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Connecting growth with gene expression: of noise and numbers

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Growth is a dynamic process whereby cells accumulate mass. Growth rates of single cells are connected to RNA and protein synthesis rates, and therefore with biomolecule numbers. Noise in gene expression depends on these numbers, and is thus linked with cellular growth. Whether these global attributes of the cell participate in gene regulation is still largely unexplored. New experimental and modelling studies suggest that systemic variations in biomolecule numbers can coordinate cellular processes, including growth itself, through global regulatory feedback that acts in addition to genetic regulatory networks. Here, we review these findings and speculate on possible implications of this less appreciated layer of gene regulation for cellular physiology and adaptation to changing environments.

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A global regulation of gene expression by cellular growth

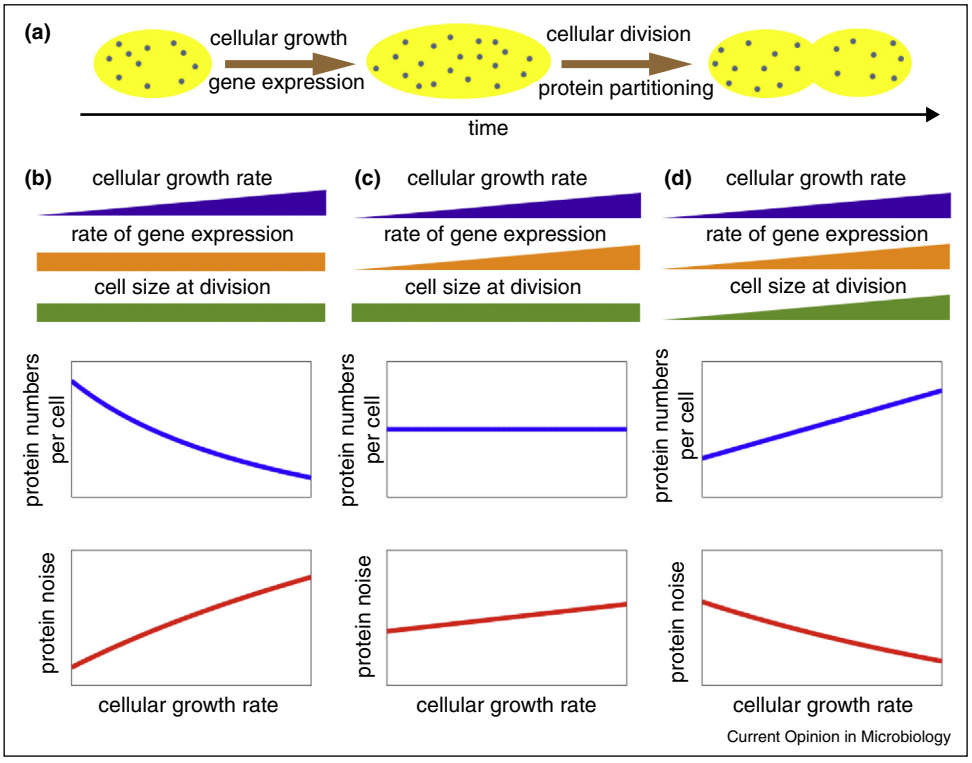
Gene expression is plastic, and is controlled by intrinsic and extrinsic cues. Signalling pathways control networks of transcription factors that regulate expression levels of defined target genes. For example, transitions between successive phases of the division cycle are controlled by a series of signalling events that result in cell cycle phase specific changes in expression of regulatory proteins. This form of gene expression is commonly called 'periodic'. In reality, the numbers of all mRNAs and proteins *per cell* exhibit periodic variation during the division cycle, because RNA and protein molecule numbers increase gradually as the cell accumulates mass and decrease sharply at cell division when molecules partition randomly between daughter cells (Figure 1a). For this reason, changes in cellular growth rate of single cells (e.g. in response to nutrient availability) are reflected by the expression dynamics of all genes (in addition to any specific regulation through signalling and gene-regulatory networks). Non-deterministic cell-to-cell variability in mRNA and protein numbers, which is commonly referred to as noise in gene expression, is also connected to cellular growth. This is because intracellular noise levels are related to cellular copy numbers of genes, mRNAs and proteins (Figure 1b–d) [1].

These systemic variations in mRNA and protein numbers and noise levels can intrinsically and globally alter the dynamics of biochemical networks involved in metabolism, signalling and gene regulation [2–4]. Interestingly, perturbed networks dynamics can in turn affect, or 'feedback', to cellular growth rates as illustrated in Figure 2. We refer to this phenomenon as 'global feedback' as it connects cellular growth dynamics of individual cells to gene expression programmes through modulation of all genes independently from, or in addition to, dedicated signalling pathways or transcription factors. Recently, pioneering studies have started to shed light on the mechanisms underlying this systemic layer of regulation and its impact on cell physiology. In this short review, we highlight some of these works with a particular focus on the role of noise in gene expression, and discuss their impact on our understanding of how cells integrate environmental conditions through growth (for a recent perspective on the subject with a more deterministic focus see Ref. [5]). For clarity and to avoid confusion, the key terms used in this review are defined in Box 1.

Mathematical models of global feedback

As described above, regulation through global feedback arises from systemic interconnected processes and is therefore challenging to study experimentally (Figure 2). Using mathematical models of cellular physiology has been a powerful approach to bring the mechanisms of global feedback regulation to light. These models take into account aspects of metabolism, gene expression, and cellular resource allocation to predict growth in single cells and populations. Two main modelling strategies have been explored. Whole-cell detailed models are designed to include as much information as possible of the system they describe [6]. These models can capture different aspects of global feedback and phenotypic variability but are very complex and difficult to implement even

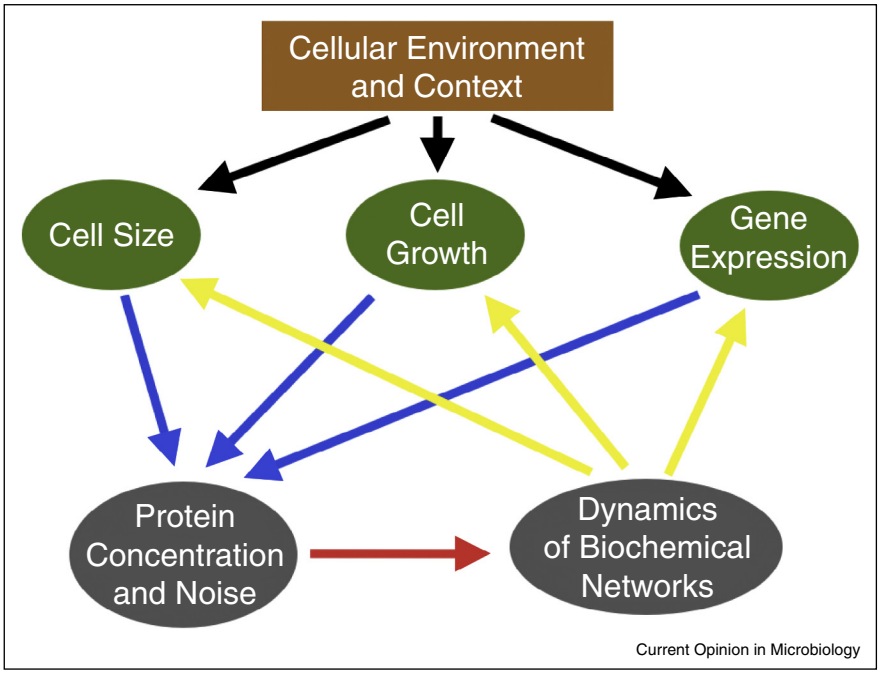
Figure 1



Relationship between cellular growth and gene expression. **(a)** Illustration of cellular growth, cellular division and gene expression. The expression of a typical protein is illustrated as it accumulates in the cell during cellular growth. The protein molecules are randomly partitioned between the daughter cells during cell division. Therefore protein numbers and noise are modulated during cellular growth and the cell division cycle. To explore the effect of cellular growth rate on protein numbers and noise three hypothetical scenarios are considered in the panels **(b)**, **(c)** and **(d)**. The plots show qualitative changes in protein numbers and noise at a given point in the cell division cycle (e.g. midpoint in the cell cycle) and are in arbitrary units. In (b) the rate of gene expression and cell size at division remains constant as cellular growth rate is increased. As the dilution rate of proteins increases at faster growth rate protein numbers go down with growth rate. As protein noise is inversely related to protein numbers, it increases sharply with growth rate. In (c) gene expression perfectly matches dilution (and cellular growth), so protein concentrations remain fixed. As cell volume at division is assumed to be fixed in this case as well, protein numbers remain constant as a function of cellular growth rate. In this case, protein noise may still increase slowly with cellular growth rate because cells experience more partitioning events per unit time. This contribution may increase protein noise at faster growth. In (d) both the rate of gene expression and the cell volume at division increases with cellular growth rate. In this case it is expected that protein numbers increase with cellular growth rate, while noise in gene expression decreases with cellular growth rate. This is the scenario that matches observation in *E. coli* [3•]. Protein concentrations are reduced at faster cellular growth rate as increase in the rate of gene expression does not perfectly match increase dilution rate. Exact stochastic simulations that capture the effect of cellular growth and partitioning using the experimental parameters of gene expression in *E. coli* [3•] reveals that indeed noise in gene expression is reduced at faster growth rates [39].

for the simplest model organisms [7]. Promising alternatives use coarse-grained approaches that model only specific aspects of the cell's physiology, such as metabolic processes, or regulation of large groups of proteins like the ribosome [8•]. These models have succesfully described cellular resource allocation and economy, and are particularly powerful when built from quantitative data as in recent models of proteome partitioning and growth [9•,10,11••]. For instance, they were found to recapitulate phenomenological population 'growth laws' without modelling detailed molecular processes [5]. They could also predict shifts in population growth strategies [8•], scaling and distribution of promoter activities in response to external conditions [12,13••], cellular growth bi-stability of single cells through positive global feedback [14••,15], gene-dosage compensation for growth related genes [11••], and suppressive drug interactions in different environments [16]. Current coarse-grained models have been developed for studying exponential growth of cell populations, and (with few exceptions) ignore unbalanced population growth dynamics, cell size, cell-to-cell phenotypic variability and gene expression noise. These are all features that are likely to play important roles in regulation through global feedbacks, and it may be useful to consider them in future models. Nevertheless, as described in the rest of this review, coarse-grained models have been instrumental in revealing regulation through global feedback and its mechanisms.

Figure 2



Schematics of global feedback routes. During adaptation to environmental conditions cell growth, size and gene expression impact on protein concentration and noise that in turn affect the dynamics of different biochemical networks inside the cell. In the absence of significant degradation, protein numbers, concentration and noise are determined by the balance of production (gene expression), dilution (cell growth) and cell volume (size). Protein levels determine the dynamics of all cellular processes (biochemical networks) that in turn control, or 'feedback' to, cellular phenotypes (including cell size, growth and gene expression). The colours used in the diagram are just to guide the eye.

Global feedback and molecular concentrations

In a growing cell, the balance between production, degradation and dilution of biomolecules determines their concentrations. Gene expression is tuned globally to growth conditions and cell size [17]. Yet, unless rates of gene expression are perfectly in tune with the cellular growth rates, overall mRNA and protein concentrations (and numbers) could vary due to the changes in cell volume that accompany cellular growth (Figure 1). As the rate of biochemical reactions depends on molecular concentrations, this imbalance can then affect cell physiology globally and be a source of global feedback (Figure 2). In line with this, the concentration of a constitutively expressed gene in *Escherichia coli* was indeed reduced at a faster population growth rate (shorter population doubling time), and growth-dependent changes in protein concentration were found to impact on small regulatory networks behaviour [3•]. Imbalance between molecule production or degradation and increase in cellular volume can give rise to coordinated change in large fractions of proteome. Recent analysis from the Hwa laboratory revealed that the bacterial proteome can be partitioned in three fractions: one fraction that does not change with the population growth rate, and two fractions that do, one dedicated to protein synthesis, which includes ribosomes, and one containing metabolic enzymes [9•,10]. The ribosomal fraction was found to increase in proportion during faster population growth, resulting in a decrease in proportion of the metabolic fraction [9•,10]. In the case where total protein concentration remains unchanged across growth conditions, growth-related relative changes in proteome fractions are directly linked to protein concentrations [3•]. Indeed, recent absolute measurements of protein numbers in *E. coli* combined with coarse-grained modelling revealed that concentrations of a number of large proteome fractions are regulated with population growth [18••]. These coordinated changes in concentrations of large portions of the proteome in response to external conditions could affect behaviour of gene regulatory networks and be an intrinsic part of global regulatory feedback from cellular growth rate to gene expression. The molecular processes that underlie these changes or that are affected by them remain largely elusive. Interestingly, global changes in proteome resource allocation can result from regulation by a single molecule as in the case of cAMP regulation in response to catabolic and anabolic limitations [19]. This example provides a simple mechanism that connects the cellular environment and relative changes in proteome fractions. A coarse-grained model was instrumental in revealing the feedback mechanisms

Box 1 **Biomolecule:** refers to proteins, mRNAs or metabolites.

Cell size: the total volume of a cell, which is related to its dimensions and surface area.

Cell division rate: the increase in cell number per unit of time. It is also called population doubling rate. It is inversely proportional to the population cell division time and population doubling time. In the literature, cell division rate and growth rate have sometimes been used interchangeably, but they do not refer to the same thing as population growth rate refers to the rate of increase in total cell mass.

Cellular concentration: the number of molecules per unit of volume in a cell.

Cellular economy: Allocation of cellular resources in different conditions by balancing supply and demand.

Coarse-grained cell model: a mathematical model of some aspect of cell physiology that focuses on a small number of key factors or processes.

Detailed whole-cell model: a computer model of a particular cell type with as much detail as one can handle, for example, it includes all the genes.

Fitness: ability of a single cell with a given phenotype and genotype to survive and reproduce as part of a cell population.

Gene expression: the processes that mediate expression of a gene into a protein or another gene product, which includes transcription, translation, or mRNA and protein degradation.

Global feedback: describes processes by which global regulation of gene expression by growth (or cell size) can feedback to cell physiology and in turn affect growth (or size) (see Figure 2). Crucially, this kind of global regulation operates at a different level than the signalling and gene-regulatory networks and connects cellular context to global features of gene expression. Although their existence is more and more recognised, the molecular mechanisms at play remain vastly mysterious.

Growth laws: phenomenological and quantitative relations between growth and gene expression, in particular linear relations observed in proteome resource allocation (see Ref. [9•]).

Cellular growth rate: the mass increase of a cell per unit of time. We use the term 'cellular growth rate' when referring to increase in mass or volume of a single cell. In the literature, the cell growth rate is sometimes expressed as the rate of exponential mass increase in a population. This is problematic because the cellular growth rate of a cell and of mass increase in a population are often different. This is especially the case when cellular growth rate differ between cells of a population for instance.

Cellular interdivision time: the time between two cell division for a single cell. Also called cell cycle time or generation time. This is inversely related to cellular growth rate.

Noise: non-genetic stochastic fluctuations in biomolecule numbers due mainly to random timing of biochemical reactions. Noise levels can be measured by temporal fluctuations in single cells or cell-to-cell variability across an isogenic populations (for ergodic systems these measures are equivalent). We refer to noise in mRNA and protein number as noise in gene expression.

Phenotypic variability: non-genetic cell-to-cell variability in a given phenotype. As gene expression levels are also phenotypes, noise is an example of phenotypic variability. However, the term noise is usually specifically used to refer to gene expression and molecular variability. Examples of phenotypic variability, relevant to this review, are cell size variability and cellular growth rate variability.

Population growth rate: the rate of exponential mass increase in a cell population.

at work in this case [19]. Absolute quantification of molecular concentration under different conditions is rare in eukaryotic systems, yet it is known that total mRNA levels are reduced by a factor greater than the decrease in volume when fission yeast cells exit the division cycle to enter quiescence, while global protein concentrations remain relatively constant [20•]. In summary, molecule concentrations do vary with the population growth rate providing an underlying mechanism for global feedback propagation. Absolute measurements of mRNA/protein numbers across growth conditions will be essential to study further the impact of this variability on biochemical networks and the resulting global feedback.

Global feedback and cell size

Cell size homeostasis is an interesting example of a process that could be affected by global feedback on protein concentration. Cell size is tightly regulated as a function of cellular growth and division. Although, mechanisms controlling cell size homeostasis are not entirely understood, several genes have been identified that are involved in size regulation in bacteria and yeast (for review see [21,22]). The number of biomolecules in a cell is linked to its size, as at equal molecular concentrations bigger cells have on average higher copy numbers of molecules. Therefore, changes in overall cellular molecule numbers associated with an imbalance between molecule production/degradation and increase in volume (as described above), could affect concentrations of regulators of cell size homeostasis and feedback to size itself. Such global feedback could impact on cellular 'sizing' mechanisms that are required for correct cell size homeostasis. In the fission yeast *Schizosaccharomyces pombe*, the protein kinase Cdr2p accumulates at the medial cellular cortex proportionally to the cell surface area and contributes to size control [23]. Mathematical modelling that assumes constant concentration of Cdr2p during cell elongation explains the above experimental observation. Therefore it is crucial that Cdr2p expression levels precisely scale with cell volume to ensure proper sizing [23]. Cdr2p acts in a network of other factors that includes Pom1p [24,25]. A polar concentration gradient of Pom1p regulates Cdr2p activity [26,27]. Altogether, these homeostatic mechanisms are potentially susceptible to regulation via global feedback because global effects that would affect 'sizer' molecule concentrations would in turn affect cell size. Recent data propose an alternative mechanism of size control distinct from the 'sizer' principle. Microfluidic measurements of cell size in bacteria has revealed that the slope of the relation between cell size and cellular growth rate depends on whether quantification is based on single cells or population averages [28]. These data combined with mathematical modelling hint at a new 'adder' size homeostasis principle, in which cells add a constant mass at each generation, irrespective of their newborn size [29]. As for 'sizer' molecules, 'adder' regulators could be affected via global feedback on molecule

concentrations. Altogether this raises the hypothesis that multiple fine tuning mechanisms of cell size homeostasis could have evolved in order to confer robustness against global regulation of protein concentration through global feedback. To investigate this possibility, models of cellular size control should take into account global regulation of size sensing factors, since non-trivial feedback are likely to occur (Figure 1). In summary, we propose that global feedback of cell size and cellular growth on protein concentrations could be an integral element of size sensing mechanisms.

Global feedback and noise in gene expression

Noise in gene expression scales with the inverse square root of protein copy numbers in bacteria and yeast [30,31]. Therefore size and cellular growth related changes in biomolecule numbers in single cells can affect protein noise levels. Changes in noise levels of regulatory or metabolic factors can in turn impact, or feedback, to cellular growth itself (see below). Noise has an important role in regulating cell fate, and such variations could also have consequences for cell survival or differentiation. Indeed, phenotypic variability and gene expression noise are thought to adversely affect population growth and division rates. Accordingly, genome scale data in yeast suggest that noise is minimized in genes affecting population growth [32]. Together, these observations suggest that noise and growth are functionally connected at the level of a cell population. In single cells, growth kinetics could impact on noise levels in several ways. Recent evidence suggests that stochastic promoter bursting of constitutively expressed genes varies between cell cycle phases [33]. Cellular growth (i.e. increase in cellular mass of individual cells) can occur in different cell-cycle phases depending on the organism and/or environmental conditions. Because stochastic transcriptional bursting is a potent source of noise, these observations taken together provide a potential mechanistic link between cellular growth and gene expression noise through cell-cycle specific features of transcription. Growth-related variability in the energetic status of the cell can affect gene expression and noise as well. For instance, cell-to-cell variability in numbers of mitochondria propagate to transcription through cellular ATP levels resulting in variability in RNA polymerase II elongation rates [34]. More generally, variability in cellular growth rates of single cells contributes to gene expression noise as suggested by modelling [35–37] (see also discussion of Ref. [48••]). Another phenomenon that links cellular growth and division of single cells with gene expression noise is the random partitioning of biomolecules at cell division (Figure 1a). Cellular pools of mRNA and proteins are distributed binomially between daughter cells and each partitioning event generates noise [38]. When cellular interdivision time decreases, a single cell experiences more partitioning events per unit time, potentially increasing noise levels (Figure 1c). Interestingly, a shorter cellular interdivision time is connected with an increase in cell size at division in many unicellular organisms including yeast and bacteria [21,22]. It is not completely understood whether reaching a larger size at division is advantageous at a faster cell division rate, and if it is, why? We hypothesize that this regulation of cell size with cell division rate may have evolved to minimize protein noise during fast doubling, because copy numbers of mRNAs and proteins are higher in larger cells (Figure 1d). Indeed, mathematical modelling performed in our group shows that increased size at faster population growth rates can control noise in gene expression [39]. In summary, gene expression noise and growth kinetics, in populations or in single cells, are tightly interconnected. As changes in global noise levels of biomolecules have the potential to affect large number of genes, noise could be a mediator of the systemic form of gene regulation discussed in this review. This is indeed the case as in several examples discussed below.

Global feedbacks and metabolism

In cell populations, the rates of growth and division are complex phenotypes emerging from metabolic and environmental cues. Even at constant population cell division rates, the budding yeast transcriptome, proteome and metabolome are dynamic, and cover a continuum of physiological states [40]. For example, yeast oxygen metabolic cycles involve thousands of genes, and are tightly linked to cell culture dynamics [41,42]. Cycling is particularly evident in conditions where cell division is slow, and cycle frequencies are affected by culture density and population doubling time [41,42]. Interestingly, single cells from a metabolically asynchronous population were found to be in different phases of the metabolic cycle suggesting that cycling can occur also in absence of metabolic synchrony in the culture [43]. Moreover, recent *in silico* simulations predict a broad distribution of metabolic phenotypes and cellular growth rates among individual cells due to expression noise in metabolic genes [44]. Altogether, this illustrates how metabolic states and growth dynamics can vary from cell to cell and are heterogeneous traits within isogenic populations. Recent data show that metabolic heterogeneity and noise have implications for cellular decision making. Metabolic flux is used as a controlling factor of phenotypic bi-stability on gluconeogenic substrates [45•,46]. In yeast, the design of the glycolysis pathway gives rise to two glycolytic states, of which only one is compatible with cellular growth. Modelling has revealed that variability in metabolic enzyme concentrations can generate both states [47]. This indicates that noise in metabolic enzyme expression underlies metabolic heterogeneity in a cell population growing in a constant environment. An intriguing recent study revealed that quantitative features of the cell connect its metabolic status to cellular growth through noise and global feedback. In *E. coli*, time-lapse imaging measurements of cellular growth of single cells, *lac* enzyme

concentrations and *lac* production rates suggest that noise in gene expression is transmitted to cellular growth and generates cells with variable cellular growth rates [48••]. Interestingly, noise also propagates from cellular growth back to gene expression and affects levels of genes unrelated to the *lac* operon. Noise transmission was maintained when specific gene expression regulators were modified and could affect reporter constructs using a range of different promoters [48••]. Thus metabolic enzymes can connect cellular growth with gene expression of many genes in single cells without the need of specific regulators. In other words, the metabolic state of the cell spreads from its cellular growth dynamics to gene expression, and back, through global non-specific feedback mechanisms. Taken together, these data demonstrate how cellular growth and central metabolism are connected via global feedback mechanisms involving gene expression noise.

Global feedback on genes that affect fitness

We have seen in the previous section how global feedback through noise and metabolic enzymes can orchestrate gene expression responses independently of the cognate promoters or regulators of a gene. Another example of this comes from regulation of genes that directly affect the cell fitness. This provides an elegant mechanism by which cells can adapt to unknown environments in absence of dedicated signalling pathways. In a synthetic bi-stable system where fluctuating environments favour one of two stable gene expression states (attractor), *E. coli* cells switch to the state supporting the higher fitness [49]. Mathematical modelling revealed that condition-dependent global feedback through modulation of gene expression noise is responsible for making the state with the lower cellular growth rate unstable [49]. This phenomenon is not restricted to bi-stable circuits as global feedback was also found to affect expression of a mono-stable gene required for population growth even in absence of its cognate promoter [50]. In this case, regulation seems to correlate with cell size and noise in gene expression but the specific mechanisms at play remain unclear [51]. Yet, global feedback through noise in gene expression is a possible candidate that should be tested with detailed modelling. In another scenario, positive global feedback on gene expression induces bi-stability in a cell population by generating cell-to-cell variability in cellular growth rates [3•,15]. For instance, expression of an antibiotic-resistance gene becomes bi-stable due to a global positive feedback through cellular growth on its own expression [14••]. Similar feedback on toxin-antitoxin systems could also mediate antibiotic persistence in bacteria [3•,52,53]. Although, in this case global feedback is not necessarily required as slow fluctuations in toxin expression alone, without feedback from cellular growth, can result in a population of persister-like cells [54]. In summary, as in the case of metabolic genes, mutual global feedback between protein noise and cellular growth propagate signals without the need of specific gene regulation enabling survival in changing environments.

Mechanisms of global scaling of gene expression with growth

Regulation of gene expression occurs at multiple levels including transcription, translation, mRNA or protein degradation. Defining how different regulatory layers respond to quantitative features of the cell is key to the understanding of the molecular mechanisms underlying global feedback. We have seen above that regulation by global feedback can be maintained when gene promoter sequences are altered or when genes specific regulators are removed [48••,55••]. Intuitively, this observation points towards mechanisms either acting at the post-transcriptional level or affecting transcription from a large variety of promoter sequences. Recent studies shed more light on this aspect of global regulation by demonstrating that changes in population growth rate affect global promoter activities [13••,55••,56••]. A study into the arginine biosynthesis pathway in *E. coli* revealed population growth rate dependent global regulation alongside specific regulatory circuits [55••]. Global regulation seems to serve the function of setting maximal promoter activity during adjustment to new conditions, whereas, specific regulation controls metabolic activity at steady state [55••]. A larger scale study of around 900 yeast, and 1800 *E. coli* promoters reached similar conclusions suggesting global scaling to growth conditions is common with 60-90% of promoters affected [13••]. Interestingly, this phenomenon is well explained using a simple passive allocation of cellular resources that assumes the overall promoter activity is a fixed resource available to the cell per division cycle [13••]. Coordinately regulated genes under given conditions preserve proportionality, suggesting that global regulation contributes to ensuring stoichiometry. Expression of genes with similar functions adjust to growth conditions by similar scaling factors suggesting that levels of systemic responsiveness to growth conditions is linked to function. Therefore, in cell populations, growth-dependent global regulatory mechanisms acting at the level of transcription can shape gene expression programmes. In *E. coli*, the observation that pools of free and active RNA polymerase vary with population growth rates provides a possible mechanism that can connect population growth rates with global transcription [57]. Moreover, recent data demonstrate that an increase in cell volume can regulate transcription directly [58•]. Together, these data provide evidence that population growth dynamics and quantitative features of the cell impact globally on gene promoter activities enabling global feedback between population growth and gene expression. Further work will be required to uncover how other layers of gene expression control, such as mRNA stability and protein degradation for instance, are integrated into this process.

In this context, genome-wide absolute measurements of biomolecules production and degradations rates in cell populations and ideally in single cells would be especially powerful resources. Finally, coarse-grained models that take into account these additional layers of regulation will be instrumental in shedding light on their respective contributions.

Conclusion

Complex global regulatory feedback controls gene expression beyond classical gene regulatory circuits. These processes are governed at least in part by quantitative features of the cell. They simultaneously trigger, and respond to, changes in single cell and population growth dynamics. Appreciating their impact on complex traits such as growth will help reveal genetic design principles that allow cellular networks to function robustly across environmental conditions [59•]. Quantifying absolute protein numbers and noise across environmental conditions will be used to inform the next generation of coarse-grained models of cellular resource allocation that will include gene expression noise and cell size. The quantitative data on different aspects of cell physiology will be used to constrain the topology and parameters of these models and will increase their predictive power. We believe that mathematical models of cellular processes should be built on top of appropriate coarse-grained models of cell physiology to help capture the effect of global feedback. Ultimately, this kind of multi-scale modelling will enable effective designs of synthetic biological systems that function robustly in changing environments.

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