Leading Edge
Review

Cell

Passive Noise Filtering by Cellular Compartmentalization

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Chemical reactions contain an inherent element of randomness, which presents itself as noise that interferes with cellular processes and communication. Here we discuss the ability of the spatial partitioning of molecular systems to filter and, thus, remove noise, while preserving regulated and predictable differences between single living cells. In contrast to active noise filtering by network motifs, cellular compartmentalization is highly effective and easily scales to numerous systems without requiring a substantial usage of cellular energy. We will use passive noise filtering by the eukaryotic cell nucleus as an example of how this increases predictability of transcriptional output, with possible implications for the evolution of complex multicellularity.

Introduction

The single cell is highly complex. Most cellular processes involve the action of hundreds of different molecular species. Given the inherent stochastic nature of molecular diffusion and interaction (Figure 1A), and the small copy number with which some molecules are present, it seems at first sight impossible that a single cell could display highly controlled behavior. Indeed, early studies argued that because of this inherent uncertainty, single cells that are genetically identical and grow under identical conditions must display a large degree of molecular and phenotypic variability (Gusella et al., 1976; Ko, 1991; Till et al., 1964). This was supported by experimental observations in prokaryotic cells (Elowitz et al., 2002; Ozbudak et al., 2002) and subsequently extrapolated to cells from many different organisms, including yeast and mammals (Eldar and Elowitz, 2010; Raj and van Oudenaarden, 2008).

Clearly, variability in biology is highly advantageous. It gives molecular and cellular systems more flexibility and easier adaptability. Moreover, many biological systems require an initial variegated behavior of its individual components for certain properties to emerge. For instance, symmetry breaking in an individual cell during cell polarization or cell migration is an emergent phenomenon of the collective action of polarity factors within the cell and depends on their initial fluctuating behavior (Altschuler et al., 2008). Also, emerging phenomena at the multi-cellular level, such as increased population robustness from bet-hedging strategies in prokaryotes (Veening et al., 2008b) to robust patterning in developing embryos (Collier et al., 1996), rely on phenotypic variability between individual cells.

Basic chemistry states that a system that relies on chemical reactions and molecules, no matter how complex, has a limit in its controllability and, at its steady state, will display at least minimal stochasticity as described by a single Poisson process (Thattai and van Oudenaarden, 2001). In this case, the likelihood for the occurrence of a single chemical reaction event remains constant over time and is independent of the occurrence of prior reactions. At the single-cell level, the variance in the abundance of a molecular species will be equal to its mean (Figure 1B). Concordantly, the few known cellular structures that are absolutely invariant in number, such as centrioles or the primary cilium, are all polymers, whose rare replication is controlled by multiple checkpoints and kinetic proofreading steps that push the chemical system far away from its steady state (Costa et al., 2013; Doxsey et al., 2005; Ishikawa and Marshall, 2011). On the other hand, if no control would exist, random fluctuations can become amplified during complex multi-step processes, which could lead to stochastic variation much larger than the Poisson limit (Figure 1C) (Blake et al., 2003; Raj and van Oudenaarden, 2008).

Single-cell distributions of molecular or phenotypic measurements often show variability much greater than the Poisson limit (Figure 1D) and are often described with multi-state stochastic models, which mimic the complex interplay of multiple inherently stochastic reactions that underlie the involved biochemical process (Figure 1D). However, in recent years it became clear that, at least in mammalian cells, such distributions can also be described by deterministic models based on features that quantify a cell's position along the cell cycle, its shape, or the extent of local cell crowding it experiences (Figure 1D), demonstrating that the outcome of a cell-fate decision, the level of a cellular activity, or the abundance of a molecule can actually be predicted to a high extent in single cells (Figure 1D) (Battich et al., 2015; Snijder et al., 2009). Accounting for such "hidden variables" in single-cell distributions has shown that the remaining variability often approaches a limit of minimal stochasticity (Figure 1D) (Battich et al., 2015; Gut et al., 2015; Islam et al., 2014; Padovan-Merhar et al., 2015). Thus, the largest fraction of the variability that identical cells display reflects the homeostatic ability of single cells to

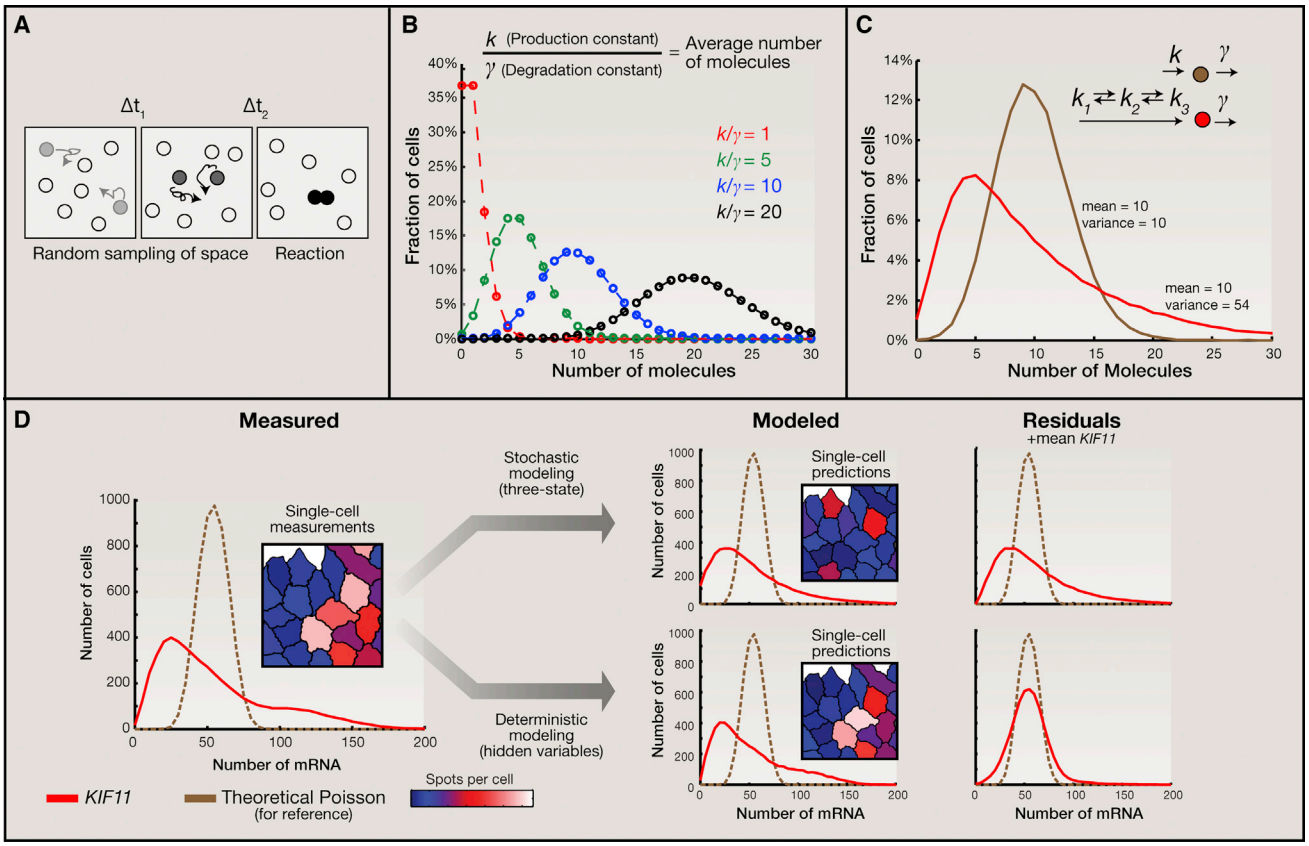

Figure 1. Chemical Noise and Regulated Variability in Single Cells

(A) Individual molecules (circles) sample a finite amount of space within a discrete interval of time (Δt). Radom behavior of molecules, such as thermal noise affecting their movement, renders the occurrence of a chemical reaction within a given Δt inherently stochastic.

(B) Due to this probabilistic behavior, the abundance of a molecule will vary between single cells even when it has a constant production and degradation rate. In this case, single-cell distributions of molecule abundance will fall on the limit of minimal stochasticity as described by a Poisson process, according to the ratio of production and degradation rates.

(C) A multi-state process, where the production rate (*k*) varies between multiple defined values (red), results in cell-to-cell variability (variance) that is much larger than the Poisson limit (brown).

(D) Stochastic models predict distributions, but not actual single-cell abundance, while deterministic models predict both, as shown for the example of *KIF11* mRNA in the cytoplasm (adapted from Battich et al., 2015). Measured (left) and modeled (center) single-cell distribution of *KIF11* mRNA abundance in the cytoplasm (red lines). Residuals show difference between single-cell measurements of *KIF11* mRNA abundance and modeled *KIF11* mRNA abundance. As a reference, Poisson distributions with a mean equal to the average abundance of *KIF11* mRNA are shown (golden lines).

adapt their activities and outcomes to a range of differences in their cellular state and microenvironment. As cell populations grow, these differences inherently emerge due to the physical, spatial, and chemical influences that cells have on each other (Snijder and Pelkmans, 2011). Consequently, we will use the term "noise" strictly to describe the variability that emanates from the inherent stochasticity of chemical reactions, rather than any variability observed between isogenic single cells.

This raises the question of how individual cells can avoid the amplification of noise in complex multi-step processes and allow them to display minimal stochasticity, such that regulated variability dominates the total variability observed. To understand this problem, we believe that it is helpful to borrow the distinction between active and passive noise control systems from engineering, where this has been actively researched for many years (Dolce et al., 2000; Franchek et al., 1996; Ver and Beranek, 2006). Like in biology, such systems differ in their energy requirement, but also in their intrinsic limitations and application scenarios (Bies and Hansen, 2009). While we will use the term noise "filtering" when we refer to the effect that a noise control system has, namely to filter our inherent stochastic fluctuations, but not regulated variability (Battich et al., 2015). As we will outline below, the mechanism by which this effect is achieved can be different, and we will use the mechanistic term (e.g., insulation, buffering, time-averaging) when referring more specifically to the mode of action.

In recent years, cellular noise control has mostly been addressed from the perspective of network theory, by studying the noise-filtering capacity of specific regulatory motifs (Balázsi et al., 2011). As we will discuss below, network motifs can be considered as forms of active noise filtering. Because active noise filtering has limitations in its effectiveness and scalability, it has been argued that noise cannot be effectively suppressed in single cells. We will point out that this assumption is not

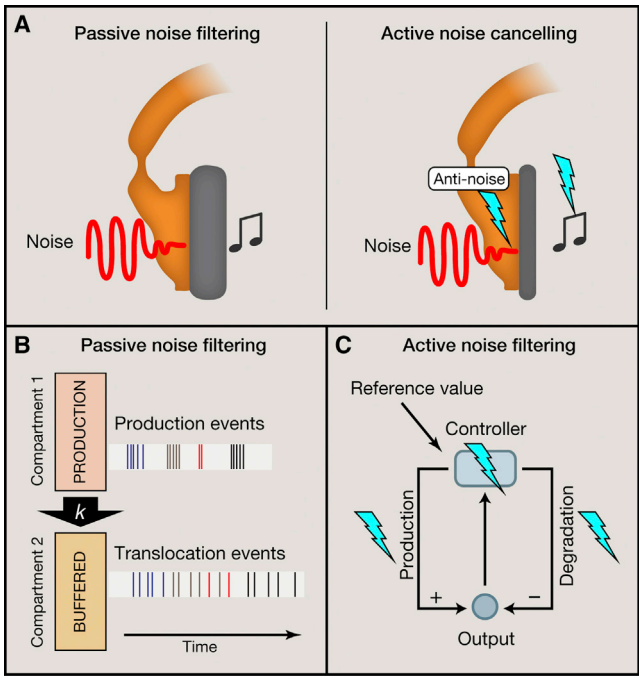

Figure 2. Active Noise Filtering Requires Energy and Passive Noise Filtering Is Based on Compartmentalization

(A) Passive (left) and active (right) strategies for ambient noise reduction (red wave) in headphones. In passive noise filtering, noise is dampened by insulation. In active noise cancellation, the energy-dependent generation of an anti-noise signal (blue flash) superimposes and thereby cancels the ambient noise.

(B) Passive noise filtering by cellular compartmentalization. If the translocation rate (*k*) becomes limiting, production events are averaged over time, leading to a low variability in the buffered compartment.

(C) Active noise filtering in the abundance of a molecule requires additional energy (blue flash) for molecule production, molecule degradation, and comparison of the present number of molecules with a reference value.

necessarily true, since there exists another class of noise filters in cells, which relies on passive noise filtering. In contrast to active noise filtering, passive noise filters can be highly effective, act broadly, and scale easily. Passive noise filtering relies on a hallmark (Knoll, 2011), but not exclusive (Diekmann and Pereira-Leal, 2013), property of eukaryotic cells, namely their physical compartmentalization.

In this review, we will at first outline differences between passive and active noise filtering in the context of biochemical reactions occurring within living cells. We will subsequently discuss the variety of membranous and non-membranous compartments for which evidence exists that they passively filter noise in molecular systems. We, however, emphasize that studies on passive noise filtering in biology are rare and that, for most forms of cellular compartmentalization, this has not yet been explicitly studied, with the exception of nuclear compartmentalization. We will thus dedicate a full section to the ability of the nucleus to filter noise in transcription while preserving regulated variability between single cells. Finally, we will place the role of cellular compartmentalization in noise filtering in the context of evolution and the emergence of multi-cellular organisms.

Active versus Passive Noise Filtering in Cells

A modern-day and well-known example of both active and passive noise filtering can be found in noise-canceling headphones. In such a device, active noise filtering is achieved by using the noise as the input for a controller that creates a signal to counteract the noise. Passive noise filtering is simply achieved by creating a separate compartment around the ear from which noise is prevented to enter (Figure 2A). Although the latter seems less sophisticated, it requires no extra energy, and in contrast to active noise cancelling, it does not introduce artifacts into the audio signal.

Mechanisms that rely on feedback or feedforward control can be considered as active noise filtering. In biology, these mechanisms are of a chemical nature, meaning that the information exchange to filter noise relies on biochemical reactions, which limits their efficiency (Figure 1B). Formally, feedback loops belong to the general class of closed-circuit control systems, in which the regulated entity generates a signal for the controller, which in turns, generates a signal for the regulated entity (Hopgood, 2012). Based on a theoretical framework to estimate the energy requirements that control systems would impose on cells, it was demonstrated that, while reducing stochasticity below the variability of a Poisson process is mathematically possible, the reduction of variability would maximally scale with the quadric root of the increase of signal-birth events in the controller. This scaling would soon render the energy requirements for a reduction of variability prohibitively high. Furthermore, this amount of energy would have to be spent for each cellular process independently (Lestas et al., 2010; Sun and Becskei, 2010). In feedforward control, the regulated entity is in part receiving its signal directly and in part indirectly through a relay via a controller, which can modify the signal that it forwards to the regulated entity. Such control schemes come at the cost of an additional intermediate control activity and often with an increased turnover of regulated molecules (Herranz and Cohen, 2010; Lan and Tu, 2013). This also limits their efficiency and scalability to globally cancel noise in cells.

Passive noise filtering has received less attention in biology, which stands in strong contrast to fields such as engineering (Bies and Hansen, 2009), in which it is considered the most effective and dominant mechanism (Rao, 2003). However, some early discussions on noise in biology did specifically point toward the potential of passive noise filtering (Vinogradov, 1998). In fact, it was noted relatively early that passive noise filtering in biology might be the predominant mechanism. This is a natural consequence of the involvement of cellular structures in signal processing, because most physical systems inherently attenuate high-frequency noise on input signals, which manifests itself as fluctuations occurring on short timescales, by imposing time lags and delays (Rao et al., 2002). In eukaryotic cells, the extensive sub-compartmentalization and the abundance of intracellular membranes offers ample passive noise-filtering opportunities (Figure 2B). Avoiding crosstalk of independent cellular processes and the spreading of molecules beyond one compartment already has an inherent noise-buffering effect (Chen and Silver, 2012). However, cellular compartmentalization can also physically influence the dynamics of molecular interactions. Such influences act on the

transition dynamics of molecules between compartments and on the probability of molecules to interact inside or on the surface of compartments, which can have strong effects on the propagation of fluctuations within cellular systems (Figure 2B). In fact, no matter how much noise may have been pre-amplified within a molecular system, as soon as the output of that system is moved from one to another compartment in a constant manner by a mechanism that delays the occurrence of individual molecules, the noise will be efficiently reduced toward a limit of minimal stochasticity (Figure 2B). The introduction of a rate-limiting step will, to some extent, uncouple the output of a system from the kinetics of its earlier chemical reactions. When a certain molecular species, which serves as the output of a reaction scheme, exerts its function in a buffered compartment, whereas the noisy multi-step process leading to the production of this molecular species occurs in another compartment, then the number of molecules in the buffered compartment will be given by their translocation rate into and their turnover within the buffered compartment. As a result, the stochastic variability in the buffered compartment will approximate the variability of a time-invariant formation and degradation process and, thus, the minimal stochasticity of a Poisson process. This contrasts a scenario in which noise resulting from the inherent stochasticity of a multi-step production process occurs in the same compartment where the formed molecule exerts its function. In such a case, filtering of pre-amplified noise would require control schemes that monitor the number of molecules and subsequently increase or decrease the number of molecules (Figure 2C). This increases the consumption of cellular energy by requiring additional synthesis and degradation events of the molecule and by the additional biochemical reactions that constitute the control system. In contrast, passive noise filtering requires no extra energy once the compartment exists. And even though the energy costs for building and maintaining compartments may be high, compartments have many purposes in cells, not just noise filtering. Their inherent ability to passively buffer noise can thus be seen as a highly beneficial consequence of their existence. In addition, a single compartment may achieve noise buffering for many processes simultaneously. For instance, nuclear compartmentalization has the potential to buffer noise in the transcriptional dynamics of every gene transcript in the cell that moves from the nucleus to the cytoplasm.

Types of Compartmentalization that May Passively Filter Cellular Noise

A first experimental indication that passive noise filtering by cellular compartmentalization occurs comes from observations that the total variability of protein levels between single isogenic yeast cells grown under identical conditions is particularly low for proteins that are translated at the endoplasmic reticulum and then transported to the cell surface or to compartments of the endocytic and exocytic membrane system (Newman et al., 2006). While this study did not fully separate noise from regulated variability, the relatively low variability of those proteins suggests that noise during protein production might have been filtered by passive mechanisms and compartmentalization. In the subsequent sections, we will focus on recent findings that more directly show the ability of non-membranous, as well as membranous, compartments to act as passive noise filters.

Inhomogeneous Fluids, Dynamic Aggregation, and Liquid Unmixing

Theoretical work on inhomogeneous fluids has shown that concentration-dependent transient multimerization of molecules can create short-lived micro-compartments that render chemical gradients robust to noise (Saunders, 2015). In addition, work on artificial cellular nanosystems revealed that molecular crowding agents increase the robustness of gene expression to perturbations in the concentrations of required ions and reagents such as potassium (K^+), magnesium (Mg^{2+}), ammonium (NH_4^+), spermidine, and folinic acid (Tan et al., 2013). Clearly, the cytoplasm of every cell, prokaryotic and eukaryotic, can be considered an inhomogeneous crowded fluid (Luby-Phelps, 2000, 2013). In particular, it recently became clear that intrinsically disordered domains in proteins can have a weak and reversible aggregating tendency that drives phase separation, or liquid unmixing, within the cyto- and nucleo-plasm of single cells (Kato et al., 2012; Weber and Brangwynne, 2012). Thus far, only one study has directly demonstrated the potential of weak and reversible aggregation to buffer intrinsic noise in cells. In *S. pombe*, the dual-specificity kinase Pom1 associates with the plasma membrane at cell tips and then diffuses on the membrane while displaying weak and reversible aggregation (Figure 3A). This creates a two-state gradient, which buffers against fluctuations in monomeric protein concentration and, together with the inherent time averaging of such a mechanism, allows robust specification of positional information (Figure 3A) (Saunders et al., 2012). Since genes coding for proteins with intrinsically disordered domains are abundant in eukaryotic genomes and enrich for genes that control cellular activities (Ward et al., 2004), the plausible role of these domains in passive noise buffering of both cellular information processing and spatial patterning of cellular components warrants further investigation.

Membranes as Semi-Permeable Barriers between Two Compartments

The hallmark property of eukaryotic cells is their extensive sub-compartmentalization by membranes (Diekmann and Pereira-Leal, 2013; Kirschner and Gerhart, 2005; Knoll, 2011). All membranous organelles in cells have the ability to allow transport of molecules across their membranes and, as such, may thus act as passive noise filters in cellular processes that involve these molecules. Although transport of molecules across membranes of exocytic and endocytic organelles does occur, in ions particularly, but also proteins (especially in the endoplasmic reticulum), the two compartments that show most extensive traffic across their membranes are mitochondria and the nucleus. All this traffic is funneled through, respectively, mitochondrial translocases and nuclear pore complexes. Regulatory mechanisms and signaling pathways that involve the movement of molecules between such compartments are, thus, strongly affected by the retention time of molecules within these compartments. Importantly, it is long known that the nuclear envelope has a tightly controlled density of nuclear pores (Maul et al., 1972) and also the density of mitochondrial translocases is tightly regulated (Maul et al., 1972; Wurm et al., 2011). This has the potential to

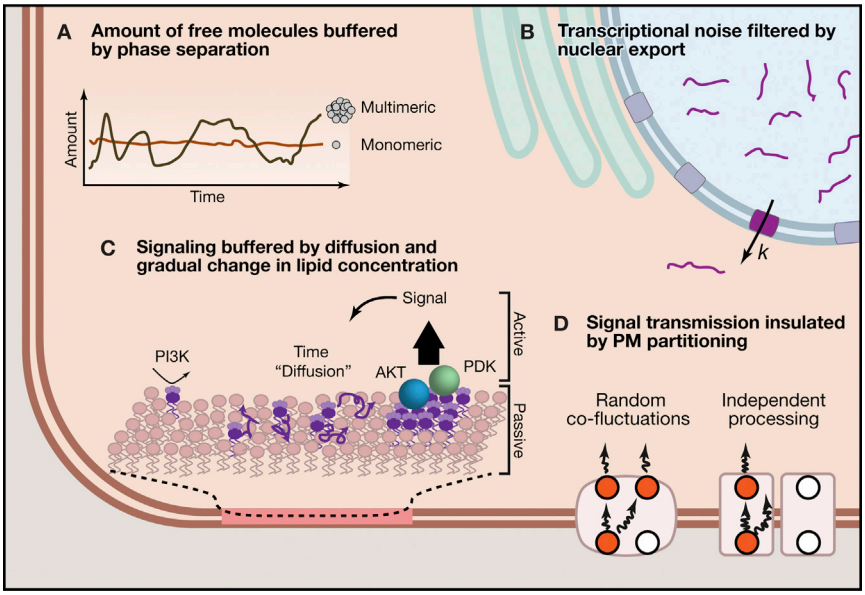

Figure 3. Examples of Cellular Compartmentalization that Can Passively Filter Noise

(A) Saturation-induced clustering through weak reversible aggregation buffers the amount of monomeric molecules.

(B) Compartmentalization of the nucleus and cytoplasm allows buffering of transcriptional noise (see also Figure 4).

(C) The diffusing phosphoinositide pool in the plasma membrane (violet) acts as a capacitor that integrates upstream signals over time.

(D) Spatial partitioning of the membrane eliminates noise in the propagation of signals by lowering random correlations between molecules during signal processing.

turn a highly fluctuating process on one side of the compartment boundary into a constant process with minimal stochasticity on the other side (Figure 3B). For mitochondria, the role of compartmentalization in passively buffering noise has not yet been directly demonstrated, but the importance of mitochondrial integrity for the emergence of non-stochastic cell-to-cell variability in the timing of apoptosis (Spencer et al., 2009) may suggest that it plays such a role. For nuclear compartmentalization, some of its roles in passive noise filtering have recently been explicitly addressed by multiple studies (Figure 3B) and currently serves as a paradigm of passive noise filtering by compartmentalization. These studies will be discussed more extensively in the next section.

Membranes as Capacitors and Inhomogeneous 2D Fluids

Besides creating boundaries between compartments, membranes have some unique properties in offering a two-dimensional inhomogeneous fluid for signal integration (Kusumi et al., 2012). Most cellular processes involve some molecules that are membrane integrated, lipid anchored, or membrane associated. These include transmembrane receptors, palmitoylated and GPI-anchored subunits of G-proteins, prenylated GTPases such as Ras, and importantly, also specific lipid species, such as various forms of phosphoinositides, to which multiple kinases can bind via their lipid-binding domains. The movement of these molecules is restricted to diffusion within a two-dimensional plane, which is influenced by the extent of lipid ordering in the membrane. This allows membranes to accumulate information from signaling events occurring at one timescale and to pass this information on at a different timescale, which can be tuned by lipid transporters that alter membrane-lipid composition (Figure 3C) (Frechin et al., 2015). This property of membranes can be compared to tunable capacitors in electronic circuits, which passively store and integrate information from upstream signals, and whose total capacity can be modulated in order to adapt signal transmission to different timescales. While tunable capacitors are thus potent noise-buffering devices, the concept of membranes as tunable capacitors has just emerged (Figure 3C) (Frechin et al., 2015), and their roles in noise filtering within living cells remain to be investigated. Such tunable capacitors may have highly interesting and unusual properties, since they combine passive noise filtering (time integration through diffusion) and active noise filtering (using enzymes that alter the diffusion coefficient in a feedback mechanism) (Figure 3C). Here, the active component scales well, since it controls the noise filtering properties of the passive component, which can physically affect all processes that rely on membrane diffusion. Besides these global properties, biological membranes are also inhomogeneous and allow the transient and reversible coalescence of lipids and proteins with a somewhat higher affinity for each other (Simons and Toomre, 2000), possibly aided by the underlying cytoskeleton (Kusumi and Sako, 1996). Besides the physical effects of such dynamic spatial partitioning on noise, as pointed out above for liquid unmixing in the cytoplasm, it may improve the reliability of biochemical signaling in membranes by suppressing random co-fluctuations between molecules (Figure 3D) (Mugler et al., 2013).

The Role of Nuclear Compartmentalization in Passive Noise Filtering

Buffering Stochastic Bursts in Gene Transcription

Transcript synthesis occurs in bursts, in which a large number of mRNA molecules are transcribed in short periods during "on" times, followed by relatively long inactive periods due to chromatin remodelling, or "off" times (Figure 4A) (Raj et al., 2006; Suter et al., 2011). Such discontinuous transcript synthesis, which can give rise to fluctuations in transcript abundance in the nucleus far exceeding the Poisson limit, can in principle be efficiently buffered in the cytoplasm by imposing a time delay in nuclear export (Bahar Halpern et al., 2015; Battich et al., 2015; Singh and Bokes, 2012; Xiong et al., 2010). The time it takes for a nascent RNA transcript to appear in the cytoplasm is the convolution of multiple processing and quality-control steps in the nucleus and the transport rate into the cytoplasm, which generates probabilistic nuclear retention times (Battich et al., 2015). Intuitively, if mRNA export is not itself a burst-like

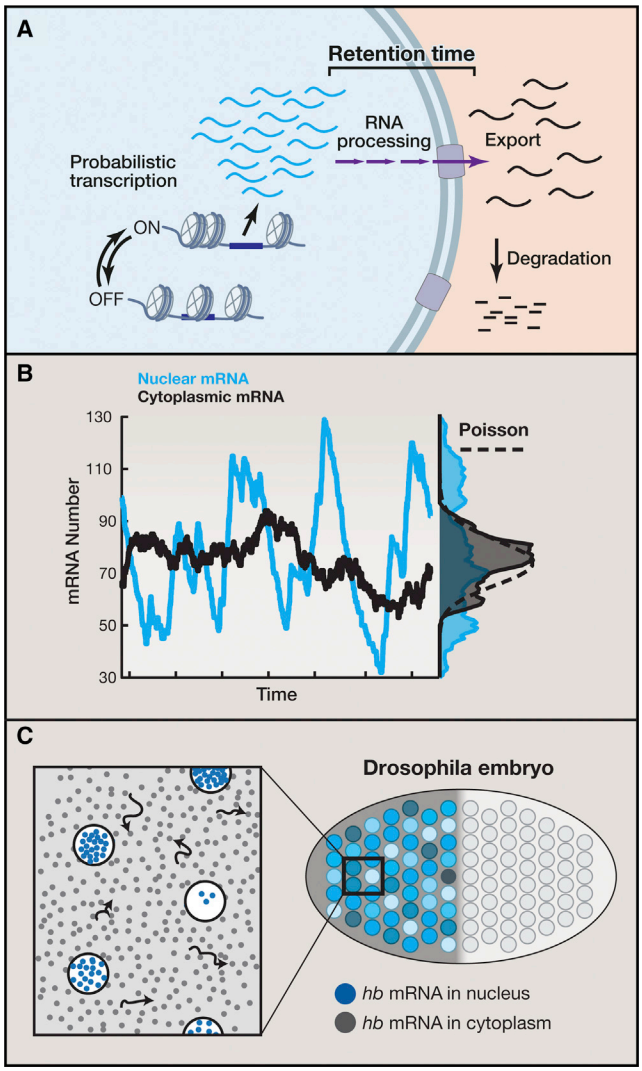

Figure 4. Examples of Passive Noise Filtering in Transcript Abundance by Nuclear Compartmentalization

(A) Transcription is a discontinuous and multi-state stochastic process, which introduces noise in the levels of mRNA. RNA processing following synthesis results in a probabilistic delay in the export of the mRNA molecules from the nucleus to the cytoplasm, which can buffer noise introduced during transcription.

(B) Stochastic simulation of nuclear and cytoplasmic transcripts in the same single cell. Cytoplasmic variability approaches the Poisson limit of variability.

(C) Spatial precision of the *hunchback* (*hb*) mRNA in the *Drosophila* embryo, despite the fact that transcriptional noise is achieved by low degradation rates and diffusion of mRNA molecules in a shared cytoplasm (adapted from Little et al., 2013).

process, i.e., the mRNA export probability is constant over time, and the mean retention time approaches or exceeds the promoter "off" time, the appearance of transcripts in the cytoplasm would be buffered against burst-like synthesis events and would display minimal stochasticity of a Poisson process (Figure 4B). Because time integration due to low cytoplasmic degradation rates of transcripts can also attenuate noise, the effect of buffering fluctuations by nuclear retention would be mostly observed in the cytoplasm of cells if the degradation rate of transcripts is faster or within the same order of magnitude as the nuclear retention time (Bahar Halpern et al., 2015; Battich et al., 2015). One important characteristic of a buffer by nuclear retention is that, unlike modulating the degradation rate, and when assuming no degradation of mRNA in the nucleus, at the steady state, retention has a minor impact on the mean expression levels.

Indirect support to the idea of buffering variability in cytoplasmic transcript abundance by nuclear retention is given by the finding that a major fraction of the transcriptome of mammalian cells resides in the nucleus, pointing to significant retention times (Bahar Halpern et al., 2015; Djebali et al., 2012). Such retention time can be viewed as the integrated effect of multiple probabilistic events including chromatin dissociation, nuclear diffusion, RNA splicing, polyadenylation, binding to proteins and export factors, binding to the nuclear pore, and successful transport across the nuclear pore (Battich et al., 2015). Each of these steps can add to retention time, including the last step of nucleo-cytoplasmic transport of mRNA molecules, which has been shown to be a process of relatively low probability, sometimes involving the scanning of several nuclear pore complexes by the mRNA prior to successful export (Grünwald and Singer, 2010). From published measurements on the amount of newly transcribed transcripts associated with chromatin, present in the nucleoplasm and in the cytoplasm of human macrophages at multiple time points after stimulation with LPS (lipopolysaccharide) (Bhatt et al., 2012), it was estimated that even under conditions of strong acute gene induction, the nuclear retention time of human transcripts is on average 20 min (Battich et al., 2015). Importantly, even for immediate early response genes such as *JUN*, *FOS*, and *NR4A2*, which are known to show fast RNA induction and degradation rates, the nuclear retention time of their transcripts was, in different mammalian cell types, about 6–10 min, which is strikingly similar to their induction and degradation rates (Battich et al., 2015). Under such conditions, nuclear retention is the largest contributor to buffering fluctuations in gene transcription caused by stochastic bursts and has the ability to reduce this noise by a factor of three to four. Thus, when transcripts are synthesized and degraded at a high rate, nuclear retention does not need to be long to allow efficient noise buffering. As a consequence, these genes show lower cell-to-cell variability in transcript abundance in the cytoplasm than in the nucleus. Importantly, a direct experimental test could confirm a role for nuclear retention in buffering noise in the cytoplasm. Overexpression of the nuclear pore complex protein NUP153, which slows down nuclear export of transcripts (Bastos et al., 1996), resulted in a further reduction of cytoplasmic variability of *JUN* transcripts without affecting mean abundance (Battich et al., 2015). Furthermore, long-term (5 hr) time-lapse imaging of tetracyclin-induced transcription of a synthetic construct in human cells showed that, upon a burst of transcription, transcripts display transient accumulation on the inside of the nuclear envelope and a higher autocorrelation over time in the cytoplasm than in the nucleus (Battich et al., 2015). A second, independently conducted study confirmed that, also within mammalian tissues, many RNA transcripts are retained for a considerable time in the nucleus before being released and also indicated that this may contribute to buffering noise in gene transcription (Bahar Halpern et al., 2015).

Noise Buffering in D. melanogaster *Syncytia*

The effect of nuclear compartmentalization on noise in gene transcription has also been documented in an entirely different model system, namely during the syncytial stages of *D. melanogaster* development (Figure 4C). In this system, mathematical analysis and simulation studies suggested that when the synthesis of *hunchback* (*hb*) gene products has a super-Poissonian noise component, as a consequence of stochastic bursts in gene transcription, sufficient precision in the *hb* anterior-posterior expression boundary of the *Drosophila* syncytium can only be achieved by some form of noise buffering (Erdmann et al., 2009). Indeed, experimental measurements confirmed that the nuclear content of *hb* nascent transcripts varies by 22% between the individual nuclei of an embryo, while variation of transcript content of cytoplasmic regions around each nucleus in the syncytium is only about 6% (Figure 4C) (Little et al., 2013). The major part of this reduction was attributed to temporal averaging of multiple bursts in the cytoplasm as a result of low cytoplasmic degradation rates, and a small part was attributed to spatial averaging (Little et al., 2013). Nuclear retention was not specifically addressed in this system but could also play a considerable role.

Nuclear compartmentalization enables regulated variability to dominate. All mechanisms described above that relate to nuclear compartmentalization depend on passive processes, such as nuclear retention, temporal integration, and spatial averaging. These processes are generally applicable to multiple mRNA species without the need of evolving specific active buffering mechanisms for each of these species separately. Importantly, such probabilistic processes can maximally reduce the noise in a system to one of minimal stochasticity as given by the Poisson limit. This corresponds with our finding that, while transcript abundance in the cytoplasm of genetically identical human cells grown under identical conditions can show large cell-to-cell variability, it can be accurately predicted at the single-cell level by a multivariate set of features that describe the cellular state and its microenvironment (Battich et al., 2015). What is left unpredicted reaches the limit of minimal stochasticity, indicating the importance of such general noise-attenuating mechanisms in the control of cell-to-cell variability in mammalian cells. It allows predictable variability to dominate for most transcripts, stemming from regulatory mechanisms that scale transcript abundance with cell volume, cell surface area, cell shape, local cell crowding, the activity of neighbors, or position in the cell cycle.

Although the exact molecular mechanism by which this scaling is achieved is in many cases still unknown, it can act at the level of gene transcription. Nuclear retention will namely only affect regulatory changes in transcription that occur at timescales shorter than the retention time, which are irrelevant for these transcript homeostatic mechanisms. Such mechanisms may also act at the level of transcript degradation in the cytoplasm, although a recent study has indicated that this is not the case for scaling transcript abundance to cell volume (Padovan-Merhar et al., 2015). While continued research will be necessary to elucidate the specific molecular mechanisms for single-cell transcript homeostasis, nuclear compartmentalization might also be an essential component in these scaling mechanisms. For instance, under varying concentrations of extracellular calcium, pulsatile shuttling of the calcineurin-responsive zinc-finger transcription factor Crz1 between the cytoplasm and the nucleus of *S. cerevisiae* enables a proportional scaling of multiple target genes with different promoter kinetics without the need of active control mechanisms (Cai et al., 2008). Similarly, simulations show that nuclear compartmentalization and passive transport by diffusion have the potential to largely eliminate noise in the nuclear concentration of a protein during its induced nuclear accumulation (Albert and Rooman, 2015). Importantly, this does not imply that the amount of cell-to-cell variability in the induced nuclear accumulation of transcription factors would approach the Poisson limit. On the contrary, such variability can be very high, as studies in mammalian cells on induced nuclear translocation of NFkB, Myc, and Erk have shown (Albeck et al., 2013; Tay et al., 2010; Wong et al., 2011). It, however, suggests that most of this cell-to-cell variability does not stem from the amplification of noise in the cytoplasm (the non-buffered compartment) prior to transcription factor translocation into the nucleus (the buffered compartment) but from extrinsic sources, which act at longer timescales. In support of this view, multiple hidden variables in single-cell distributions of transcription-factor accumulation in the nucleus are now being identified (Buganim et al., 2012; Gut et al., 2015; Sero et al., 2015), revealing previously unappreciated biology.

Compartmentalization and the Rise of Multicellularity during Evolution

The role of cellular compartmentalization in evolution has been extensively discussed from many angles (Bogorad, 1975; López-García and Moreira, 2006; Madhani, 2013; Martin and Koonin, 2006). Intriguingly, obligate complex multicellularity, in which only some cells are in direct contact to the environment (Knoll, 2011), only evolved from eukaryotic cells and did so multiple times independently within different clades. This suggests that cellular compartmentalization gave some unique properties that favored an obligate cooperation between single cells. Compartmentalization is generally regarded as a means to allow more complex processes and more extensive regulation to evolve, which is considered necessary for the development of complex multicellular life forms (Kirschner and Gerhart, 2005; Knoll, 2011). A paradigm example of additional complexity is nuclear compartmentalization, a major evolutionary transition that distinguishes eukaryotes from archaea and prokaryotes. It is considered to have been necessary for managing more complex and larger genomes, to allow abundant RNA splicing to emerge, to allow higher levels of gene regulation, and to protect the integrity of the genome (Mekhail and Moazed, 2010). Nevertheless, some prokaryotic genomes are larger than small eukaryotic genomes, and an increase in genome size is not strictly correlated with a higher complexity in life form. In addition, some prokaryotes, such as Planctomycetes, enclose their chromosomes in a membranous subcellular compartment. However, unlike eukaryotic nuclei, these compartments do not separate gene transcription from translation (Fuerst and Webb, 1991). We propose that another consequence of cellular compartmentalization lies in its ability to make phenotypic variability more robust against molecular noise by acting as a passive noise filter of stochastic,

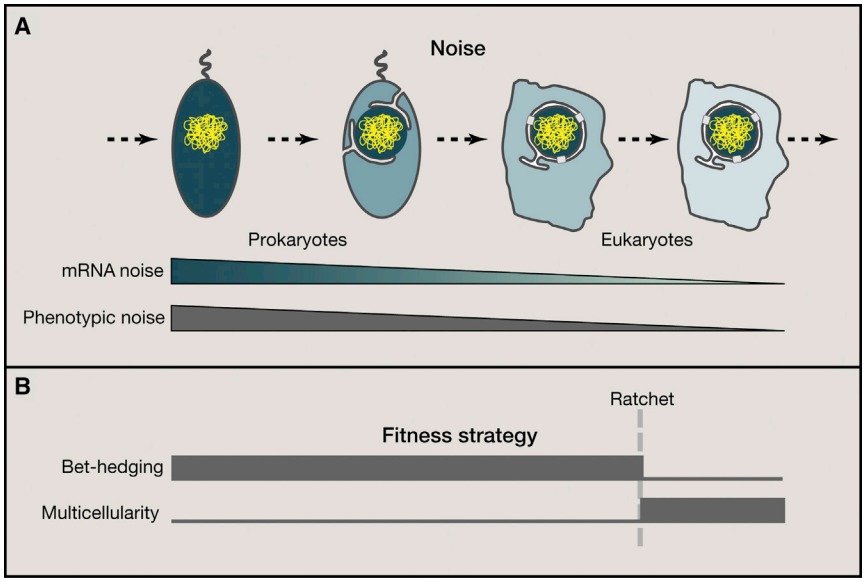

Figure 5. Passive Noise Filtering by Nuclear Compartmentalization in the Evolution of Multicellularity

(A) Analogously, during the acquisition of a nucleus in evolution, the abundance in mRNA molecules shows a higher level of noise close to the production site, the genome (yellow). In small cells without any genome compartmentalization, this high level of noise will be similar throughout the cell. Since translation into proteins is not spatially separated from transcription, this noise can propagate into the phenotype. As the genome becomes more enwrapped by membranes and protein translation becomes more separated from gene transcription, fluctuations in mRNA abundance may propagate less into the phenotype. When a complete eukaryotic nucleus with nuclear pores is acquired, this effect is largest.

(B) The idea of filtering noise by nuclear compartmentalization acting as an evolutionary ratchet toward obligate multicellularity. High phenotypic noise favors the fitness of organisms relying on random bet-hedging strategies but reduces the fitness of an organism relying on tight coordination between single cells. As phenotypic noise reduces, it favors the fitness of organisms relying on tight coordination between single cells and on complex multicellularity, including differentiation, but reduces the fitness of an organism relying on random bet-hedging strategies.

high-frequency fluctuations in transcription (Figure 5A). Although this may have reduced the response time due to inherent delays of passive noise filtering, it came with increased predictability of single-cell behavior, which is essential for stable cooperation (Axelrod, 1997). This could be explained by considering passive noise filtering through nuclear retention as an evolutionary ratchet, which has opposing effects on the fitness of a species in a unicellular and multicellular context and thus stabilizes multicellularity (Libby and Ratcliff, 2014). In unicellular organisms, cell-intrinsic phenotypic variability that emerges from an unregulated variability of the cellular phenotype can confer a selective advantage, since such bet hedging can increase the chance that a small fraction of cells survives variable environmental conditions (Blake et al., 2006; Veening et al., 2008a). On the other hand, in complex multicelluar organisms, phenotypic variabilty of single cells is coupled to extrinsic sources, such as spatial differences between single cells, and subsequently reinforced by cell differentiation. And while some level of intrinsic fluctuation in molecular reactions may be beneficial for multicellular pattern formation and collective cell behavior (Altschuler et al., 2008), an amplification of it would result in uncontrolled phenotypic variability, reducing the fitness of an obligate multicellular organism (Figure 5B) (Ben-David and Benvenisty, 2011; Gilbert et al., 2007). The notion that passive noise filtering through nuclear retention of transcripts acted as an evolutionary ratchet in the emergence of multicellularity seems supported by several findings that eukaryotic genes quickly reacting to external stress evolved unusual properties, which aim to minimize the extent of nuclear retention of their transcripts (Culjkovic et al., 2006; Lei et al., 2011; Taddei et al., 2006).

Although circumstantial, some experimental support for the importance of cellular compartmentalization during evolution in the context of noise filtering comes from work done in the field of artificial intelligence. A genetic algorithm was used to automatically evolve a physically existing electronic circuit on a programmable gate array that could distinguish a high-frequency (comparable to molecular stochastic fluctuations in biology) from a low-frequency signal (comparable to regulated variability in biology). Even though energy supply was not limiting, the resulting best-performing circuits had evolved elements beyond conventional engineering motifs (e.g., feedback or feedforward motifs) and relied on physical and spatial properties of the implementation medium, which passively influence signal processing (Mellis and Raj, 2015; Thompson, 1997).

General Conclusions and Outlook

In this review, we have made an attempt to bring together various lines of evidence to argue that cellular compartmentalization allows passive noise filtering in molecular and cellular processes. While this notion has only recently been explicitly addressed in two studies on the role of nuclear compartmentalization to filter out stochastic bursts in gene transcription (Bahar Halpern et al., 2015; Battich et al., 2015), it is implicitly present in a multitude of quantitative studies on cellular compartmentalization and the inhomogeneity of biological fluids and membranes.

Passive noise filtering may well be the dominant mechanism of noise reduction in biology, at least down to a level of minimal stochasticity as given by the Poisson limit, which can be strikingly easily achieved once compartments exist. Active noise filtering may be in place when cellular outcomes need to have a certainty greater than the Poisson limit, but these cases are, as far as we currently know, rare. This suggests that the prime reason for the ample presence of feedback and feedforward motifs in regulatory networks is not their potential noise filtering capacity but rather their generally known ability to drive emerging properties such as transient, oscillatory, or sustained outputs.

Enabling cellular processes to achieve minimal stochasticity increases the predictability of their outcomes. This does not imply that processes are invariable between single cells, but that cell-to-cell variability in molecular readouts or cellular activities is largely determined by extrinsic sources, which allows the prediction of single-cell behavior once these sources are known. Through its passive noise-filtering capacity, cellular compartmentalization thus reconciles the stochastic and the deterministic worlds in quantitative biology. It also explains that cell-to-cell variability among prokaryotic cells grown under identical conditions can be more stochastic than cell-to-cell variability among eukaryotic and, in particularly, among mammalian cells (Johnston and Desplan, 2010). However, some forms of compartmentalization that we discussed also occur in prokaryotes, implying that the assumption that prokaryotic cell-to-cell variability is largely stochastic may also be wrong (St-Pierre and Endy, 2008). This is a more balanced view on measurements obtained from large numbers of single cells and an important one to stress in the current time of mass adoption of single-cell technologies. Finally, we hope that by the long overdue placement of cellular compartmentalization in the limelight of noise buffering, more research will be directed to passive noise filtering in biology, which will contribute to unraveling how systems properties such as robustness of cellular processes can arise from cellular compartmentalization.

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