circuits—including translational repression—involved in autoregulation of RNA binding proteins (RBPs).

In late erythroblasts (i.e., red blood cells) there is an increased global intron retention in the later stages of development, that regulates some of the major spliceosome genes.[75] For example, Splicing Factor 3b subunit 1 (SF3B1) is auto-regulated via intron retention. This is achieved by the presence of six decoy exons, thus inhibiting splicing of SF3B1 and

promoting SF3B1 i4 retention,[76] resulting in an mRNA auto-depletion feedback that could efficiently attenuate noise. The appearance of this negative feedback (i.e., an upregulation of intron retention) coincides with the last two differentiation stages.[75] Interestingly, extensive intron retention has been shown to occur during development in other systems, and is proposed to be a post-transcriptional mechanism to downregulate gene expression by inducing degradation via nuclear surveillance machinery.[77]

The serine/arginine-rich splicing factor 1 (SF2/ASF) also has 6 alternatively spliced isoforms discovered to date, of which only one is translationally active. The other 5 are responsible for two different regulatory mechanisms.[78] First, regulation through unproductive splicing and NMD - a regulatory mechanism that is commonly observed in conjunction with retained introns.[69,74,79–82] Second, the translationally active transcript of SF2/ASF contains an RNA recognition motif (RRM2) in the 3'UTR. The presence of the RRM2 has been shown to be both necessary and sufficient for autoregulation via translational repression.[78] The regulatory mechanism acts by downregulating the ribosomal occupancy on the mRNA upon binding of the splicing isoforms. Similar translational repression motifs mediated by microRNAs have previously been reported to attenuate noise.[44]

Poly(A)-binding protein nuclear 1 (PABPN1) is a protein required for efficient polymerization of poly(A) tails that regulates its own expression by exploiting a protein auto-depletion circuit similar to HIV. It produces two transcript classes, one unspliced and one spliced, with the unspliced isoform being retained in the nucleus,[83] suggesting post-transcriptional splicing. PABPN1 negatively controls its own expression by binding to the A-rich region of the 3'UTR in its pre-mRNA. This binding promotes intron retention and clearance of these intron-retained pre-mRNAs by nuclear exosomes, causing an overall downregulation of the PABPN1 protein.[83] Analogous to the HIV's Rev-mediated negative feedback,[47] PABPN1 auto-depletion also requires inefficient splicing of the 3'-terminal intron.[83]

In summary, many cellular subsystems may show post-transcriptional autoregulation motifs —including translational repression. This appears particularly true for autoregulation of RNABPs, implying that they are a protein class that require temporal noise-control.

EFFICIENCY OF POST-TRANSCRIPTIONAL FEEDBACK IN NOISE CONTROL

To analyze the potential noise attenuation properties of post-transcriptional and translational feedback motifs compared to classic transcriptional auto-repression feedback, we performed stochastic numeric simulations[84] of a conventional model of eukaryotic gene-expression expanded to include the individual feedback architectures (Fig. 4a). In these models, ON and OFF represent the active and repressed promoter states, respectively; Rn represents nuclear mRNA; Rc represents cytoplasmic mRNA; P represents protein; and all parameters are lumped rate parameters representing promoter toggling between ON and OFF states (k_{on} and k_{off}), transcription (α), nuclear export (λ), translation (β), mRNA degradation (δ), protein degradation (γ), and feedback strength (ε). [47] Feedback by transcriptional auto-repression

(Fig. 4c) is modeled by the protein interacting with the promoter to reduce transitioning from OFF to ON.[72] Feedback by all post-transcriptional motifs is modeled by introducing a "pre" state variable (representing a pre- mRNA) that is spliced into Rn (or Rs for mRNA auto-depletion and translational repression) at rate θ. In short: (i) for the mRNA precursor auto-depletion model (Fig. 4d), pre-mRNA can be removed upon a second-order interaction with the non-translated alternate splicing product Rs; (ii) the translational repression model (Fig. 4e) acts by Rs downregulating translation upon a second-order interaction with Rc; and (iii) the protein precursor auto-depletion model (Fig. 4f) acts by removing pre-mRNA upon a second- order interaction with the protein product P.

Mean (μ) and variance (σ^2) in protein counts were then determined for each feedback motif, allowing for subsequent noise (σ^2/μ) quantification for both high noise (i.e. bursty) promoters (Fig. 4g) and low noise (i.e. constitutive) promoters (Fig. 4h). Strikingly, all feedback motifs acting post-transcriptionally—including the translational repression motif—are more efficient at noise attenuation than transcriptional auto-repression and hold for both high noise (i.e. bursty) promoters and low noise (i.e. constitutive) promoters, enabling the use of noise as an assay for post-transcriptional feedback detection (Box 1). Analytically[47,64] and intuitively, this efficient noise attenuation can be explained by focusing on the number of molecules each respective feedback can act on. The classic negative-feedback will act on a single-molecule DNA, which can only toggle between active (ON) and repressed (OFF). On the other hand, mRNA and protein auto-depletion can act on a large number of molecules (i.e., pre-mRNA) that can be reduced in an analog-like fashion (from x molecules to x - n molecules), greatly increasing their efficiency in noise reduction. Finally, translational repression will reduce variability by time-averaging the fluctuations generated by transcriptional bursts.[19,30]

Notably, for constitutive promoters ($k_{on} > k_{off}$), presumed to be mostly house-keeping genes,[2,3,12] the transcriptional negative feedback increases the protein variability while decreasing the mean (Fig. 4h). This phenomenon occurs because transcriptional repression acts on the promoter, inevitably affecting promoter toggling which is a large contributor to protein noise. Therefore, to remain low in noise constitutively expressed genes appear to require post-transcriptional or translational autoregulation.

Interestingly, the post-transcriptional feedbacks we analyzed here reflect the architectures that regulate the expression of RBPs. Many RBPs, including splicing factors, regulate their expression through alternative Splicing-coupled NMD (AS-NMD)[82] and 10–30% of mammalian genes are thought to be regulated by AS- NMD,[81,88,89] which has been proposed to play a role in noise attenuation.[90] Notably, post-transcriptional auto-regulation effectively attenuates noise even in the presence of intermediate species (unspliced mRNA) that typically act as additional noise sources. Thus, post-transcriptional autoregulatory motifs that appear to regulate generalized alternative splicing suggest a functional reason is noise-control. Finally, the tight regulation of splicing networks is critical for organismal development and disease[78,91,92] further implicating post-transcriptional autoregulation's function in noise-control.

CONCLUSIONS

Here, we reviewed recent findings that (i) transcriptional noise is generally amplified downstream of promoter toggling[56] and (ii) due to this generalized amplification, efficient noise attenuation needs to be implemented in situations where noise is detrimental to the system.

To achieve both specificity for an expressed gene, and allow for temporal control of gene-expression variability, noise attenuation through feedback or regulatory circuits[72] is an attractive mechanism. We show that post-transcriptional or translational auto-regulatory motifs are more efficient at noise attenuation than classic negative-feedback and hypothesize that they provide a powerful strategy for genes to attenuate noise in both a gene-specific and temporal way. Therefore, genes may have selected post-transcriptional regulatory architectures due to their ability to control noise in addition to mean.

The criteria for post-transcriptional autoregulation exist in many cellular subsystems. Retained introns are thought to affect over 80% of coding genes in mammalian cells,[93] with their prevalence ranging from about 2260 introns in mouse cells to almost 5650 introns in human cells.[94] Post-transcriptional autoregulation suggests a potential function for the widespread occurrence of retained introns and their involvement in post-transcriptional splicing and post-transcriptional autoregulation.[93–95] Interestingly, intron retention is upregulated during later stages of development,[75] indicating that by inducing or up-regulating their post- transcriptional autoregulation, genes can temporally switch from high to low expression variability when variability is harmful. Moreover, the ability of neurons to post-transcriptionally regulate splicing and nuclear export[96] and the delayed splicing of introns in multiple other systems[97–99] indicate that post- transcriptional autoregulation could be a ubiquitous phenomenon. We therefore propose that temporal noise-control is the functional reason for the widespread occurrence of post-transcriptional and translational feedback motifs.

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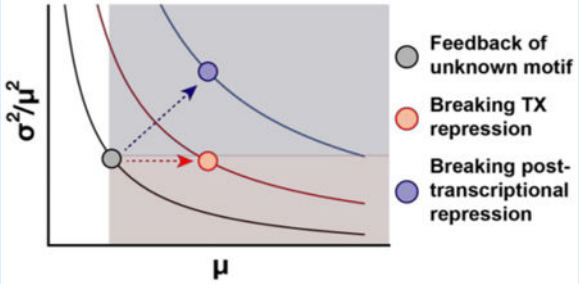
Box 1: Using noise as an assay for post-transcriptional negative feedback

To determine if a protein (P) is negatively autoregulated via transcriptional feedback as opposed to post-transcriptional feedback, the protein CV^2 (σ^2/μ^2) can be utilized, as previously outlined.[47]

First, the CV^2 of the protein within the putative feedback motif (black circle, below) is quantified. Then, provided that feedback can be interrupted to generate a relatively large (e.g., >2-fold) change in mean (μ), transcriptional and post- transcriptional feedback can be differentiated based on the change in CV^2.



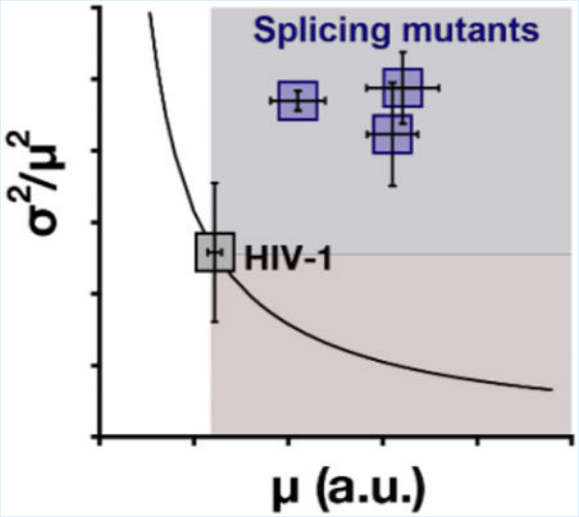
In the transcriptional-bursting case ($k_{off} > k_{on}$), the intrinsic variance is given by $\sigma^2 = b_R b_P \mu$[85] where b_R is the transcriptional burst size and b_p is the translational efficiency, such that the mean (μ) and CV^2 are:

$$\mu = \frac{b_R b_P f}{\gamma} \quad \text{(Equation 1)}$$

$$\frac{\sigma^2}{\mu^2} = \frac{\gamma}{f} \cdot (1 + |T|)^{-1} \quad \text{(Equation 2)}$$

where f is the transcriptional burst frequency $(1/k_{on} + 1/k_{off})^{-1}$, γ is the protein degradation rate, and T is the modification due to net-feedback strength.

Typically, transcriptional auto-repression reduces μ by decreasing f, which from Eqs. 1–2, can in fact increase the CV^2 if T is not sufficiently strong. For a substantial change in mean (e.g., ~2-fold) the CV^2 will decrease or remain relatively unchanged if the underlying feedback motif is transcriptional auto-repression. So, if feedback on P is broken, μ increases while CV^2 decreases or remains unchanged (red circle). Notably, for constitutive promoters (i.e., $k_{on} > k_{off}$) transcriptional auto-repression can increase noise,[64,86,87] resulting in a substantial noise decrease when the feedback is broken. As a result, depending on the promoter architecture, the CV^2 of P when transcriptional auto-regulation is broken can lie anywhere in the red shaded area.



In contrast, post-transcriptional feedback acts on b_p (and possibly b_R) without perturbing *f*. Thus, if post-transcriptional negative feedback is broken, the CV^2 of P increases substantially as mean increases. In the case of HIV-1, negative feedback strength was reduced by increasing splice-acceptor efficiency, which resulted in a ~3- fold increase in μ accompanied by an increase in CV^2.[47] This result shows that CV^2 changes can be used to identify post-transcriptional auto- regulatory motifs.

Instead of genetic manipulation to disrupt negative feedback, small molecule inhibitors of translation such as cycloheximide could also be used. Inhibiting translation in this manner would result in a decrease in production of P, which in turn would decrease feedback strength. In this case, the change in CV^2 of RNA (i.e., Rc), instead of P, should be analyzed.

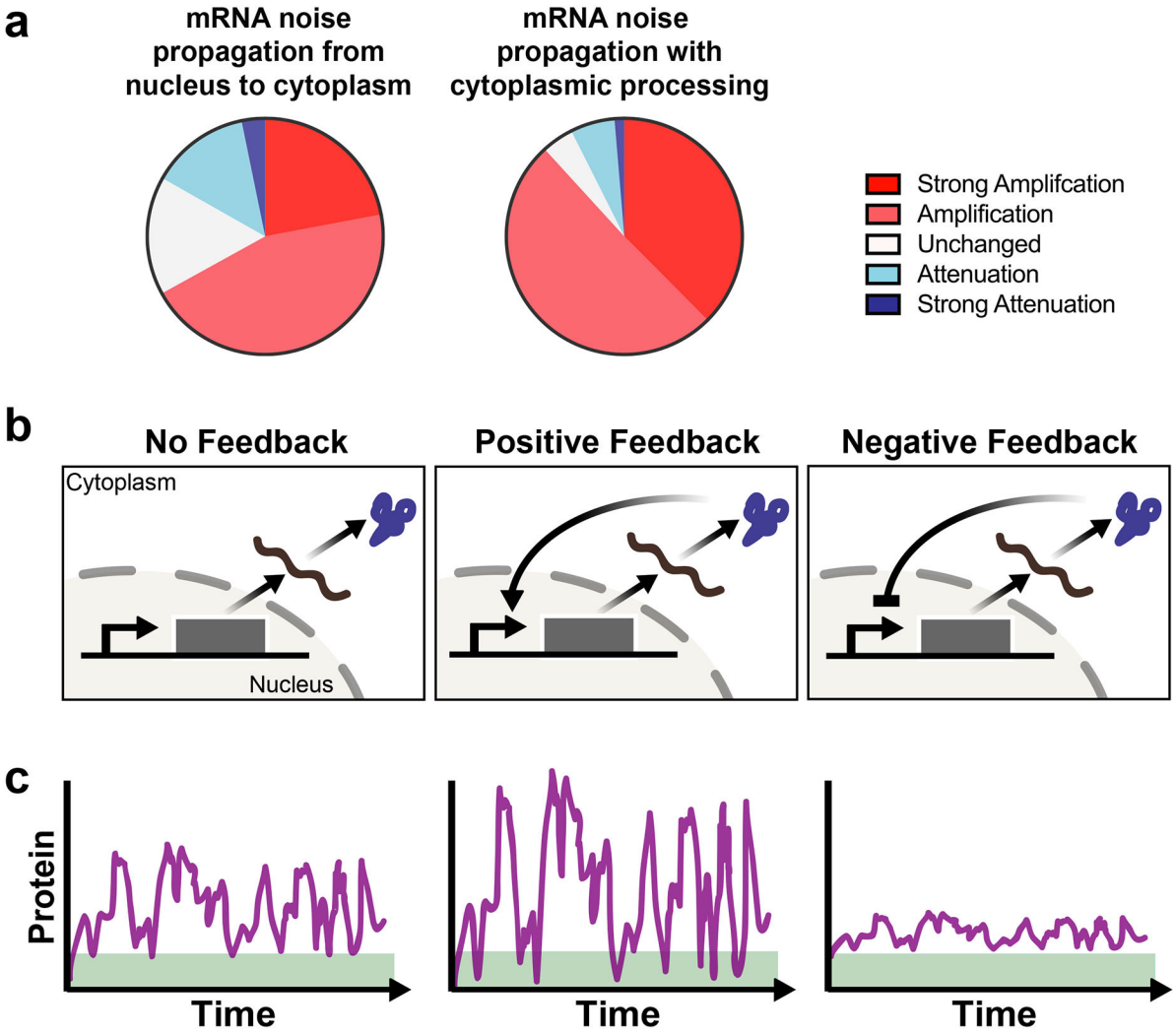


Figure 1. Transcriptional fluctuations are generally amplified by nuclear export and cytoplasmic mRNA processing, requiring feedbacks to modulate noise.

(a) Probability that a gene will show amplification versus attenuation of noise when comparing the nuclear- to-cytoplasmic noise (σ^2/μ) ratio in the absence (left) and presence (right) of bursty cytoplasmic mRNA processing. Increasing red represents increasing noise amplification, while increasing blue represents increasing noise attenuation, and white represents no change in noise from nucleus to cytoplasm. Adapted from [56].

(b and c) Large fluctuations in protein expression levels in the absence of a feedback (left) can be amplified with a positive feedback (center) or attenuated with a negative feedback (right).

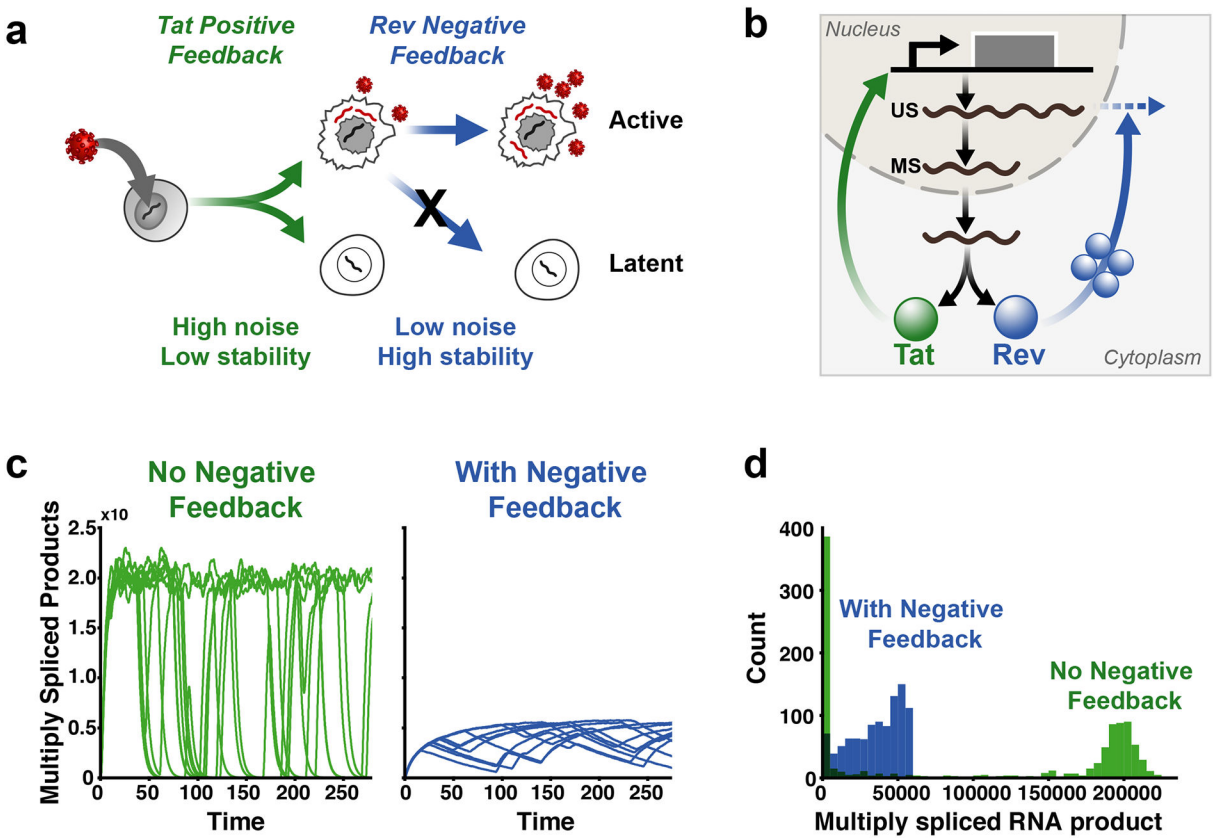


Figure 2. Two separate feedbacks, one transcriptional, one post-transcriptional, temporarily regulate noise in HIV to control and stabilize viral fate.

(a) Tat positive feedback amplifies transcriptional fluctuations generating high noise (i.e., low stability) to probabilistically select HIV's alternate fates early in the viral life cycle (left, green). The Rev negative feedback attenuates noise to drive viral commitment (i.e., high stability) to a specific fate, at later stages in HIVs life cycle (right, blue).

(b) In the nucleus unspliced (US) transcripts are post-transcriptionally spliced into multiply spliced (MS) transcripts. Tat and Rev are both produced from MS transcripts. Tat is responsible for a positive feedback acting on the HIV long terminal repeat (LTR) promoter. Rev acts by exporting US transcripts to the cytoplasm, thereby depleting nuclear US mRNA and reducing the amount of MS mRNAs that can be produced, generating an auto-regulatory negative feedback loop.

(c) Gillespie simulations in the absence (left, green) and presence (right, blue) of the Rev negative feedback show that the negative feedback efficiently attenuates noise and stochastic ON-OFF switching of the LTR is minimized, stabilizing active gene expression. In the absence of the Rev negative feedback, there is higher gene expression noise and increased stochastic ON-OFF switching of the promoter despite an ~3- fold higher mean expression-level of the ON state. Adapted from [47].

(d) Histograms of 1000 simulations of the HIV precursor auto-depletion model (blue) and absent this negative feedback (green) at the end of the simulation run (i.e., t=300hr from c). In the absence of the Rev- negative feedback, substantially more trajectories are expected to be in the GFP OFF state compared to the simulations with the Rev-negative feedback. In

other words, the absence of the negative feedback substantially destabilizes commitment to the active state. Adapted from [47].

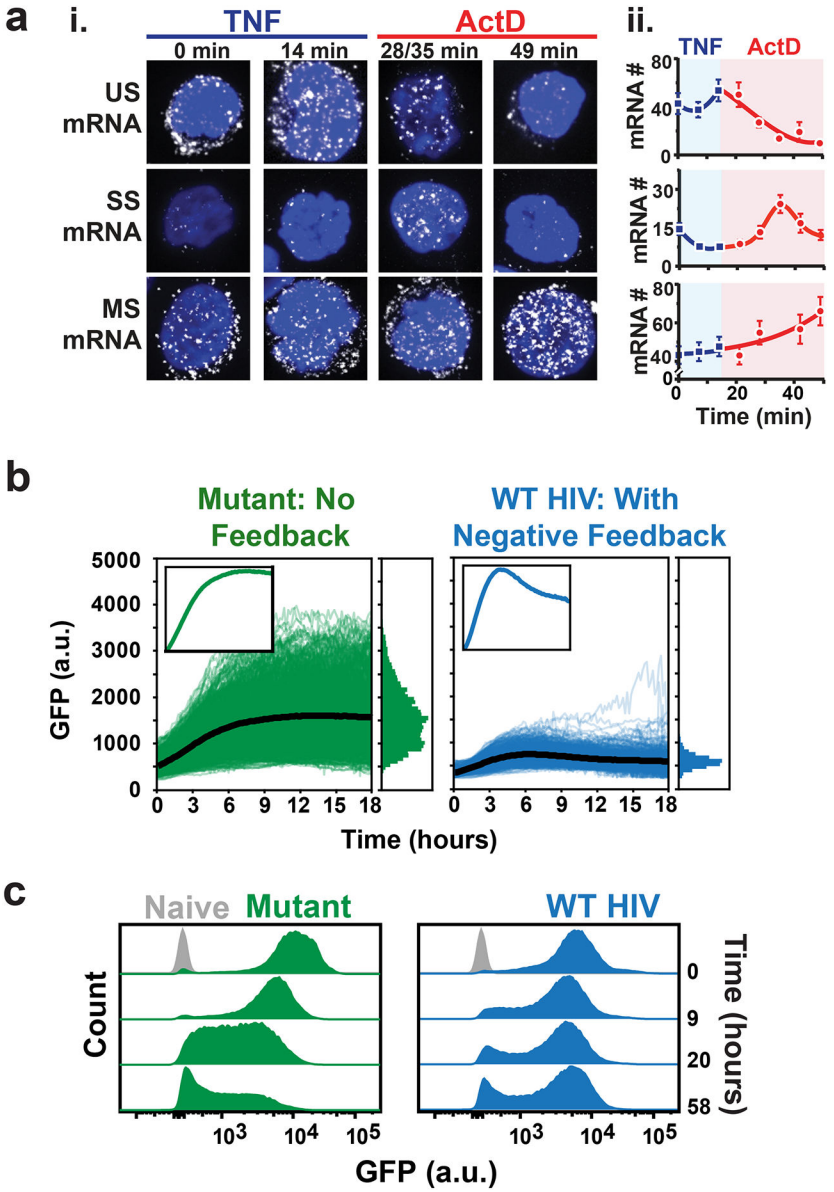


Figure 3. Post-transcriptional architecture of HIV's negative feedback suppresses noise to stabilize fate.

(a) i. Representative smFISH images of transcriptional pulse-chase in fixed HIV-infected Jurkat cells. The nucleus is DAPI stained (blue) and the mRNA is visualized using smFISH (white). Across: TNF activation of the HIV promoter was chased 14 min later with the transcriptional elongation inhibitor ActD. Down: smFISH probes were designed to visualize unspliced (US), singly spliced (SS), and multiply spliced (MS) mRNA respectively. ii. Quantification of nuclear mRNA molecules during the TNF pulse (blue) and the ActD chase (red) from i. Adapted from [47].

(b) Time-lapse microscopy of WT HIV d2GFP containing the Rev negative feedback (right, blue) and a mutant with enhanced splice-acceptor efficiency lacking the Rev negative feedback (left, green). Insets: mean trajectories normalized to max (to examine overshoot). Adapted from [47].

(c) Flow cytometry analysis of active-state stability following a pulse of TNF reactivation for WT HIV d2GFP and splicing mutant lacking the Rev negative feedback; cells were removed from TNF induction at time 0. As predicted in Fig. 2d, after 58 hours, the mutant lacking the Rev negative feedback shows substantially more cells in the GFP OFF (i.e., naïve) state compared to WT. Adapted from [47].

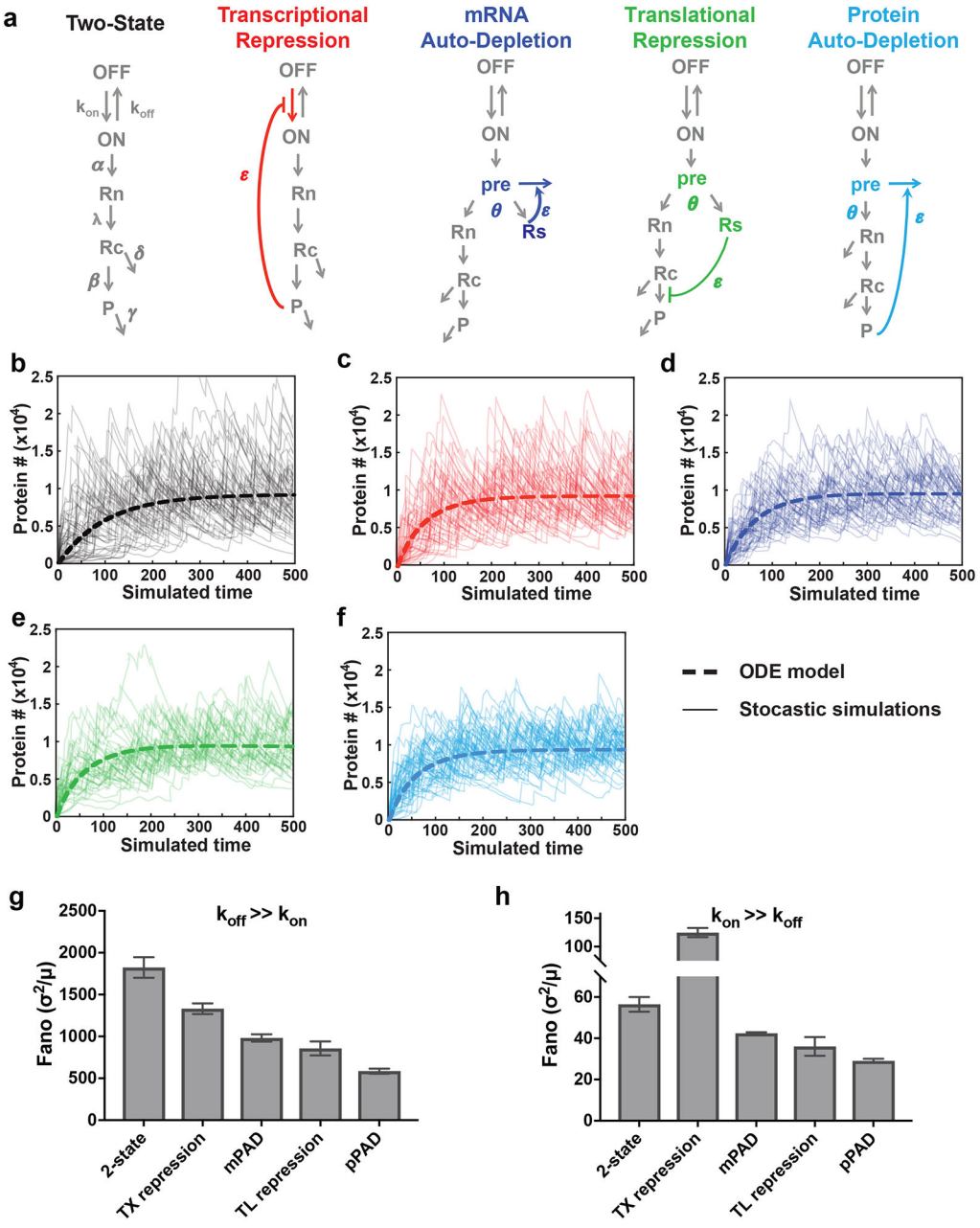


Figure 4. Several feedback architectures can lead to post-transcriptional noise control

(a) Schematics of simplified gene-circuit models used for comparing effects of different negative feedback motifs on noise suppression.

(b-f) Outputs of Gillespie simulations for each model shown in (a): (b) two-state model; (c) transcriptional repression; (d) mRNA precursor auto-depletion (mPAD); (e) translational repression; and (f) protein precursor auto-depletion (pPAD). (g) Fano factor (σ^2/μ) of stochastic simulations comparing models shown in (a) for bursty promoter regime ($k_{off} >> k_{on}$). Left to right: two-state model; transcriptional (TX) repression; mRNA precursor auto-depletion (mPAD); translational (TL) repression; and protein precursor auto-depletion (pPAD). Mean and standard deviation shown for three simulation (200 iterations each).

(h) Fano factor (σ^2/μ) of stochastic simulations comparing models shown in (a) for constitutive promoter regime ($k_{on} >> k_{off}$). Left to right: two-state model; transcriptional (TX) repression; mRNA precursor auto-depletion (mPAD); translational (TL) repression; and protein precursor auto-depletion (pPAD). Mean and standard deviation shown for three simulation (200 iterations each).