Trends in
Cell Biology

CellPress

Review

Mechanisms of cellular mRNA transcript homeostasis

Scott Berry [1,2,*] and Lucas Pelkmans [1,*]

For most genes, mRNA transcript abundance scales with cell size to ensure a constant concentration. Scaling of mRNA synthesis rates with cell size plays an important role, with regulation of the activity and abundance of RNA polymerase II (Pol II) now emerging as a key point of control. However, there is also considerable evidence for feedback mechanisms that kinetically couple the rates of mRNA synthesis, nuclear export, and degradation to allow cells to compensate for changes in one by adjusting the others. Researchers are beginning to integrate results from these different fields to reveal the mechanisms underlying transcript homeostasis. This will be crucial for moving beyond our current understanding of relative gene expression towards an appreciation of how absolute transcript levels are linked to other aspects of the cellular phenotype.

Highlights

mRNA abundance is coordinated with cell size to achieve mRNA concentration homeostasis.

Absolute transcript production rates increase with cell size.

RNA polymerase II activity and abundance are key points of control underlying transcript homeostasis.

Feedback mechanisms lead to quantitative interdependence of mRNA production, nuclear export, and degradation rates, making mRNA concentration homeostasis remarkably robust to perturbation.

RNA metabolism, cell size, and the cell cycle are closely linked, so interpretation of the outcome of perturbations must account for changes in cell size and cell-cycle distribution, and carefully distinguish concentration from abundance.

mRNA concentration homeostasis in single cells

Homeostatic mechanisms provide cells with an ability to maintain constant concentrations of biochemical reactants and are therefore crucial for proper cellular function. One example of this is the setting and maintaining of constant concentrations of mRNA transcripts. This is likely to be important for all life forms. In multicellular organisms, cells have a wide range of sizes while often having the same amount of DNA. In unicellular organisms, cell sizes vary according to environmental conditions and cells can grow and divide at different rates. In both cases, the transcriptome arising from a constant DNA template must be coordinated with cell size (Figure 1A).

The topic of mRNA transcript homeostasis has been studied sporadically over the past 60 years and overlaps with the question of how cell size is determined (reviewed in [1–3]). Whereas historical work on scaling of transcript abundance with cell size focused mainly on bulk biochemical comparisons of differently sized cells (reviewed in [4,5]), more recent studies have combined approaches from genetics, quantitative imaging, next-generation sequencing, and mathematical modeling. These studies have begun to provide glimpses of the mechanisms underlying transcript concentration homeostasis in single cells.

We begin by briefly reviewing the evidence for scaling of mRNA abundance with cell size and then delve deeper into how **mRNA production rates** (see Glossary) and **mRNA degradation rates** are coordinated with cell size. We then discuss how the coordination of these rates with one another has been revealed through perturbation studies, and finally describe the current state of knowledge regarding the mechanisms underlying mRNA concentration homeostasis.

mRNA abundance scales with cell size

Single-molecule RNA fluorescence *in situ* hybridization (smFISH) together with quantification of cell size allows gene-specific measurement of mRNA concentration at the single-cell level. This has been performed in unperturbed *Schizosaccharomyces pombe* [6], *Saccharomyces cerevisiae*

[1]Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
[2]European Molecular Biology Laboratory (EMBL) Australia Node in Single Molecule Science, School of Medical Sciences, University of New South Wales, Sydney, Australia

*Correspondence:
scott.berry@unsw.edu.au (S. Berry) and
lucas.pelkmans@mls.uzh.ch
(L. Pelkmans).

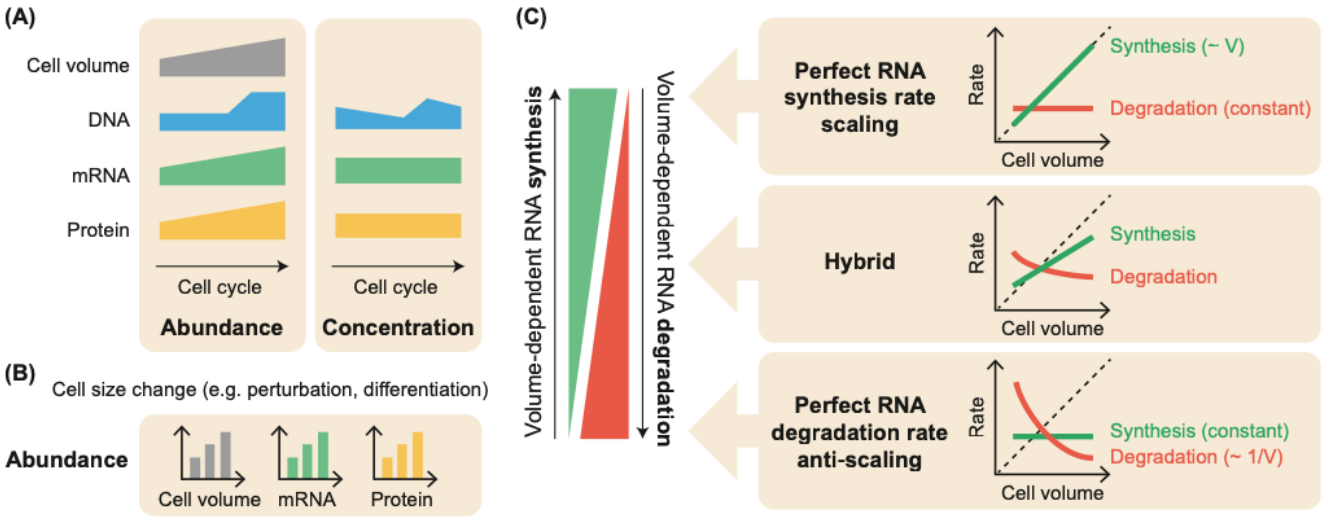

Figure 1. Cellular mRNA concentration homeostasis. (A) Unlike the DNA template, the amount of mRNA in a cell increases over the cell cycle in proportion to the absolute abundance of protein and the volume of the cell. Total mRNA and protein concentrations are typically constant. (B) mRNA and protein abundance increase with cell volume when cell size is perturbed or changes during differentiation. (C) mRNA concentration homeostasis requires that mRNA production rates increase with cell size or that mRNA degradation rates decrease with cell size, or some combination of the two ('hybrid') (Box 2).

[7], *Arabidopsis thaliana* [8], *Caenorhabditis elegans* [9], and human cells [9–12]. With a few notable exceptions (Box 1), these experiments have revealed that gene-specific transcript abundance is generally coordinated with cell size – known as **size-scaling**. This is in agreement with bulk studies of total cellular RNA content in rat, which showed that differences in average cell size roughly reflect differences in RNA to DNA ratios over a 16-fold range, indicating that the amount of RNA produced from the DNA template scales with cell size over a large range *in vivo* [13].

In *S. pombe*, cell size can be altered in mutants that uncouple cell growth from division. In these cases, mRNA abundance changes are coordinated with changes in cell size [14–16], indicating that mRNA concentration is maintained despite changes in cell size. Moreover, a recent image-based screen in human cells across hundreds of genetic perturbations also revealed that total cellular RNA and mRNA abundances typically remain stable even when cell size or bulk RNA production rates are dramatically altered [12,17]. mRNA abundance is therefore closely and robustly linked to cell size to maintain constant concentrations of mRNA transcripts (Figure 1B).

Coordination of mRNA synthesis rates with cell size

The average lifetimes of mRNA molecules are typically an order of magnitude shorter than the timescale over which cells grow (reviewed in [18]). For example, in *S. cerevisiae*, which divides approximately every 90 minutes, the median mRNA half-life is 11 min [19]. By contrast, in mammalian cells that divide approximately once per day, the median mRNA half-life is 3.4 h [20]. These short mRNA lifetimes underlie the ability of cells to rapidly change concentrations of specific transcripts by simply changing the production rate (and not necessarily the degradation rate) of these transcripts. However, such short lifetimes also imply that mRNA molecules are typically replenished several times within a cell cycle. mRNA synthesis and/or mRNA degradation rates must therefore be controlled according to cell size to achieve mRNA concentration homeostasis (Box 2).

In principle, cells could coordinate mRNA abundance with cell size by either increasing mRNA production rates or decreasing mRNA degradation rates in larger cells – or by a combination of

Box 1. How pervasive is transcriptome size-scaling?

Profiling the transcriptomes of *S. pombe* mutants of different cell sizes revealed that the relative expression levels of individual genes remain constant over a wide range of cell sizes even though the absolute mRNA abundance increases proportionally to cell size changes [16]. This suggests that size-scaling is a transcriptome-wide phenomenon. Combining imaging and single-cell RNA-seq of unperturbed *S. pombe* cells also led to similar conclusions, although a few genes that escape size-scaling were reported [93]. In agreement, comparing populations of G1-arrested *S. cerevisiae* cells of different sizes, as well as G2/S/M cells sorted into different size fractions, indicated that most transcripts scale with cell size [7]. In this case, notable exceptions were mRNAs encoding histone proteins, which scale with DNA content rather than with cell size, and also *WHI5* – a gene involved in cell-cycle control and cell size regulation [7]. Evidence so far suggests that the promoter sequences of these genes underlie their ability to escape transcriptional scaling, resulting in reduced mRNA concentration in larger cells [7,94].

In human cells, smFISH of 923 genes revealed that cytoplasmic transcript abundance increased with cell size for all genes studied [11]. However, although many genes showed close coupling with cell size, other genes showed additional coordination with other cell features such as cell-cycle stage or local cell crowding. One striking example was a set of genes that showed more precise coordination with cell area than with cell volume. Intriguingly, the proteins encoded by these area-scaling genes tended to have functions associated with membranes, or to encode membrane-associated proteins – providing a biological rationale for why they should scale with surface area. This suggests that precise control of protein abundance in cells of different shapes is at least partially controlled at the level of mRNA abundance. Similar results were obtained in *S. cerevisiae* where ploidy-associated changes in cell size were found to be associated with changes in the relative levels of transcripts encoding cell-surface proteins [95]. In this case, changes to cell volume alter the surface-area-to-volume ratio, potentially affecting the relative abundances of transcripts that scale with surface area or volume. In mouse hepatocytes, cytoskeletal and mitochondrial genes also increase their relative mRNA abundance when cell size is increased *in vivo* [96]. Moreover, levels of ATP-binding cassette (ABC) transporter transcripts in mouse cells have been shown to be continuously regulated by local cell crowding via a cell-intrinsic process involving signaling from focal adhesions to adapt lipid composition [97]. In addition to these transcriptional mechanisms, recent proteomic studies [85,87] suggest that there are also post-transcriptional mechanisms that change the concentrations of specific proteins as cell size changes (without necessarily changing the concentrations of their respective mRNA transcripts).

Histone transcripts scaling with DNA content, membrane-protein transcripts scaling with surface area, and ABC-transporter transcripts scaling with local cell crowding all suggest that there is more than one mechanism of 'scaling'. Although volume-scaling is certainly the most commonly observed and accounts for the majority of transcript abundance variability in cell populations, transcript homeostasis is clearly multilevel – with each gene coupled to different aspects of the cellular phenotype. It is currently unclear whether these other mechanisms operate in addition to a generic volume-scaling or whether they are independent.

Glossary

Gene expression heterogeneity: variability in transcript abundance between cells in a population or tissue.
mRNA buffering: (also sometimes known as mRNA crosstalk) refers to coordinated changes in mRNA production and degradation rates when one or the other of these processes is perturbed.
mRNA degradation rate: also known as the mRNA decay rate, the probability per unit time of an mRNA transcript being degraded. Equal to the inverse of the mean lifetime of an mRNA transcript.
mRNA production rate: also known as the mRNA synthesis rate, the probability per unit time of an mRNA transcript being produced (either at a single gene or for an entire cell). Note that there are alternative definitions [92].
Poisson process: a model for a series of discrete events (for example, RNA synthesis events) that occur with a fixed average rate but are independently and randomly distributed in time.
Size-scaling: relates to the observation that mRNA and protein abundance increases proportionally with cell size.
Transcriptional burst: a period of high transcriptional activity during which multiple transcripts are produced from a single gene in a short time. Bursts are interspersed with periods of inactivity during which no transcripts are produced. A gene that undergoes transcriptional bursting may be said to be 'bursty'.

both mechanisms (Box 2 and Figure 1C). This has been studied using bulk measurements to examine how mRNA production rates vary across the cell cycle in synchronous cultures, or to compare mRNA production and degradation rates in populations of cells with different sizes. More modern approaches have focused on these rates in single cells. Mathematical modeling has also been important in guiding understanding in this field. We now review each of these approaches.

Bulk studies

Metabolic pulse labeling in synchronous *S. pombe* [15,21–23] and human cell cultures [24,25] revealed that mRNA synthesis rates increase throughout the cell cycle as cells grow. However, different studies (even on the same organism) show conflicting results as to whether the increase in mRNA synthesis occurs in a step-like manner coupled to DNA replication, or instead scales more continuously with cell size changes. Some of these differences likely relate to different synchronization procedures and/or growth conditions (reviewed in [4]).

The isolation of an *S. pombe* cell-cycle mutant with reduced cell size [14] allowed the first studies connecting RNA production rates to altered cell size. These mutants, which have the same DNA content but reduced protein and RNA abundance [14,22], were found to have reduced RNA synthesis rates [22]. Later work extended these studies to consider different mutants that result in DNA-to-protein ratios which vary over a fivefold range [16]. Similarly, they found that RNA production rates for both total and mRNA are coordinated with changes in cell size, provided

Box 2. Volume-dependence of mRNA synthesis or degradation rates

Considering Pol II-mediated transcription as a simple birth–death process, mRNA is produced with probability per unit time, k_s, and degraded with probability per unit time, k_d We refer to k_s and k_d as mRNA synthesis and mRNA degradation rates, respectively. The evolution of the number of molecules of mRNA over time, n, is given by:

$$\frac{dn}{dt} = k_s - k_d n \quad [I]$$

where n is related to the mRNA concentration ρ, by $n = \rho V$, where V is the cell volume. In terms of mRNA concentration, this equation can therefore be written as:

$$\frac{d\rho}{dt} = \frac{k_s}{V} - \left(k_d + \frac{1}{V}\frac{dV}{dt}\right)\rho \quad [II]$$

When mRNA degradation is much faster than mRNA dilution due to cell growth $\left(k_d \gg \frac{1}{V}\frac{dV}{dt}\right)$, we can neglect the last term. In this case, to achieve mRNA concentration homeostasis ($d\rho/dt = 0$), the quantity $\frac{k_s}{Vk_d}$ must be independent of volume. This is true when mRNA synthesis rates scales perfectly with volume ($k_s = \alpha V$, $k_d = \beta$, for some constants $\alpha, \beta \geq 0$) or when mRNA degradation rates scale inversely with volume $\left(k_s = \alpha, k_d = \frac{\beta}{V}\right)$. However, there are also other possibilities (e.g. 'hybrid' in Figure 1C in main text).

that the changes in cell size are within a twofold range of the wild-type value. Likewise, in mammals, pulse labeling in rat tissues [13] and human cell culture [12] showed that increased RNA content of larger cells was accompanied by a proportional increase in RNA production. Moreover, using an inhibitor of cyclin-dependent kinases to disrupt cell-cycle progression in human cells leads to larger cells [26] with proportionally increased RNA production rates [12].

Single cells

Quantitative measurements at the single-cell level allow direct comparisons of molecular features, such as transcript abundance or RNA production rates, with phenotypic features such as cell size or cell-cycle stage. This offers two advantages over bulk approaches: First, it allows the study of gene-expression scaling in the absence of experimental perturbations by using the natural variation in cell size and cell-cycle stage. Second, it allows one to observe and account for multiple changes to the cellular phenotype when interpreting perturbations.

Pioneering work in cultured mouse fibroblasts using label-free imaging revealed that RNA accumulates throughout interphase as cells grow – doubling between cell divisions and therefore remaining at a constant concentration [27]. In these data, the ratio of RNA to cell mass remained constant throughout the cell cycle, even though the ratio of DNA to cell mass was not constant [28].

smFISH using probes targeting intronic pre-mRNA sequences can be used to visualize transcription sites in fixed cells. This has been used to study the scaling of mRNA production rates for individual genes [6,8,9]. In human fibroblasts it was observed that smFISH signal intensity at the transcription site increases with cell size (for three of four genes measured), whereas the number of active transcription sites was independent of cell size (after normalizing for differences in gene copy number over the cell cycle) [9]. The conclusion drawn from this was that increases in mRNA production rates in larger cells are achieved by increasing the number of transcripts produced in a **transcriptional burst** rather than by increasing the frequency of bursts (transcriptional bursting is discussed in Box 3). However, it is unclear whether the observed changes in burst size are sufficient to explain transcript abundance scaling and whether these conclusions can be generalized to other genes or to other biological systems. In particular, mathematical modeling together with smFISH of nascent transcripts in *A. thaliana* [8] and *S. pombe* [6] indicate that transcription initiation rates increase with cell size. However, these studies also indicate that transcription occurs as a continuous

Box 3. How noisy is gene expression? Stochasticity versus deterministic variability

The number of mRNA transcripts of each gene varies considerably between cells, even in clonal cell populations. This has been observed across the kingdom of life and has been referred to as **gene expression heterogeneity** (reviewed in [98,99]). In many cases, this has been attributed to stochasticity or 'noise' in transcription. However, more recent work has indicated that mRNA abundance at the single-cell level is instead precisely regulated according to the cellular state [6,8,11,100–102].

The production of an mRNA transcript requires several steps, including binding of RNA polymerase and other general transcription factors to the gene promoter, transcriptional initiation, elongation, and termination. These events are governed by the inherent stochasticity of molecular interactions (also referred to as intrinsic noise [103]), and can be modeled as a Poisson process. If all processes leading to transcript production have only this minimal level of molecular variability, then the distribution of transcript abundances between cells is expected to follow a Poisson distribution [104] (the same distribution that is observed for the length of a supermarket queue when arrivals and departures of customers are uncorrelated). However, it has also been observed that individual genes switch between periods of high activity, in which multiple transcripts may be produced in a short time, and periods of low activity, in which no transcripts are produced. This is referred to as transcriptional bursting (reviewed in [105]). The terms 'burst length', 'burst size', and 'burst frequency' are commonly used to quantify the duration, number of transcripts produced, and fraction of time a gene is active, respectively.

Poissonian transcription [30,106] and transcriptional bursting [30,107–109] have been observed in live cells across diverse organisms. Transcriptional bursting kinetics have also been inferred from fixed cell data [110–113]; however, this relies on the assumption that measuring transcript levels in a single cell over time is the same as measuring transcript levels in a cell population at one timepoint. That is, it assumes that all cells can be considered to be the same. To appreciate the problem with this assumption, consider a population of cells that exist in two states A and B, which both transcribe gene *X* in a non-bursty manner. For each cell state, the variability in transcript abundance between cells is Poissonian, with state B having slightly higher expression of gene *X* (Figure I). If cell states A and B are considered together, then the distribution of transcript abundance is super-Poissonian. One may be tempted to conclude that transcription switches randomly between periods of low activity (such as state A) and high activity (such as state B). However, it could also be the case that cells are merely in two different states that may not interconvert, or may do so slowly or in a deterministic manner (e.g., according to position in the cell cycle or metabolic state).

Considering cell volume and cell-cycle stage as a continuous series of such states that globally affect gene expression levels, it is clear that one must incorporate these aspects of single cells when interpreting snapshots of transcript abundance. Such an approach has been taken when fitting kinetic models of transcription to smFISH data in *A. thaliana* [8] and *S. pombe* [6]. In both cases the authors found that, although transcript abundance distributions were super-Poissonian, once cell size and cell-cycle stage were taken into account the remaining variability was consistent with non-bursty transcription. Indeed, cell size alone typically accounts for 50–80% of the cell-to-cell variability in transcript abundance in unperturbed populations [6,8,9,11,12]. In human cells, the multivariate cellular state (e.g., cell-cycle stage, size, shape, local cell crowding, mitochondrial abundance, capacity to respond to Ca^{2+}) can explain even more variability than size alone [11,102]. Similar to other organisms, the unexplained variability of these models approached the limit of minimal stochasticity imposed by a Poisson process.

It should be noted that these findings do not contradict the phenomenon of transcriptional bursting. However, they do suggest that cell-to-cell variability in transcript abundance is often not caused by stochasticity of transcriptional bursts. The danger of explaining the observed gene expression heterogeneity in a population of cells by 'stochasticity' is that it can miss interesting biology – for example, the size-scaling of transcript abundance.

Poisson process rather than in bursts (Box 3), suggesting that mechanisms to scale transcriptional output with cell size may have additional adaptive properties depending on whether transcription is continuous or not.

Metabolic pulse labeling has been applied at the single-cell level in human cells [9,12] and in *Rana pipiens* motoneurons [29]. This approach labels all RNA synthesized during the pulse and thereby provides a measure of the bulk RNA production rate of the cell. Image-based approaches combining this with measurement of cell volume and cell-cycle stage in human cells revealed that overall RNA production rates increase almost proportionally with cell size, but also suggested differences between cell-cycle stages – S-phase and G2-phase cells showed increased RNA synthesis rates compared to G1-phase cells of the same volume [12]. This is reminiscent of earlier

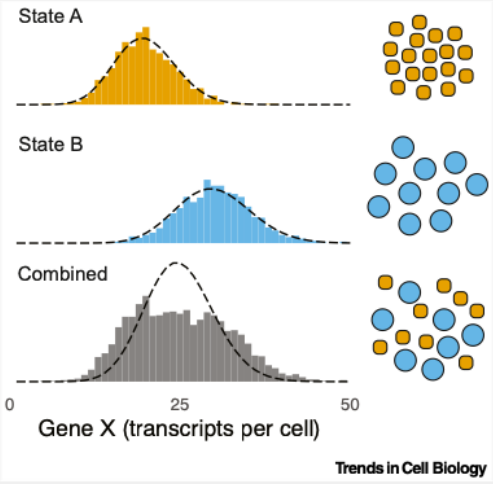

Figure I. Quantifying transcript count distributions over multiple cell states. If cells in state A or state B synthesize mRNA of gene *X* in a non-bursty manner, the resulting distribution of mRNA transcripts per cell is a Poisson distribution. However, if those states are considered together (combined) the distribution is super-Poissonian, despite there being no transcriptional bursts. Histograms show transcript count distributions for the populations in each state. For comparison, dotted lines show a Poisson distribution with mean equal to the population mean.

work on synchronized HeLa cell populations (and also other organisms) that showed a step-increase in RNA production during or after S-phase (reviewed in [4]); however, it does not completely agree with the picture in which increases in gene dosage caused by DNA replication are immediately buffered by reducing transcription rates at each allele to one-half of the pre-replication levels [30–32]. These different studies measured bulk RNA production versus gene-specific transcription using different techniques and used different organisms. Further work will therefore be necessary to resolve the origins of this apparent discrepancy. Multivariate approaches that quantify both cell-cycle stage and cell size in the same assay will be essential.

mRNA degradation rates

Observations across diverse eukaryotes that mRNA synthesis rates increase with cell size have been consistent. This, together with the relative difficulty of accurately measuring mRNA decay rates compared to synthesis rates, has resulted in less attention being paid to the regulation of mRNA decay rates in relation to cell size. In *S. pombe* [6,16], *Arabidopsis thaliana* [8], and mammalian cells [9,12], mRNA decay rates in relation to cell size were measured for several genes using transcription inhibition. It was concluded that cell size had little, if any, generic effect on mRNA decay rates. In *S. cerevisiae*, however, comparing G1-phase populations at different times after α-factor release, or different *S. cerevisiae* mutants and polyploid strains, indicated that mRNA degradation rates are reduced in larger cells [33]. This agrees with measurements made using metabolic changes to switch off the expression of specific genes [34,35]. Regulation of mRNA decay rates according to cell size may be specific to *S. cerevisiae*, but it is important to note that studies in other eukaryotes have universally measured mRNA stability using transcription inhibition. Such inhibition results in vastly different estimates of stability compared to pulse-labeling approaches [36], and is considered by many to be unsuitable for mRNA half-life determination [37] – possibly because of coordination between mRNA synthesis and decay (discussed later). It is also interesting to note that bulk transcript production rates measured in larger cells were slightly less than proportional to increases in cell volume for some

human cell lines [12]. Therefore, it may be worth revisiting this in other organisms through more sophisticated measurements of mRNA stability.

Mathematical modeling

Mathematical modeling has been extensively employed to understand the control of the cell cycle (reviewed in [38]) and cell size (reviewed in [2]). Modeling has also been employed together with experiments to understand size-scaling of mRNA transcript abundance [6,8–10,12,35], and there has also been purely theoretical work on this topic [39–42]. Because cell growth, mRNA synthesis, and degradation rates, as well as cell volume and DNA template concentrations, can all vary over time, and because feedback mechanisms can often lead to counter-intuitive phenomena when perturbations are made, mathematical modeling continues to be a useful tool for exploring hypotheses. The further integration of modeling with experiments will be important for quantitatively evaluating proposed mechanisms and suggesting key experiments.

Coordinated control of mRNA synthesis and degradation

Cell size-scaling of transcript abundance seems to be predominantly mediated through control of transcription rather than degradation. However, it has also been shown that global inhibition of mRNA degradation is generally not associated with increased mRNA abundance because mRNA production rates are reduced to compensate for these changes [43,44]. The converse is also true: reduction of transcription, for example by genetic disruption of Pol II cofactors, does not generally lead to reduced mRNA concentration because mRNA degradation rates are reduced to compensate [12,45–48]. These findings have led to a model referred to as **mRNA buffering** in which mRNA degradation and production rates are kinetically coupled to achieve homeostasis of mRNA concentration (Figure 2) (reviewed in [49]). Most studies on mRNA buffering have been performed on *S. cerevisiae*, but mRNA buffering has recently also been comprehensively demonstrated in human cells [12,48]. In both yeast and human, the mechanistic basis remains unclear (reviewed in [49]).

Given the wealth of data showing that transcript abundance and production rates are coordinated with cell size, it is striking that studies of mRNA buffering have generally not considered changes in cell size. It is also striking that the size-scaling field has generally not considered mRNA buffering – with a few notable exceptions [12,33]. However, because both size-scaling and mRNA buffering directly impinge on mRNA concentration, it is important to determine the relationships between these two phenomena. Indeed, it is possible that both might arise from the same mechanism.

One hypothesis that has been pursued to explain the coupling of mRNA synthesis and decay in *S. cerevisiae* is that subunits of Pol II (Rpb4/Rbp7) are physically associated with mRNA transcripts from the moment of transcription until their decay in the cytoplasm – linking transcription with mRNA export and decay [50]. However, although *rpb7* is essential, cells lacking *rpb4* still grow similarly to wild-type cells in optimal conditions [51] and reduced transcription in *rpb4* mutants occurs together with a reduction in the mRNA decay rate [52], indicating that Rpb4 is not strictly required for mRNA buffering. Moreover, in optimal conditions, Rpb4 and Rpb7 are localized predominantly in the nucleus [53] where they are assembled into Pol II complexes [54,55]. A major cytoplasmic role for Rpb4 in global mRNA homeostasis in the absence of stress therefore remains controversial [52,56].

Other hypotheses to explain mRNA buffering in *S. cerevisiae* center on the major cytoplasmic RNA exonuclease, Xrn1. However, initial studies disagreed as to whether mRNA buffering occurs in *xrn1* mutants: one study reported global transcriptional increases in *xrn1* mutants that resulted in 3.2-fold increase in mRNA levels [43], whereas another study found that *XRN1* deletion resulted in decreased mRNA decay as well as decreased mRNA synthesis (indicating buffering) [44]. The

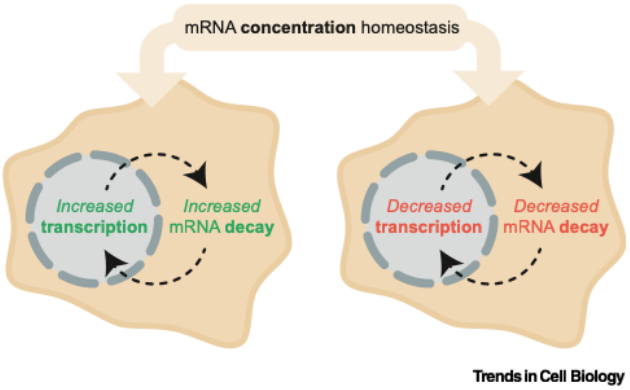

Figure 2. mRNA buffering. Increased or decreased mRNA decay elicits a compensatory change in transcription rate, and vice versa for perturbation of transcription. This allows mRNA concentration homeostasis to be robust to perturbations of mRNA metabolism.

latter (functional mRNA buffering in *xrn1* mutants) is supported by more recent experiments using orthogonal techniques [57,58]. However, none of these studies measured cell size, and mRNA production was normalized to cell number (using spike-ins) rather than to total protein or mRNA content [57–59]. This is important because haploid *xrn1* mutants have been reported to be among the top 5% of *S. cerevisiae* mutants by cell size [60]. If we extrapolate conclusions from the size-scaling field to these larger *xrn1* mutant cells, then an increased mRNA content and mRNA synthesis rate (but not mRNA concentration) may in fact be caused by an increase in cell size, and not by a disrupted buffering mechanism.

This is not only an issue for *xrn1* mutants. In *S. cerevisiae*, genetic perturbation of general transcription factors or Pol II itself often results in changes to average cell size [60] and to changes in the distributions of cell sizes in a population [61]. In *S. pombe*, perturbing the *xrn1* ortholog *exo2* also results in elongated cells [62] and Pol II subunits have been shown to be limiting for cell growth [63]. In *Drosophila melanogaster*, perturbation of mRNA processing and transcription also results in cell size changes [64]. This highlights the importance of considering the effect of cell size changes together with the effects of perturbed mRNA production and degradation on transcriptome abundance.

In human cells, such an approach was recently taken in a genome-wide genetic screen for perturbations that affect bulk RNA synthesis rates (determined using metabolic labeling) [8,12]. Crucially, this screen combined quantification of cell size and cell-cycle stage together with the measurement of RNA synthesis rates in thousands of single cells for each of >20 000 genetic perturbations. This allowed comparison of the rate of RNA synthesis in perturbed cells with that of unperturbed cells of the same size and cell-cycle stage. In this way, only perturbations whose changes in RNA production rate cannot be explained by changes to cell size and/or cell-cycle stage were considered as having altered RNA synthesis. Using this measure, thousands of perturbations resulted in altered RNA synthesis rates, demonstrating that RNA synthesis rates are easily perturbed. However, the vast majority did not result in changes in RNA concentration, indicating that mRNA buffering is a widely observed phenomenon in human cells [12].

Mechanistic basis of mRNA concentration homeostasis

Limiting factor model

A popular model for scaling of mRNA production with cell size is the so-called 'limiting factor' model [6,9,35]. In this model, the level of a particular factor that is essential for transcription is

precisely coordinated with cell size. That is, it has a constant concentration. If this limiting factor has a high affinity for DNA, then its local concentration on DNA will be proportional to the size of the cell despite having a constant global concentration [5,9] (Figure 3A). Another variant of this model has been proposed in which the limiting factor does not need to be DNA-bound and only needs to be localized to the nucleus [9]. This model requires that the nuclear volume does not scale with cell volume, but this is typically not the case: nuclear volume is known to scale tightly with cell volume in many cellular systems (reviewed in [65]).

In both *S. cerevisiae* and *S. pombe*, Pol II occupancy on chromatin increases with cell size [6,16,35], and in *S. pombe* and human cells Pol II abundance in the nucleus increases with cell and nuclear size [6,12]. Pol II itself, as well as components of the Pol II preinitiation complex (PIC), are therefore prime candidates as the limiting factors for transcription. The effects of a rapid 50% reduction in nuclear levels of several different PIC components on the chromatin occupancy of Pol II were recently measured in *S. cerevisiae* [35]. This revealed that none of the PIC components had a large effect on Pol II genome occupancy, except for Rpb1 (the largest subunit of yeast Pol II) itself. Moreover, it was found that overexpression of the 12-subunit Pol II complex resulted in increased Rpb1 levels on chromatin [35], suggesting that Pol II itself shows characteristics expected of a limiting factor in the sense of the model. However, although Pol II abundance doubled (>100% increase), Pol II loading on chromatin only increased by ~20%, suggesting that additional factors limit Pol II loading or the assembly of functional Pol II complexes. Moreover, in this study it remains unclear whether this minor increase reflects a functional engagement that results in increased mRNA production rates.

Although the limiting factor model can explain how a constant concentration can lead to a size-dependent mRNA production rate from a size-independent DNA template (Figure 3A), it does not explain how the concentration of the limiting factor (e.g., Pol II) is determined or maintained.

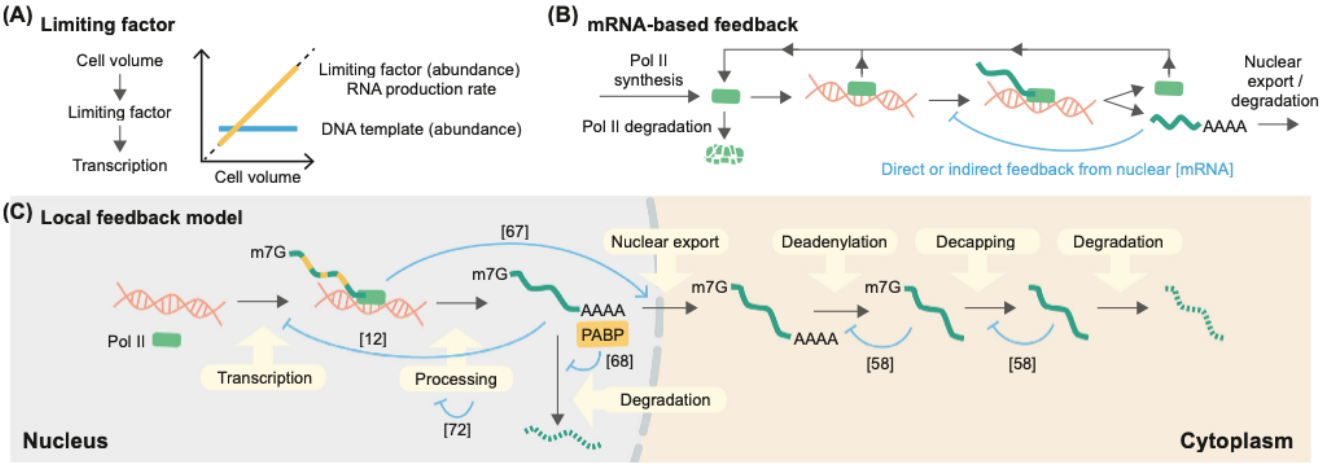

Figure 3. Mechanisms of transcriptional size-scaling. (A) The limiting factor model posits that a factor that is limiting for transcription (e.g., Pol II) is closely coordinated with cell volume and thereby leads to increased mRNA production in larger cells, despite a fixed amount of DNA. (B) The mRNA-based feedback model of mRNA transcript concentration homeostasis [12] comprises preferential degradation of inactive Pol II together with negative feedback from nuclear mRNA concentration on the global rate of transcriptional activation. (C) The local feedback model of transcript concentration homeostasis [58] in which many different feedback loops stabilize different parts of the mRNA metabolic pathway. A common motif is product inhibition in which the rate of a particular step in the pathway is sensitive to the concentration of its product. Numbers in square brackets are citations of studies on the indicated regulatory loops. Note that the mechanistic details of the feedback loops remain unknown and also that the model depicted here combines results from several different model organisms. Many more local feedbacks may exist, and in fact should exist if such a model is able to coordinate the kinetics of the entire mRNA metabolic pathway. Abbreviations: m7G, 7-methylguanine; PABP, poly(A)-binding protein; Pol II, RNA polymerase II.

This is crucial because, in this 'simple' model, the concentration would directly set the mRNA synthesis rate. Moreover, with Pol II as the limiting factor, the model could potentially have a problem with runaway positive feedback wherein a random fluctuation of transcription could lead to increased abundance of Pol II mRNA transcripts and thereby increased abundance of Pol II protein. With Pol II as the limiting factor, this would lead to increased transcription of all mRNA (including transcripts encoding Pol II itself) and therefore even further increased abundance of Pol II protein, and so on [9]. For both reasons, it seems unavoidable that there must be feedback mechanisms in place to control the levels of the limiting factor. Recent work in human cells suggests that this is indeed the case and could be mediated through the effects of nuclear mRNA concentration on Pol II transcription [12].

Regulatory feedback in mRNA metabolism

In a study of genome-wide perturbations that affect cell-size-corrected RNA production rates in human cells it was found that disruption of nuclear mRNA metabolic pathways downstream of transcription initiation, including transcript processing, nuclear degradation, and nuclear export, often result in reduced bulk RNA synthesis [12]. Moreover, long-term depletion of these factors leads to reduced concentrations of Pol II. This suggests that transcription rates and Pol II levels are reduced when cells are perturbed in their ability to remove mRNA from the nucleus. In many cases, the reduction in transcription was seemingly sufficient to buffer the perturbation so that the nucleus did not accumulate mRNA. However, acute depletion of the nuclear RNA exosome (a protein complex responsible for mRNA decay) via auxin-mediated degradation of the catalytic subunit DIS3 did lead to nuclear mRNA accumulation. Strikingly, this first resulted in reduced levels of actively transcribing Pol II (as measured by Pol II serine-2 phosphorylation levels) whereas total levels of Pol II were less affected over short timescales [12]. Similar reductions in transcription and Pol II levels were observed when forcing nuclear retention of mRNA [12], indicating that this is not specific to nuclear RNA exosome perturbation.

These experiments suggest that transcription is inhibited by nuclear mRNA (Figure 3B). Such a mechanism is an example of enzyme product inhibition, a common module in almost every biosynthetic pathway [66]. This type of negative feedback is probably one of many regulatory links that couple the kinetics of the various stages of mRNA processing and transport to ensure mRNA concentration homeostasis (Figure 3C). Indeed, the dynamics of transcriptional shutdown in *S. cerevisiae* in response to acute perturbation of the different steps of cytoplasmic mRNA degradation are consistent with a model of 'local feedback' along the life cycle of the mRNA [58]. Within the nucleus, it has been shown that nuclear export in human cells depends on continuous transcript production because Pol II inhibition results in nuclear retention of nuclear-injected mRNA [67]. This implies that nuclear export rates are coordinated with transcript production rates. In *S. cerevisiae*, the poly(A)-binding protein Nab2 is required for protection of nascent mRNA [68] and its levels are strongly controlled by the nuclear RNA exosome [69]. This suggests that nuclear mRNA concentration homeostasis is also achieved via regulation of nuclear mRNA decay. Nab2-mediated mRNA stabilization is also linked to nuclear export because acute inhibition of export leads to nuclear decay of newly transcribed RNA (when the Nab2 pool becomes exhausted due to an elevated nuclear mRNA concentration) [70]. In human cells, release of nascent transcripts from the chromatin template after transcription can also depend on splicing factor availability [71]. This is important because splicing factors, SR proteins, and RNA export factors are all known to be under feedback regulation to ensure their homeostasis (reviewed in [72]). This often takes the form of autoregulation in which a protein negatively feeds back to generate a non-functional version of its own mRNA, for example through regulation of alternative splicing (e.g., SRSF1) or nuclear export (e.g., NXF1). Similar to the example of Nab2 regulation by the exosome in *S. cerevisiae* mentioned earlier [69], there are also examples of cross-

regulation in human cells: SRSF3 regulates the splicing of other SR-protein family members [73], and highly abundant HNRNPD and HNRPNL cross-regulate their own paralogs [74,75].

Many of these regulatory links can contribute to homeostatic control of mRNA metabolism (Figure 3C). This, together with the observation that Pol II levels are modulated (in both directions) by perturbation of processes downstream of transcription [12], has important consequences for the limiting factor model of transcriptional scaling. Although this model can explain how a constant concentration of Pol II is converted to a size-dependent mRNA production rate from a size-independent DNA template, its most essential element, namely the precise size-scaling of Pol II levels, may be a consequence, and not a cause, of altered transcriptional activity. It is well known that Pol II protein is rapidly degraded by the ubiquitin-proteasome system when it is transcriptionally inhibited and not bound to chromatin [76–81]. Therefore, any control system that regulates Pol II association with chromatin may result in an altered Pol II concentration. From classic *in vitro* experiments we know that RNA inhibits transcription by dissociating Pol II from the DNA [82], and recent studies on transcriptional condensates indicate that this can also act locally inside the nucleus, where a local increase of newly synthesized mRNA in the condensate results in condensate dissolution and dissociation of Pol II from the chromatin [83]. In other words, the exact parameter that both the mRNA-buffering and cell size-scaling systems attempt to keep constant, namely mRNA concentration, may directly control transcriptional activity. In the case of cell size-scaling, Pol II abundance would then scale with cell size because transcription rates scale with cell size – rather than the other way around. Importantly, this model would still predict that short-term depletion of Pol II results in less Pol II on chromatin in the short term, and that overexpression of Pol II would result in increased Pol II loading, as recently observed in *S. cerevisiae* [35] (unless homeostasis of Pol II concentration is rapid and perfect).

When scaling breaks down

During prolonged cell-cycle arrest in *S. pombe*, cells continue to grow until they become extremely large (~fivefold larger than wild-type). At a certain point, RNA synthesis rates fail to continue to increase proportionally with cell size unless DNA content is also increased [16]. Similar observations have been made in *S. cerevisiae*, where it was further shown that RNA and protein concentrations are actually reduced in very large cells – demonstrating that cell volume can continue to increase despite dilution of RNA and protein [84]. In *S cerevisiae*, it was suggested that this reduced RNA concentration is due to activation of a stress response. Increased cell size is also associated with cellular aging and senescence in *S. cerevisiae* [84,85], mouse hematopoietic stem cells [86], and human cell lines [87]. It has been proposed that cell size may even be causative for these changes in cell physiology [84–86]. Possibly related to this, it has been suggested that high RNA concentrations in the nucleus may be necessary to maintain the solubility of intrinsically disordered proteins [88].

Concluding remarks

Bringing together the fields of transcriptional size-scaling and mRNA buffering holds enormous promise for moving beyond our current understanding of how relative gene expression levels are controlled towards understanding the kinetic basis that allows coordination of the absolute transcriptome of a single cell to the cellular phenotype (see Outstanding questions). Recent observations that nuclear Pol II levels are limiting for transcription [35] and are coordinated with both cell size [6,12,16,35] and mRNA output across perturbations [12] indicate that mRNA buffering and transcriptional scaling with cell size both converge on the regulation of Pol II abundance. Understanding the mechanisms that control Pol II abundance is therefore of utmost importance. Evidence that Pol II abundance can be controlled in response to disruption of splicing or by blocking nuclear export suggests a fundamental coupling of mRNA production, export, and

Outstanding questions

Which factors change in abundance to buffer the changes in bulk RNA production rates produced by perturbations and thereby allow robust mRNA concentration homeostasis? How do cells bring about these changes in abundance?

How is Pol II abundance regulated according to cell size? To what extent is this achieved by feedback?

Are the mechanisms underlying transcript abundance scaling conserved among eukaryotes?

Is the total mRNA concentration of a cell under homeostatic control (and the abundance of each transcript determined as a fraction of this total)? Or is mRNA transcript homeostasis gene-specific (and the abundance of each transcript determined according to cell volume)? Are there some transcripts with more precise concentration homeostasis than others?

Beyond volume-scaling: how do some genes scale with different aspects of cellular state, for example cell-surface area or DNA content? Is this additive to, or independent from, volume-scaling?

degradation rates. Two important aspects of experimental design and interpretation may help to achieve the goal of understanding this coupling: first, if mRNA buffering and transcriptional size-scaling are part of the same mechanism, then the timescales of transcriptional adaptation in response to perturbations should be shorter than the timescale over which cells grow. The traditional tools of genetics are therefore likely to be too blunt to understand causes and consequences in perturbation experiments. We must instead focus on acute perturbations measured with timecourse experiments that evaluate both the short-term (direct) and long-term (adaptation) effects of perturbations. In most model organisms there are several methods to rapidly control the localization and abundance of targeted proteins [89]. Second, perturbations must be evaluated using multiple readouts to more fully capture phenotypic changes – ideally at the level of individual cells. As a minimum, it is essential to consider cell size changes to draw conclusions about RNA concentration homeostasis, and one must be careful to distinguish between abundance and concentration. This is readily achieved by using quantitative cellular imaging where spatially resolved readouts of multiplexed cellular phenotypes for both RNA and protein are now possible [12,90,91]. Third, we must embrace the idea of feedback and embed studies of transcriptional regulation within the context of cellular metabolism – where multiple regulatory feedbacks are the expectation rather than the exception. After all, although transcription is often thought of as expression of the genome, the synthesis and degradation of mRNA as a biomolecule is also a fundamental part of cellular metabolism.

Acknowledgments

We thank Martin Howard for comments on the manuscript, and all members of the groups of S.B. and L.P. for discussions. S.B. is supported by an EMBO long-term fellowship (ALTF1175-2016), a Human Frontier Science Program (HFSP) long-term fellowship (LT000238/2017-L), and the University of New South Wales. L.P. is supported by the European Research Council (ERC-2019-AdG-885579) and the Swiss National Science Foundation (SNSF grant 310030_192622). Both authors are supported by the University of Zurich.

Declaration of interests

L.P. has filed a patent on the iterative indirect immunofluorescence imaging (4i) technology (patent WO2019207004A1). The other authors declare no conflicts of interest.

References

1. Schmoller, K.M. and Skotheim, J.M. (2015) The biosynthetic basis of cell size control. *Trends Cell Biol.* 25, 793–802
2. Facchetti, G. *et al.* (2017) Controlling cell size through sizer mechanisms. *Curr. Opin. Syst. Biol.* 5, 86–92
3. Björklund, M. (2019) Cell size homeostasis: metabolic control of growth and cell division. *Biochim. Biophys. Acta Mol. Cell Res.* 1866, 409–417
4. Mitchison, J.M. (2003) Growth during the cell cycle. *Int. Rev. Cytol.* 226, 165–258
5. Marguerat, S. and Bähler, J. (2012) Coordinating genome expression with cell size. *Trends Genet.* 28, 560–565
6. Sun, X.-M. *et al.* (2020) Size-dependent increase in RNA polymerase II initiation rates mediates gene expression scaling with cell size. *Curr. Biol.* 30, 1217–1230
7. Swaffer, M.P. *et al.* (2021) Transcriptional and chromatin-based partitioning mechanisms uncouple protein scaling from cell size. *Mol. Cell* 81, 4861–4875
8. Ietswaart, R. *et al.* (2017) Cell-size-dependent transcription of FLC and its antisense long non-coding RNA COOLAIR explain cell-to-cell expression variation. *Cell Syst.* 4, 622 635
9. Padovan-Merhar, O. *et al.* (2015) Single mammalian cells compensate for differences in cellular volume and DNA copy number through independent global transcriptional mechanisms. *Mol. Cell* 58, 339–352
10. Kempe, H. *et al.* (2015) The volumes and transcript counts of single cells reveal concentration homeostasis and capture biological noise. *Mol. Biol. Cell* 26, 797–804
11. Battich, N. *et al.* (2015) Control of transcript variability in single mammalian cells. *Cell* 163, 1596–1610
12. Berry, S. *et al.* (2022) Feedback from nuclear RNA on transcription promotes robust RNA concentration homeostasis in human cells. *Cell Syst*. https://doi.org/10.1016/j.cels.2022.04.005. Published online May 24, 2022
13. Schmidt, E.E. and Schibler, U. (1995) Cell size regulation, a mechanism that controls cellular RNA accumulation: consequences on regulation of the ubiquitous transcription factors Oct1 and NF-Y and the liver-enriched transcription factor DBP. *J. Cell Biol.* 128, 467–483
14. Nurse, P. (1975) Genetic control of cell size at cell division in yeast. *Nature* 256, 547–551
15. Fraser, R.S. and Nurse, P. (1978) Novel cell cycle control of RNA synthesis in yeast. *Nature* 271, 726–730
16. Zhurinsky, J. *et al.* (2010) A coordinated global control over cellular transcription. *Curr. Biol.* 20, 2010–2015
17. Müller, M. *et al.* (2021) High content genome-wide siRNA screen to investigate the coordination of cell size and RNA production. *Sci. Data* 8, 162
18. Pérez-Ortín, J.E. *et al.* (2019) Homeostasis in the Central Dogma of molecular biology: the importance of mRNA instability. *RNA Biol.* 16, 1659–1666

19. Miller, C. *et al.* (2011) Dynamic transcriptome analysis measures rates of mRNA synthesis and decay in yeast. *Mol. Syst. Biol.* 7, 458
20. Tani, H. *et al.* (2012) Genome-wide determination of RNA stability reveals hundreds of short-lived noncoding transcripts in mammals. *Genome Res.* 22, 947–956
21. Fraser, R.S. and Moreno, F. (1976) Rates of synthesis of polyadenylated messenger RNA and ribosomal RNA during the cell cycle of *Schizosaccharomyces pombe*. With an appendix: calculation of the pattern of protein accumulation from observed changes in the rate of messenger RNA synthesis. *J. Cell Sci.* 21, 497–521
22. Fraser, R.S. and Nurse, P. (1979) Altered patterns of ribonucleic acid synthesis during the cell cycle: a mechanism compensating for variation in gene concentration. *J. Cell Sci.* 35, 25–40
23. Barnes, A. *et al.* (1979) Analysis of the significance of a periodic, cell size-controlled doubling in rates of macromolecular synthesis for the control of balanced exponential growth of fission yeast cells. *J. Cell Sci.* 35, 41–51
24. Pfeiffer, S.E. (1968) RNA synthesis in synchronously growing populations of HeLa S3 cells. II. Rate of synthesis of individual RNA fractions. *J. Cell. Physiol.* 71, 95–104
25. Pfeiffer, S.E. and Tolmach, L.J. (1968) RNA synthesis in synchronously growing populations of HeLa S3 cells. I. Rate of total RNA synthesis and its relationship to DNA synthesis. *J. Cell. Physiol.* 71, 77–93
26. Cadart, C. *et al.* (2018) Size control in mammalian cells involves modulation of both growth rate and cell cycle duration. *Nat. Commun.* 9, 3275
27. Killander, D. and Zetterberg, A. (1965) Quantitative cytochemical studies on interphase growth I. Determination of DNA, RNA and mass content of age determined mouse fibroblasts in vitro and of intercellular variation in generation time. *Exp. Cell Res.* 38, 272–284
28. Zetterberg, A. and Killander, D. (1965) Quantitative cytochemical studies on interphase growth II. Derivation of synthesis curves from the distribution of DNA, RNA and mass values of individual mouse fibroblasts in vitro. *Exp. Cell Res.* 39, 22 32
29. Sato, S. *et al.* (1994) Transcription and motoneuron size. *J. Neurochem.* 63, 1609–1615
30. Yunger, S. *et al.* (2010) Single-allele analysis of transcription kinetics in living mammalian cells. *Nat. Methods* 7, 631–633
31. Voichek, Y. *et al.* (2016) Expression homeostasis during DNA replication. *Science* 351, 1087–1090
32. Yunger, S. *et al.* (2018) S-phase transcriptional buffering quantified on two different promoters. *Life Sci.* 1, e201800086
33. Mena, A. *et al.* (2017) Asymmetric cell division requires specific mechanisms for adjusting global transcription. *Nucleic Acids Res.* 45, 12401–12412
34. Begley, V. *et al.* (2019) The mRNA degradation factor Xrn1 regulates transcription elongation in parallel to Ccr4. *Nucleic Acids Res.* 47, 9524–9541
35. Swaffer, M.P. *et al.* (2021) RNA polymerase II dynamics and mRNA stability feedback determine mRNA scaling with cell size. *BioRxiv* Published online September 20, 2021. https://doi.org/10.1101/2021.09.20.461005
36. Eisen, T.J. *et al.* (2019) The dynamics of cytoplasmic mRNA metabolism. *Mol. Cell* 77, 786–799
37. Tani, H. and Akimitsu, N. (2012) Genome-wide technology for determining RNA stability in mammalian cells: historical perspective and recent advantages based on modified nucleotide labeling. *RNA Biol.* 9, 1233–1238
38. Tyson, J.J. and Novák, B. (2015) Models in biology: lessons from modeling regulation of the eukaryotic cell cycle. *BMC Biol.* 13, 46
39. Lin, J. and Amir, A. (2018) Homeostasis of protein and mRNA concentrations in growing cells. *Nat. Commun.* 9, 4496
40. Wang, Q. *et al.* (2021) Heterogeneous recruitment abilities to RNA polymerases generate nonlinear scaling of gene expression with cell volume. *Nat. Commun.* 12, 6852
41. Thomas, P. and Shahrezaei, V. (2021) Coordination of gene expression noise with cell size: analytical results for agent-based models of growing cell populations. *J. R. Soc. Interface* 18, 20210274
42. Lin, J. and Amir, A. (2021) Disentangling intrinsic and extrinsic gene expression noise in growing cells. *Phys. Rev. Lett.* 126, 078101
43. Sun, M. *et al.* (2013) Global analysis of eukaryotic mRNA degradation reveals Xrn1-dependent buffering of transcript levels. *Mol. Cell* 52, 52–62
44. Haimovich, G. *et al.* (2013) Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell* 153, 1000–1011
45. Baptista, T. *et al.* (2017) SAGA is a general cofactor for RNA polymerase II transcription. *Mol. Cell* 68, 130–143
46. García-Molinero, V. *et al.* (2018) The SAGA/TREX-2 subunit Sus1 binds widely to transcribed genes and affects mRNA turnover globally. *Epigenetics Chromatin* 11, 13
47. Helenius, K. *et al.* (2011) Requirement of TFIIH kinase subunit Mat1 for RNA Pol II C-terminal domain Ser5 phosphorylation, transcription and mRNA turnover. *Nucleic Acids Res.* 39, 5025–5035
48. Slobodin, B. *et al.* (2020) Transcription dynamics regulate Poly (A) tails and expression of the RNA degradation machinery to balance mRNA levels. *Mol. Cell* 78, 434–444
49. Hartenian, E. and Glaunsinger, B.A. (2019) Feedback to the central dogma: cytoplasmic mRNA decay and transcription are interdependent processes. *Crit. Rev. Biochem. Mol. Biol.* 54, 385–398
50. Choder, M. (2011) mRNA imprinting: additional level in the regulation of gene expression. *Cell. Logist.* 1, 37–40
51. Choder, M. and Young, R.A. (1993) A portion of RNA polymerase II molecules has a component essential for stress responses and stress survival. *Mol. Cell. Biol.* 13, 6984–6991
52. Schulz, D. *et al.* (2014) Rpb4 subunit functions mainly in mRNA synthesis by RNA polymerase II. *J. Biol. Chem.* 289, 17446–17452
53. Farago, M. *et al.* (2003) Rpb4p, a subunit of RNA polymerase II, mediates mRNA export during stress. *Mol. Biol. Cell* 14, 2744–2755
54. Mosley, A.L. *et al.* (2013) Quantitative proteomics demonstrates that the RNA polymerase II subunits Rpb4 and Rpb7 dissociate during transcriptional elongation. *Mol. Cell. Proteom.* 12, 1530 1538
55. Jasiak, A.J. *et al.* (2008) Genome-associated RNA polymerase II includes the dissociable Rpb4/7 subcomplex. *J. Biol. Chem.* 283, 26423–26427
56. Duek, L. *et al.* (2018) Dissociation of Rpb4 from RNA polymerase II is important for yeast functionality. *PLoS One* 13, e0206161
57. Begley, V. *et al.* (2020) Xrn1 influence on gene transcription results from the combination of general effects on elongating RNA pol II and gene-specific chromatin configuration. *RNA Biol.* 18, 1310–1323
58. Chappleboim, A. *et al.* (2022) Transcription feedback dynamics in the wake of cytoplasmic degradation shutdown. *BioRxiv* Published online January 30, 2022. https://doi.org/10.1101/2021.12.01.470637
59. Sun, M. *et al.* (2012) Comparative dynamic transcriptome analysis (cDTA) reveals mutual feedback between mRNA synthesis and degradation. *Genome Res.* 22, 1350–1359
60. Jorgensen, P. *et al.* (2002) Systematic identification of pathways that couple cell growth and division in yeast. *Science* 297, 395–400
61. Maitra, N. *et al.* (2018) Perturbations of transcription and gene expression-associated processes alter distribution of cell size values in *Saccharomyces cerevisiae*. *G3* 9, 239–250
62. Hayles, J. *et al.* (2013) A genome-wide resource of cell cycle and cell shape genes of fission yeast. *Open Biol.* 3, 130053
63. Weston, L. *et al.* (2017) Genome-wide screen for cell growth regulators in fission yeast. *J. Cell Sci.* 130, 2049–2055
64. Björklund, M. *et al.* (2006) Identification of pathways regulating cell size and cell-cycle progression by RNAi. *Nature* 439, 1009–1013
65. Cantwell, H. and Dey, G. (2021) Nuclear size and shape control. *Semin. Cell Dev. Biol.* Published online November 11, 2021. https://doi.org/10.1016/j.semcdb.2021.10.013
66. Pardee, A.B. and Reddy, G.P.-V. (2003) Beginnings of feedback inhibition, allostery, and multi-protein complexes. *Gene* 321, 17–23

67. Tokunaga, K. *et al.* (2006) Nucleocytoplasmic transport of fluorescent mRNA in living mammalian cells: nuclear mRNA export is coupled to ongoing gene transcription. *Genes Cells* 11, 305–317
68. Schmid, M. *et al.* (2015) The nuclear polyA-binding protein Nab2p is essential for mRNA production. *Cell Rep.* 12, 128–139
69. Roth, K.M. *et al.* (2005) The nuclear exosome contributes to autogenous control of NAB2 mRNA levels. *Mol. Cell. Biol.* 25, 1577–1585
70. Tudek, A. *et al.* (2018) A nuclear export block triggers the decay of newly synthesized polyadenylated RNA. *Cell Rep.* 24, 2457–2467
71. Hochberg-Laufer, H. *et al.* (2019) Availability of splicing factors in the nucleoplasm can regulate the release of mRNA from the gene after transcription. *PLoS Genet.* 15, e1008459
72. Müller-McNicoll, M. *et al.* (2019) Auto-regulatory feedback by RNA-binding proteins. *J. Mol. Cell Biol.* 11, 930–939
73. Änkö, M.-L. *et al.* (2012) The RNA-binding landscapes of two SR proteins reveal unique functions and binding to diverse RNA classes. *Genome Biol.* 13, R17
74. Preußner, M. *et al.* (2012) HnRNP L and L-like cooperate in multiple-exon regulation of CD45 alternative splicing. *Nucleic Acids Res.* 40, 5666–5678
75. Kemmerer, K. *et al.* (2017) Auto- and cross-regulation of the hnRNPs D and DL. *RNA* 24, 324–331
76. Lee, K.-B. *et al.* (2002) Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4239–4244
77. Mitsui, A. and Sharp, P.A. (1999) Ubiquitination of RNA polymerase II large subunit signaled by phosphorylation of carboxyl-terminal domain. *Proc. Natl. Acad. Sci. U. S. A.* 96, 6054–6059
78. Nguyen, V.T. *et al.* (1996) In vivo degradation of RNA polymerase II largest subunit triggered by alpha-amanitin. *Nucleic Acids Res.* 24, 2924–2929
79. Alekseev, S. *et al.* (2017) Transcription without XPB establishes a unified helicase-independent mechanism of promoter opening in eukaryotic gene expression. *Mol. Cell* 65, 504–514
80. Bensaude, O. (2011) Inhibiting eukaryotic transcription. Which compound to choose? How to evaluate its activity? *Biochem. Soc. Symp.* 2, 103–108
81. Steurer, B. *et al.* (2018) Live cell analysis of endogenous GFP RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4368–E4376
82. Pai, D.A. *et al.* (2014) RNAs nonspecifically inhibit RNA polymerase II by preventing binding to the DNA template. *RNA* 20, 644–655
83. Henninger, J.E. *et al.* (2021) RNA-mediated feedback control of transcriptional condensates. *Cell* 184, 207–225
84. Neurohr, G.E. *et al.* (2019) Excessive cell growth causes cytoplasm dilution and contributes to senescence. *Cell* 176, 1083–1097
85. Lanz, M.C. *et al.* (2021) Increasing cell size remodels the proteome and promotes senescence. *BioRxiv* Published online November 7, 2021. https://doi.org/10.1101/2021.07.29.454227
86. Lengefeld, J. *et al.* (2021) Cell size is a determinant of stem cell potential during aging. *Sci. Adv.* 7, eabk0271
87. Cheng, L. *et al.* (2021) Size-scaling promotes senescence-like changes in proteome and organelle content. *BioRxiv*. https://doi.org/10.1101/2021.08.05.455193. Published online August 5, 2021
88. Maharana, S. *et al.* (2018) RNA buffers the phase separation behavior of prion-like RNA binding proteins. *Science* 360, 918–921
89. Jaeger, M.G. and Winter, G.E. (2021) Fast-acting chemical tools to delineate causality in transcriptional control. *Mol. Cell* 81, 1617–1630
90. Gut, G. *et al.* (2018) Multiplexed protein maps link subcellular organization to cellular states. *Science* 361, eaar7042
91. Spitzer, H. *et al.* (2022) Learning consistent subcellular landmarks to quantify changes in multiplexed protein maps. *BioRxiv* Published online May 8, 2022. https://doi.org/10.1101/2022.05.07.490900
92. Pérez-Ortín, J.E. *et al.* (2013) What do you mean by transcription rate? *Bioessays* 35, 1056–1062
93. Saint, M. *et al.* (2019) Single-cell imaging and RNA sequencing reveal patterns of gene expression heterogeneity during fission yeast growth and adaptation. *Nat. Microbiol.* 4, 480–491
94. Claude, K.-L. *et al.* (2021) Transcription coordinates histone amounts and genome content. *Nat. Commun.* 12, 4202
95. Wu, C.-Y. *et al.* (2010) Control of transcription by cell size. *PLoS Biol.* 8, e1000523
96. Miettinen, T.P. *et al.* (2014) Identification of transcriptional and metabolic programs related to mammalian cell size. *Curr. Biol.* 24, 598–608
97. Frechin, M. *et al.* (2015) Cell-intrinsic adaptation of lipid composition to local crowding drives social behaviour. *Nature* 523, 88–91
98. Raj, A. and van Oudenaarden, A. (2008) Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135, 216–226
99. Sanchez, A. and Golding, I. (2013) Genetic determinants and cellular constraints in noisy gene expression. *Science* 342, 1188–1193
100. Zopf, C.J. *et al.* (2013) Cell-cycle dependence of transcription dominates noise in gene expression. *PLoS Comput. Biol.* 9, e1003161
101. Skinner, S.O. *et al.* (2016) Single-cell analysis of transcription kinetics across the cell cycle. *Elife* 5, e59928
102. Foreman, R. and Wollman, R. (2020) Mammalian gene expression variability is explained by underlying cell state. *Mol. Syst. Biol.* 16, e9146
103. Swain, P.S. *et al.* (2002) Intrinsic and extrinsic contributions to stochasticity in gene expression. *Proc. Natl. Acad. Sci.* 99, 12795–12800
104. Sanchez, A. *et al.* (2013) Stochastic models of transcription: from single molecules to single cells. *Methods* 62, 13 25
105. Tunnacliffe, E. and Chubb, J.R. (2020) What is a transcriptional burst? *Trends Genet.* 36, 288–297
106. Larson, D.R. *et al.* (2011) Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science* 332, 475–478
107. Chubb, J.R. *et al.* (2006) Transcriptional pulsing of a developmental gene. *Curr. Biol.* 16, 1018–1025
108. Golding, I. *et al.* (2005) Real-time kinetics of gene activity in individual bacteria. *Cell* 123, 1025–1036
109. Muramoto, T. *et al.* (2012) Live imaging of nascent RNA dynamics reveals distinct types of transcriptional pulse regulation. *Proc. Natl. Acad. Sci.* 109, 7350–7355
110. Raj, A. *et al.* (2006) Stochastic mRNA synthesis in mammalian cells. *PLoS Biol.* 4, e309
111. Munsky, B. *et al.* (2012) Using gene expression noise to understand gene regulation. *Science* 336, 183–187
112. Neuert, G. *et al.* (2013) Systematic identification of signal-activated stochastic gene regulation. *Science* 339, 584–587
113. Gómez-Schiavon, M. *et al.* (2017) BayFish: Bayesian inference of transcription dynamics from population snapshots of single-molecule RNA FISH in single cells. *Genome Biol.* 18, 164