Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation

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Abstract | Organisms are constantly exposed to a wide range of environmental changes, including both short-term changes during their lifetime and longer-term changes across generations. Stress-related gene expression programmes, characterized by distinct transcriptional mechanisms and high levels of noise in their expression patterns, need to be balanced with growth-related gene expression programmes. A range of recent studies give fascinating insight into cellular strategies for keeping gene expression in tune with physiological needs dictated by the environment, promoting adaptation to both short- and long-term environmental changes. Not only do organisms show great resilience to external challenges, but emerging data suggest that they also exploit these challenges to fuel phenotypic variation and evolutionary innovation.

Cells show a remarkable regulatory flexibility that allows them to thrive under different external conditions and to survive harsh situations. The ability to constantly sense and adapt to environmental changes is important for all organisms to maintain cellular functions (homeostasis), but is especially acute for plants and microorganisms; their sessile lifestyle leave them more exposed to the environment than animals. Modulation of gene expression has a central role in cellular adaptation to short- or long-term environmental changes, with extensive regulation occurring at both the transcriptional and post-transcriptional level. Signal-transduction pathways can translate extracellular signals into specific intracellular responses, including the launch of alternative gene expression programmes to cope with new conditions. In addition to these 'hard-wired' responses, gene expression networks show considerable plasticity to adapt to a wide range of challenges, including those not encountered during evolutionary history (for example, ectopic gene expression).

The application of genome-wide approaches is now providing a global view on gene expression responses to many different stress conditions, leading to exciting recent advances in our understanding of the cellular strategies that are used to stay in tune with environmental conditions. Many of these concepts have been developed in microorganisms, most notably yeast cells, which finely balance energy-efficient growth with the ability to rapidly adapt to sudden external challenges, and which provide ideal models to study gene expression under tightly controlled conditions. Research in yeast and in other organisms is uncovering conserved principles for regulatory strategies in response to changing environments. This Review will highlight some of the emerging principles underlying gene expression responses to environmental factors. The emphasis will be on transcriptional mechanisms, which have been most intensely studied for technical and historical reasons, and for which several recent papers have greatly advanced our understanding. The details of regulatory pathways or specific cellular responses to different stresses have been reviewed elsewhere[1–4]. We focus on the general principles by which cells are able to adjust their gene expression programmes to respond to changing environments, both in the short-term and over evolutionary timescales. Emerging data reveal that cells finely balance the expression of stress-related and growth-related genes, which are antagonistic programmes distinguished by distinct regulatory mechanisms. Stress-related genes generally contain TATA boxes, a promoter element that seems to promote both short-term variability and long-term evolvability of transcriptional responses. Maintaining cellular functionality under variable conditions thus enhances gene expression variability and is both a constraint and a driving force for evolution.

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Below, we will first give an overview of stress-related gene expression programmes, which are controlled antagonistically to growth-related gene expression programmes, followed by a description of recent insights into the mechanistic principles of gene expression tuning. We finish with an evolutionary perspective on the adaptation of gene expression to both known and previously unencountered environmental factors, which in turn seem to promote regulatory variation and evolution.

Global stress-response programmes

Cells respond to abrupt environmental changes by launching gene expression programmes that help to adjust the cellular physiology and metabolism to the new conditions and that protect against cell damage or death. The use of DNA microarrays for expression profiling has provided comprehensive insights into transcriptional responses to a wide range of stress conditions in several organisms, including yeasts[5–8], plants[9], flies[10,11] and humans[12]. These and similar studies have uncovered hundreds of genes, the transcripts of which are either induced or repressed in response to stress. Most of these gene expression responses are transient and, even with persistent stress, gene expression returns after some time to new steady-state levels that are close to those in unstressed cells. Stress responses show considerable sophistication and fine-tuning: the magnitude and duration of the response is proportional to the dose or severity of the perturbation, and different perturbations result in distinct expression signatures[7,13,14]. Moreover, when exposed to different simultaneous stresses, the cellular response approximates the sum of the responses for each individual stress, indicating that gene expression programmes can be combined for a precise response[7].

Besides specific responses to different stresses, both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* show a large response that is common to all or most stress conditions, and this core response is largely conserved between the two distantly related yeasts[5–7]. Stress-induced genes are enriched for heat-shock and antioxidant functions as well as for carbohydrate metabolism and energy-generation functions, whereas most stress-repressed genes have growth-related functions, such as translation and ribosome biogenesis, reflecting a redirection of resources from rapid proliferation to stress protection. The core stress response could explain the phenomenon of cross-protection, in which cells treated with low levels of one stress become also more resistant to other stresses[15]. Other organisms seem to launch much smaller, if any, core stress responses. For example, the pathogenic yeast *Candida albicans* shows only a limited core response to different stresses[8]. It is possible that this difference reflects the different lifestyles: unlike free-living yeasts, *C. albicans* proliferates in human hosts where it is largely shielded from environmental variations. In multicellular organisms, the core stress response seems to include only a small set of genes, which is enriched for regulatory functions[9–12]. Unlike in free-living yeasts, ribosome- and other growth-related genes are not[10,11] or are only weakly[9,12] downregulated in multicellular organisms. Some of these differences could reflect the fact that many cell types are present in multicellular organisms, most of which are differentiated, whereas the experiments in yeast have been performed with homogeneous, rapidly growing cells. In fact, quiescent *S. cerevisiae* cells (that resemble the differentiated G0 state) launch a stress response that overlaps only partially with the response in rapidly growing cells and that does not involve repression of growth-related genes[16]. Moreover, stress responses in multicellular organisms seem to differ between different cell lines and tissues[9,12,17].

Balanced control of growth and stress response

The balance between energy-efficient growth and the ability to rapidly respond to fluctuating environments is a fundamental physiological challenge, most notably for microorganisms. Cellular functions, such as metabolism, stress protection, and growth and proliferation, reflect external factors and can be dynamically adjusted to both transient and long-term environmental changes. Rapidly growing *S. cerevisiae* cells spend most of their transcriptional energy on ribosome synthesis, requiring the coordinated activity of all three RNA polymerases, and cellular economy dictates that ribosome synthesis is in tune with the growth rate[18,19]. Nutrient availability in the environment limits growth and proliferation and accordingly has a great influence on global gene expression. *S. cerevisiae* cells can survive starvation by undergoing differentiation, including quiescence[20], filamentation[21] or sporulation[22], which are all triggered by specialized gene expression programmes and culminate in highly stress-resistant cells or spores, and which can therefore be regarded as sophisticated stress responses. Rapid growth and high stress resistance seem to be mutually exclusive. As described above, the core stress response in *S. cerevisiae* redirects resources from growth to stress functions, and stress-resistant cells are non-growing. The degree of stress resistance is inversely correlated with growth rate[23]. Thus, yeast cells need to balance rapid growth and increased stress-resistance according to nutrient availability and stress conditions (FIG. 1). This balance between maximal physiological activity and stress resistance might be more universally valid. For example, nutrient limitation (caloric restriction) leads to increased stress resistance and lifespan in organisms ranging from yeast to man[24]. Moreover, cancer cells sustain rapid proliferation by upregulating ribosome- and other growth-related genes, but can also enter dormant stages with increased resistance to drugs[25].

The expression of growth- and stress-related genes as a function of growth rate has recently been studied in *S. cerevisiae* grown under steady-state conditions in chemostats[26–28]. Chemostats maintain constant growth conditions, such as oxygen levels, nutrient concentrations and pH values, while tightly controlling growth at defined rates. Different nutrient limitations have been used to distinguish the effects of specific nutrients from general growth-related effects on gene expression. Hundreds of genes are correlated with growth rate, both positively and negatively, revealing striking overlaps with the core stress response: genes that are

Core stress response
Involves genes with expression levels that are regulated in a stereotypical manner in all (or most) of the environmental stress conditions tested in yeasts. This response is also known as the environmental stress response (ESR), common environmental response (CER) or core environmental stress response (CESR).

Chemostat
A fermenter that is operated in continuous-culture mode and is used in microbiology for growing and harvesting microorganisms at defined growth rates and under tightly controlled conditions.

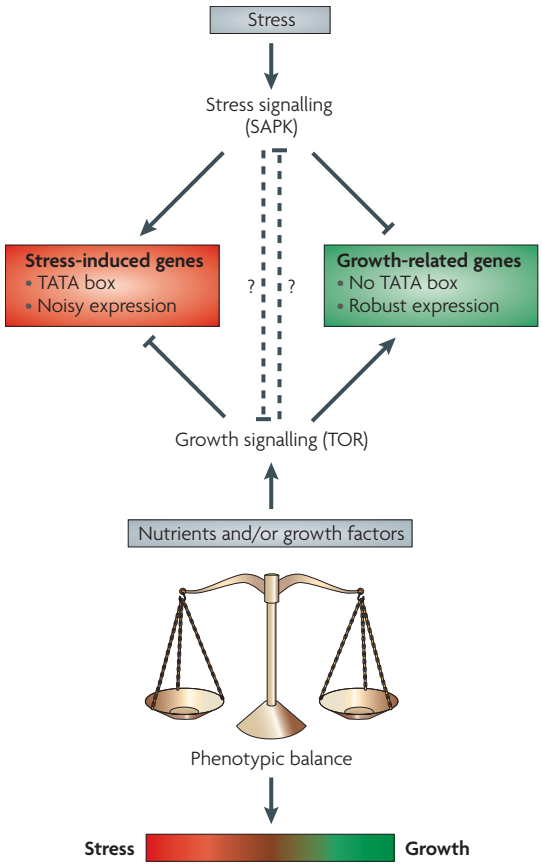


Figure 1 | **Balancing the expression of growth- and stress-related genes.** The simplified scheme shows the interplay between two major signalling pathways that antagonistically regulate the expression of growth- and stress-related genes. The target of rapamycin (TOR) pathway is upregulated in response to nutrients and growth factors, whereas the stress-activated protein kinase (SAPK) pathway is upregulated in response to stress[1,2,4]. TOR promotes growth-related genes and inhibits stress-related genes, whereas SAPK promotes stress-related genes and inhibits growth-related genes. In addition, it is possible that the TOR and SAPK pathways can also negatively regulate each other. Growth-related genes (green) are characterized by robust expression levels and the absence of TATA boxes in their promoters, whereas stress-related genes (red) are characterized by noisy transcription and the presence of TATA boxes in their promoters. Environmental conditions influence the balance between TOR and SAPK signalling, which in turn determines the balance between the expression of growth- and stress-related genes, and thus the cellular phenotype with regard to growth rate and stress resistance.

highly expressed during rapid growth are enriched for genes that are repressed during stress, whereas genes that are expressed at a low level during rapid growth (but highly expressed during slow growth) are enriched for genes that are induced during stress. These findings raise the possibility that many of the stress-response genes do not respond directly to stress but to a reduction in growth rate caused by stress[26,27].

In principle, cells could use properties of their internal state, such as growth rate, metabolite concentrations or energy levels, to coordinate gene expression with physiological needs. An alternative strategy would be to rely on signalling pathways that are responding to external conditions to deduce internal needs. To distinguish between these two possibilities, a recent study in *S. cerevisiae* asked whether the expression of ribosome-biogenesis genes and stress-related genes responds to internal or external factors[29]. Using a mutant that grows faster on non-fermentable carbon sources than on glucose (the opposite of wild-type cells, which grow faster on glucose), the actual growth rate was uncoupled from the environmentally expected growth rate shown by wild-type cells. Under these conditions, the correlation between growth rate and expression of ribosome-biogenesis genes was lost: unlike wild-type cells, the mutant cells expressed ribosome-biogenesis genes at low levels and stress-related genes at high levels during rapid growth. Thus, the mutant cells tuned gene expression to the environment rather than to their growth rate. Moreover, changes in ribosome-biogenesis gene expression and stress-related gene expression preceded changes in growth rates in chemostat cultures that were adapting to environmental perturbations, and growth rates did not correlate with ribosome-biogenesis gene expression in a large set of deletion mutants[14,29]. Together, these findings indicate that the expression of ribosome-biogenesis genes and stress-related genes mainly respond to environmental signals rather than to any internal growth-dependent feedback mechanism.

Cells use multiple signalling pathways to respond to environmental changes and to coordinate growth with stress responses. Two notable examples are the stress-activated protein kinase (SAPK) and target of rapamycin (TOR) pathways, which are conserved from yeast to man[1,2,4]. Both pathways integrate multiple functions by regulating gene expression at several levels and also by controlling proteins post-translationally. The SAPK and TOR pathways have central roles in balancing stress resistance with growth (FIG. 1). The TOR pathway promotes growth and negatively regulates stress-response genes, and possibly also promotes the SAPK pathway when activated by nutrients or growth factors[2,4]. Lowering TOR signalling by mutations or by drugs is sufficient to increase stress resistance and lifespan in *S. cerevisiae*[30]. The SAPK pathway promotes stress resistance when activated by environmental perturbations[1,3]; in *S. pombe*, it plays a central part both for the induction of stress-related genes and for the repression of growth-related genes[6,13]. It is possible that special regulatory proteins can integrate inputs from SAPK, TOR and other signalling pathways to coordinate the expression of stress- and growth-related genes.

Mechanisms of transcriptional tuning

Clearly, gene expression is regulated at multiple levels, with post-transcriptional levels of control having important roles in the cellular response to environmental factors (BOX 1). Below, we will focus on transcriptional mechanisms that allow cells to rapidly switch between growth- and stress-related gene expression programmes.

Box 1 | **Examples of post-transcriptional controls to balance growth and stress programmes**

RNA processing in P-bodies and stress granules

Following stress exposure, yeast cells reduce the production of growth-related proteins while increasing the production of stress-related proteins. Besides transcript synthesis and degradation, the reversible storage of transcripts provides an economical and rapid mechanism to regulate protein levels in response to stress. Transcripts that are not, or are no longer, translated can join big cytoplasmic structures called P-bodies[91,92]. P-bodies contain, among many other proteins, the components of the RNA-decay machinery together with transcripts targeted for degradation. As well as being a site of RNA degradation, P-bodies are a site of RNA storage. As an example, this system is used by quiescent yeast cells or during growth in human cells, which assemble P-bodies containing transcripts that can then re-enter translation when environmental conditions change[93,94].

A different type of structure, called a stress granule, is present in plant and mammalian cells. Stress granules assemble in response to environmental stress and disperse after stress recovery. Unlike P-bodies, these structures contain stalled translation initiation complexes. Stress granules are closely associated with P-bodies and might be 'triage centres' that sort, remodel and export specific transcripts for translation, decay or storage[95]. These data highlight the complex cellular strategies used to distribute transcripts in different cellular structures that control the fate of these transcripts according to environmental conditions.

Translational control of gene expression during stress

Translation provides another important regulatory layer to tune protein levels in response to environmental factors. Changes in transcription are often potentiated by homodirectional changes in translation, although some genes go against this trend[96]. Stress triggers a general translational inhibition of most transcripts, including growth-related transcripts such as those involved in ribosome biosynthesis[19,97]. This general inhibition is mediated by general control nonderepressible 2 (GCN2)-like proteins that are activated during stress, leading to inhibitory phosphorylation of the eukaryotic translation initiation factor 2 (eIF2). Additional layers of regulation are mediated by the growth and stress signalling pathways: target of rapamycin (TOR) signalling stimulates translation by activating S6 kinase (S6K)[4], and by inhibiting eIF4E-binding protein (4EBP) and, in *Saccharomyces cerevisiae*, Gcn2 (REF. 2), whereas stress-activated protein kinase (SAPK) signalling transiently inhibits translation in mammalian cells by phosphorylating the translational initiation factor eIF4E[98]. Specific transcripts that are important for the stress response, including yeast *GCN4* or human activating transcription factor 4 (*ATF4*) transcripts, escape this general translational repression by relying on special regulatory sequences upstream of their coding regions[97].

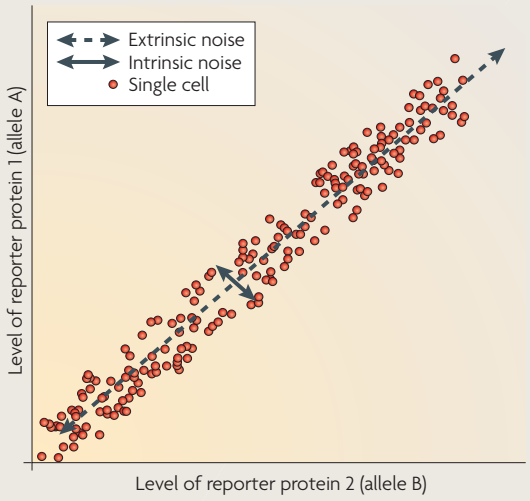
The TATA box and stress-related genes. Most eukaryotic protein-coding genes are transcribed by RNA polymerase II (pol II). Accurate initiation of transcription requires a core promoter region, as well as flanking regulatory sequences. Accessibility to promoter sequences is regulated by chromatin structure, and several steps are required to initiate transcription. First, sequence-specific DNA-binding proteins coordinate the remodelling of chromatin and the recruitment of the transcription machinery. Second, the general transcription factors and pol II assemble into a pre-initiation complex. Third, pol II and its associated elongation factors produce an mRNA transcript. The TATA box is a core promoter motif, present in approximately 20% of all *S. cerevisiae* genes[31], that is involved in the assembly of the transcriptional machinery. Interestingly, TATA-containing genes are enriched in stress-related genes, and they are extensively regulated compared with TATA-less genes in *S. cerevisiae*[31]. These data suggest that TATA boxes are associated with promoters of genes that require rapid and variable regulation, which seems to be true also in humans and plants[32,33]. The TATA box not only promotes short-term regulatory tuning but also noisy transcription (BOX 2) and the evolution of gene expression control (see below). Conversely, TATA-less genes are enriched among 'housekeeping' or growth-related genes[31]. These findings reveal a bipolar transcriptome with distinct types of core promoters being used to control growth- or stress-related genes (FIG. 1).

Distinct regulatory strategies for stress- and growth-related genes. Switching between growth and stress programmes requires the regulation of numerous genes, and cells use two separate mechanisms involving differential use of the core transcriptional machinery and TATA boxes to achieve this task. Recent studies in *S. cerevisiae* contribute to our understanding of such a global mode of regulation. An early step in transcription is the recruitment of the TATA-binding protein (TBP) to core promoters, which requires interaction with co-activator complexes such as TFIID and SAGA. Although TFIID and SAGA contribute to the expression of nearly all genes, the regulation of any given gene is usually dominated by one or the other factor. SAGA-dominated genes are enriched in stress-related functions and are highly regulated compared with TFIID-dominated genes[34]. Accordingly, TATA-containing genes show a stronger dependence on SAGA, whereas TATA-less genes are more dependent on TFIID[31]. An analysis of the dynamics of co-activator complexes during heat stress revealed that the TFIID complex disassembles from growth-related genes that are repressed, while components of the SAGA complex assemble at stress-related genes[35]. These results provide a mechanistic insight into a bipolar regulation of the transcriptome by showing that growth- and stress-related gene expression programmes rely on distinct sets of co-activators. SAGA-dominated genes are more extensively regulated than TFIID-dominated genes. First, they make abundant use of negative regulators: Bdf1 seems to block the assembly

TFIID
A complex that is composed of TBP and TAFs. Binding of TFIID to DNA is necessary but not sufficient for transcription initiation from most promoters.

SAGA
A large multi-protein complex involved in the regulation of transcription that possesses histone acetyltransferase and TBP-binding activities. The budding-yeast complex includes Gcn5, several proteins of the Spt and Ada families, and several TAFs; analogous complexes in other species have analogous compositions, and usually contain homologues of the yeast proteins.

Box 2 | **The TATA box and gene expression noise**



Whereas global studies uncovered the association of TATA boxes with stress-related genes, single-cell studies provided a mechanistic insight into this association. Such studies are based on the measurement of variation (that is, noise) in expression levels of proteins in single cells: the expression of two different reporter genes are put under the control of two identical genomic loci in diploid cells, followed by detection of expression levels of the reporters in single cells using flow cytometry or other methods. The within-cell and between-cell variability in protein expression is then analysed, leading to estimates of intrinsic and extrinsic noise, respectively[99] (see figure). Intrinsic noise is more formally defined as the variation in expression of identical proteins in the same cell (in the figure, this is seen where data points for individual cells do not lie on the diagonal, solid arrow), whereas extrinsic noise is the variation in expression of identical proteins owing to differences between cells (in the figure, this is represented by the total range of expression levels along the diagonal, dashed arrow)[100].

Unlike extrinsic noise, intrinsic noise is promoter specific[67]. Stress-related proteins that are expressed from genes with a TATA box are characterized by high levels of intrinsic noise, whereas growth-related proteins exhibit low noise[65]. This finding suggests that the growth- and stress-response programmes are characterized by different levels of intrinsic noise. Mutations that are introduced into the sequence of the TATA box decrease the intrinsic noise levels[66,67]. This behaviour could be modelled by increasing the dissociation rate of TATA-binding protein (TBP) from promoters[66]. Low TBP-dissociation rates favour the formation of a stable transcriptional scaffold, enabling repeated recruitment of RNA polymerase II — a phenomenon known as re-initiation[101] — culminating in high noise levels and sustained transcriptional 'bursts'. Conversely, high TBP-dissociation rates destabilize the transcriptional scaffold, resulting in shorter bursts and lower noise levels[66]. Chromatin regulation has a role in these differential noise patterns[102,103]. Interestingly, sustained transcriptional bursts can confer a fitness benefit in cases in which the expressed gene is necessary to combat environmental stress[66]. These data provide a mechanistic model of how the TATA box can confer a specific advantage to stress-related genes by increasing transcriptional noise and thus increasing expression variability.

of SAGA on stress-related genes in the absence of stress, whereas Mot1 is recruited with the active SAGA complex and might control the transient induction that is typical of stress-induced genes in *S. cerevisiae*[35] (see previous section). Second, SAGA-dominated genes are regulated by the coordinate action of several chromatin regulators (FIG. 2).

The *S. cerevisiae* heat-shock response provides an additional example for the use of the core transcriptional machinery to optimize adaptation to changing environments[36]. During temperature stress, cells redistribute the core transcriptional machinery to heat-response genes. Simultaneously, a large number of partial pre-initiation complexes are assembled at a different set of genes. These partial complexes contain all basal transcription factors except for pol II and TFIIH. Interestingly, some of these complexes are converted into full pre-initiation complexes following exposure to oxidative stress[36]. These findings could help to explain cross-protection[15]. It is not known whether this global mechanism is conserved for the stress responses in other organisms. However, in the next section we will see that principles similar to those described above are applied by multicellular eukaryotes during development and cell differentiation.

Related regulatory strategies for stress response and differentiation. Metazoan cells that differentiate into a mature tissue downregulate many genes that are involved in normal proliferation while inducing tissue-specific transcripts. This process is reminiscent of yeast cells deciding between growth programmes and specialized stress programmes, including quiescence, filamentation or sporulation. A recent study describes a global mechanism whereby muscle cells manage to downregulate their growth programme during differentiation into mature myotubes[37]. In these cells, differentiation is accompanied by a dramatic downregulation at the protein level of TBP and associated factors, suggesting a loss of the TFIID complex. This process could provide an effective mechanism for a global repression of growth-related genes, as their functions are needed only in undifferentiated cells. This loss of core factors is selective, leaving proteins such as TBP-associated factor 3 (TAF3) and TBP-related factor 3 (TRF3), a vertebrate-specific TBP homologue, unaffected. These proteins interact with each other and form an alternative core promoter-recognition complex that binds the myogenin promoter and activates transcription. The authors propose a model in which core promoter switching mediates the global downregulation of TFIID-dependent genes while allowing for selective activation of genes that are required for myogenesis, which depend only on the TRF3–TAF3 complex[37]. Interestingly, TRF3 is essential for embryogenesis in *Xenopus laevis* and in zebrafish[38,39], and it controls the expression of a master regulator for zebrafish haematopoiesis[40]. These findings suggest that the use of alternative core promoter-binding complexes might not be restricted to mouse myogenesis. Together, these data highlight how yeast and vertebrates exploit similar strategies, involving differential use of the core transcriptional machinery, to achieve massive reprogramming of their transcriptomes in response to external stimuli.

An important component of the core transcriptional machinery is pol II, and its recruitment to the promoter is often the limiting step for transcription. However, pol II sometimes stalls at the start of genes — this was first described for heat-shock genes in flies and later for several viral and mammalian genes[41]. This phenomenon is now widely documented also at genome-wide levels, showing that pol II stalling is widely used to regulate genes during stress response and development[42–45]. In quiescent *S. cerevisiae* cells,

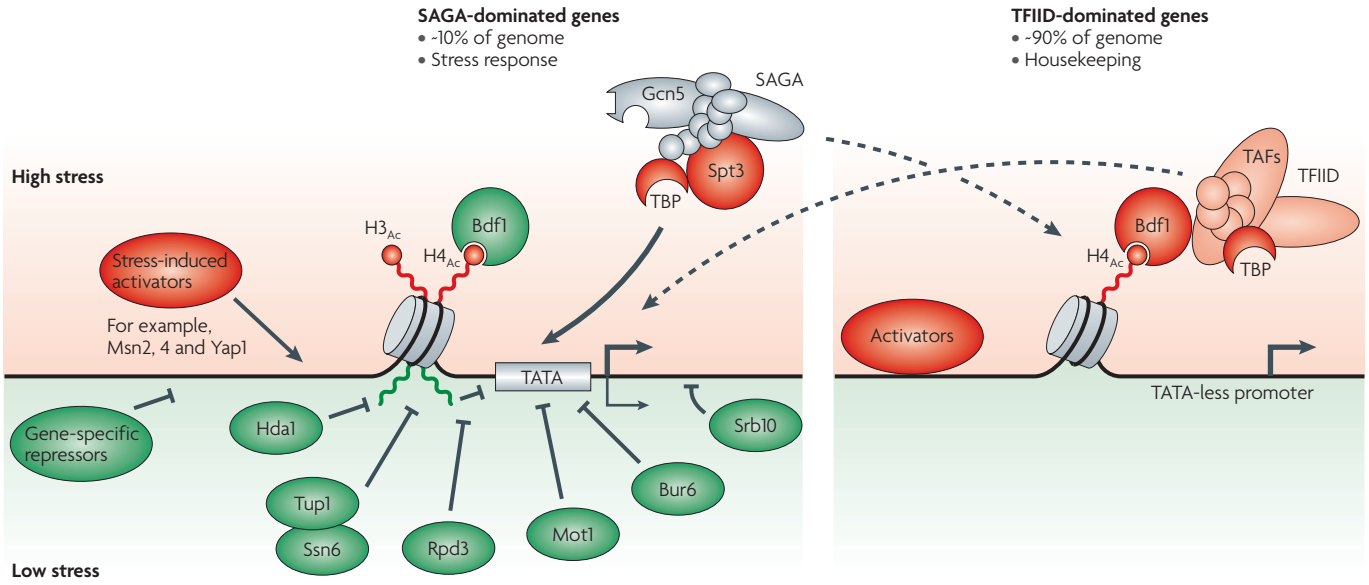


Figure 2 | **Stress- and growth-related genes show distinct regulatory features.** In budding yeast, stress-related genes tend to be extensively regulated, their control is dominated by the co-activator complex SAGA and their promoters often contain TATA boxes, whereas growth-related and housekeeping genes tend to have TATA-less promoters and their control is dominated by the co-activator complex TFIID. Factors depicted in red are negative regulators, whereas those in green are positive regulators. Stress-related genes tend to be positively regulated by the SAGA complex and by acetylation (Ac) of histones H3 and H4, and negatively regulated by histone de-acetylases (Hda1 and Rpd3), repressors and repressor complexes (Ssn6-Tup1 complex, Mot1, Bur6 and Bdf1), and a member of the mediator complex (Srb10). Growth-related genes, on the other hand, are positively regulated by histone H4 acetylation and Bdf1. The dashed arrows show that SAGA also contributes to the expression of genes targeted by TFIID, and vice versa. Gcn5, general control nonderepressible 5; TAF, TBP-associated factor; TBP, TATA-binding protein. This figure is modified, with permission, from REF. 34 © (2004) Cell Press.

for example, inactive pol II is bound upstream of hundreds of genes that are rapidly induced following exit from the quiescent state[44]. Thus, yeast seems to prepare for a rapid response by having pol II 'ready to go' on the relevant genes. Alternatively, or in addition, DNA binding of minimal amounts of pol II, which is required to exit quiescence, could protect pol II from degradation. Pol II stalling is also widespread during fly development, in which pol II is stalled on genes that are involved in development and in the response to stimuli[43,45]. A similar picture emerges in human stem cells, in which pol II is stalled on genes encoding developmental regulators[42]. These findings suggest that strategies applied by yeast to respond to changing environments are also applied by metazoans to regulate stress responses or cell differentiation.

Interplay between stress and evolution

This section deals with stress-response strategies from an evolutionary perspective. We will explore how gene expression adapts to changing environments, and how changing environments themselves provide a driving force for evolution by triggering variability in gene expression. The resulting plasticity and stochasticity of transcriptional responses in turn are instrumental in the adaptation to unforeseen challenges not previously encountered during evolutionary history.

Long-term adaptation of gene expression. Genetic variation in regulatory DNA elements provides an important basis for phenotypic variation from yeast to man, leading to heritable regulatory changes[46–51]. Transcription-factor binding sites show remarkable plasticity and rapid divergence[52–55], promoting evolvability of regulatory processes while maintaining functional robustness[56,57]. Microorganisms have been widely used to study long-term adaptation of gene expression to environmental challenges, because they can be grown under tightly regulated conditions for hundreds or even thousands of generations[58]. For example, *Escherichia coli* cells were grown under glucose limitation[59] or temperature stress[60] to analyse changes in gene expression over extended times. In both cases, the regulation of a few dozen genes was reproducibly modulated in repeated experiments, and this adaptation of gene expression led to increased fitness relative to the ancestor cells. Another study showed that protein expression from the *E. coli lac* operon is precisely optimized during evolution, reflecting the costs and benefits for different environments[61]. Experiments in *S. cerevisiae* grown under nutrient-limiting conditions also revealed characteristic gene expression patterns[62] as a long-term adaptation to the selective pressure. These studies demonstrate that long-term environmental changes lead to persistent changes in gene expression, which can occur within a short evolutionary time to support cellular adaptation to the environmental challenge.

Box 3 | **Additional examples of interplay between stress and evolution**

The examples given in this box illustrate the versatile interplay between stress and evolution. Nutrient stress in *Saccharomyces cerevisiae* triggers a large gene expression programme controlling sexual differentiation[22], which in turn might accelerate adaptation to harsh environments by increasing genetic variation[104]. Transposition is another mechanism to create potentially useful genetic variation[105,106]; as an example, oxidative stress in *Schizosaccharomyces pombe* switches Tf2 transposons from a silenced to an activated state[6,107]. Stress is also known to induce genomic rearrangements in plants[106]. A striking study in *Arabidopsis thaliana* indicates that the capacity for genetic change after stress treatment persists over successive generations, even in the absence of stress; this memory effect, which is based on an epigenetic mechanism, could increase the potential for adaptation[108]. Intriguingly, and somewhat controversially, several studies in microorganisms suggest that stress can directly induce mutations that in turn could accelerate evolution[109]. Moreover, environmental stress can also affect evolution by aggravating or alleviating the deleterious effects of mutations[110] and, in the long term, by increasing the robustness to the effect of mutations[57,111], as well as by unmasking silent genetic variation when the buffering capacity of the heat-shock protein 90 chaperone is compromised[112].

Stress promotes regulatory variation and evolvability of gene expression. Changes in external conditions trigger adaptive variation in intracellular regulation, but an excess of unpredictable regulatory variation can interfere with the robustness of cellular functions. The interplay between variability and robustness — that is, between promoting and buffering intracellular changes — is fundamental for evolution[48,57,63,64]. As discussed above, stress-related genes tend to have more noisy expression characteristics than growth-related genes, and noisy expression is associated with TATA boxes (BOX 2). Several recent studies indicate that noise levels are tuned by evolution to balance fidelity and variation of gene expression; although too much noise with respect to the regulation of processes such as cell proliferation or development could be detrimental for fitness, variable regulation of environmental responses could actually increase the chance of survival during stress[63,65–67]. Stress-related genes are also more volatile with respect to gene duplications and losses than growth-related genes in yeasts[68], and duplicated stress-related genes show higher expression divergence than developmental genes in *Arabidopsis thaliana*[69]. Between-cell variation in the expression of stress-related genes might be beneficial by enabling populations to sample multiple phenotypes, thus increasing the chance that some cells survive adverse conditions. This strategy should be particularly effective for dealing with unpredictable and rapid changes in the environment. For example, phenotypic heterogeneity within clonal bacterial populations promotes stress tolerance: *E. coli* can switch stochastically into a 'persistent' state, characterized by slow growth and increased stress tolerance, which allows it to survive antibiotic treatment[70]. A recent study in *S. cerevisiae* suggests that cells might tune the rate of stochastic switching between different phenotypes to the frequency of environmental fluctuations in order to optimize survival[71].

Variable gene expression that is triggered as an adaptation to stress in turn increases the evolvability of gene expression. Long-term evolution of gene expression changes between yeast species is correlated with short-term regulatory changes to environmental stress: genes that show high environmental responsiveness within one species also tend to show high expression divergence between species[72]. Accordingly, stress-related genes that are associated with TATA boxes show exceptionally rapid regulatory evolution, which also is true in worms, flies, plants and mammals[72]. These findings raise the possibility that the regulatory characteristics of TATA-box promoters encourage the evolvability of gene expression. Indeed, the presence of a TATA box leads to increased sensitivity of gene expression to both mutations and environmental stress[73], and TATA-containing genes tend to be regulated by more transcription factors than TATA-less genes[72,73]. TATA-containing genes are also highly variable between natural isolates[46] and experimentally evolved *S. cerevisiae* strains[31]. Together, these data not only highlight the importance of TATA-box genes for both short- and long-term regulatory adaptation, but also suggest mechanisms for their increased regulatory evolvability.

Besides promoting noisy and divergent gene expression, stress can trigger phenotypic variation and can speed up evolution by a range of additional processes (BOX 3). These two findings indicate that changing environments and stressful conditions keep organisms 'on their toes', and stress not only promotes short-term adaptations but seems to be a major driving force for evolutionary innovation.

Unspecific components of transcriptional responses to stress. As described above, environmental changes activate signal-transduction pathways to trigger extensive cellular responses in gene expression, including hundreds of genes that are either up- or downregulated. This raises questions about specificity[74]: do these global responses reflect adaptations to environmental challenges or do they include large stochastic components? Many of the genes that are regulated during stress do not seem to have any direct functional relevance to the specific perturbation. Accordingly, functional profiling in *S. cerevisiae* indicates an overall poor correlation between the stress sensitivity of mutants and the regulation of corresponding genes in the same stress[75–77]. Similarly, out of 16,000 *A. thaliana* genes that are regulated during temperature stress, only 43 genes seem to have substantial adaptive value[78].

These findings suggest that a large proportion of the gene expression response to a specific stress is not adaptive for this stress. This apparent lack of specificity could simply be an indication that a large proportion of the regulated genes are not specific for any given stress but form part of a core stress response as described above. Indeed, large stereotypical regulation of the growth- and stress-related modules dominates the gene expression responses to a wide range of chemical or genetic perturbations in *S. cerevisiae*[14,79]. A general, unspecific stress response has the advantage that it can cross protect against multiple environmental conditions, which might frequently occur together and

Box 4 | **Anticipating environmental changes: circadian clocks**

The rotation of the Earth produces one of the most influential environmental fluctuation organisms have to face. Day–night oscillations not only influence light availability but are often also accompanied by fluctuations in temperature, nutrients or other factors. These constant and predictable fluctuations led to the widespread evolution of circadian clocks (from the Latin *circa diem*, meaning 'about a day'), which control daily rhythms in gene expression and other regulatory aspects, thus permitting organisms to anticipate and prepare for predictable environmental changes[113,114]. Circadian mechanisms are not homologous and seem to have evolved independently several times in bacteria, fungi, plants and animals. Cyanobacteria and animals with altered circadian rhythms or arrhythmic mutations are outcompeted in changing environments[114]. Circadian clocks are driven by oscillators (also called pacemakers), which are regulated by transcriptional and post-transcriptional feedback loops[115] — these loops continue to fluctuate even in the absence of the environmental cues. For fine-tuning, circadian clocks are set to the actual environmental time cues (or 'zeitgebers'). Microorganisms, plants and flies have circadian pacemakers that can be reset by environmental cues, whereas in mammals and birds only the pacemaker in the central nervous system can be entrained by environmental cues, and they then synchronize peripheral oscillators by secreting uncharacterized molecules[113]. The output from circadian clocks is the periodic transcription of clock-controlled genes. The extent of circadian transcriptional control varies from organism to organism, and also between different tissues, ranging from the entire genome in cyanobacteria to ~10% of all genes in mammals. In all cases, transcriptional oscillations then influence key physiological aspects that are defined by the specific needs of organisms, including metabolism, photosynthesis, fungal spore formation and/or germination, or mammalian behaviour[113,114].

in combination with stresses that the cell cannot sense. It is also possible, however, that a portion of the stress-response programme reflects neutral evolutionary drift or large-scale connectivity and dynamic compensatory adjustment of the transcriptional regulatory network.

Gene expression tuning to unknown challenges. Some environmental changes, such as those caused by day–night cycles, are predictable, and organisms have evolved specialized periodic gene expression programmes to prepare for these expected fluctuations; these circadian-clock or rhythm programmes are maintained for some time even in the absence of the environmental cues (BOX 4). Unpredictable but known challenges, on the other hand, trigger large gene expression responses as and when required. We have seen above that these gene expression responses show limited specificity for any particular stress. This is perhaps not surprising given that the number of potential environmental conditions encountered during the lifetime of an organism and, even more so, during evolutionary history can be expected to greatly exceed the repertoire of signalling pathways and other gene regulatory processes. In this respect, it is particularly insightful to study how gene expression responds to unknown perturbations that cells could not have encountered during evolution, thus eliminating the possibility of 'pre-selected' gene expression programmes.

Recent studies are starting to address this intriguing issue. In an elegant approach in *S. cerevisiae*, the essential *HIS3* gene was placed under ectopic *GAL* control so that changing to a glucose-containing medium led to repression of *HIS3* (FIG. 3a), resulting in a severe challenge to which the cells adapted over several generations[80,81]. Although global gene expression responses were evident during adaptation to the unforeseen challenge, a large proportion of these responses was not reproducible in repeated biological experiments. This finding is surprising given that the initial changes in gene expression apparently did not reflect a population selection process but transcriptional reprogramming of most cells[81]; the differences in gene expression responses might therefore reflect physiological differences in the repeated experiments. The transient gene expression responses are not explained by metabolic logic, as would be expected for responses that had not been specifically selected for during evolution. Thus, it seems that unknown challenges can trigger stochastic, unspecific and transient gene expression responses on the basis of regulatory network plasticity, followed by selection for adaptive portions of the response[74,80]. A study on bacterial adaptation to different media led to similar conclusions: although independent *E. coli* cultures showed the same growth and metabolic phenotypes after many generations, the underlying gene expression states differed, including a large number of compensatory expression changes and only a few adaptive changes[82]. However, a related study of long-term adaptation to glucose limitation reported highly similar gene expression responses in parallel *E. coli* cultures[83]. This discrepancy could indicate that variation in glucose concentration is a familiar situation during bacterial evolution, which the cells could have adapted to by using a pre-selected response.

Two papers provide insight into a possible mechanism for the tuning of adaptive gene expression in the absence of any hard-wired and pre-selected regulatory responses. Using a synthetic bistable gene switch with mutually inhibitory operons directing the expression of genes that are required for alternative nutrients (FIG. 3b), *E. coli* cells reliably selected the adaptive state among two stable attractor states without the help of signal transduction[84]. The authors propose that this selection indicates the presence of stochastic gene-network dynamics: in the non-adaptive state, low cellular activity leads to high gene expression noise, making this state less stable than the adaptive state. This prediction was tested by mathematical modelling of the bistable gene switch[84]. A recent theoretical study expanded the model to a more general situation in which adaptive states with optimal growth rates are spontaneously selected, this is because cells are easily 'kicked out' of slow-growth states owing to higher gene expression noise in these states[85]. Gene expression changes by attractor selection are less efficient and slower than changes by signal transduction, but this simple and robust principle could be an ancient mechanism for gene expression adaptation that precedes the evolution of dedicated regulatory mechanisms. Moreover, it seems likely that attractor selection still contributes to cellular robustness and to the survival of rare or unknown challenges.

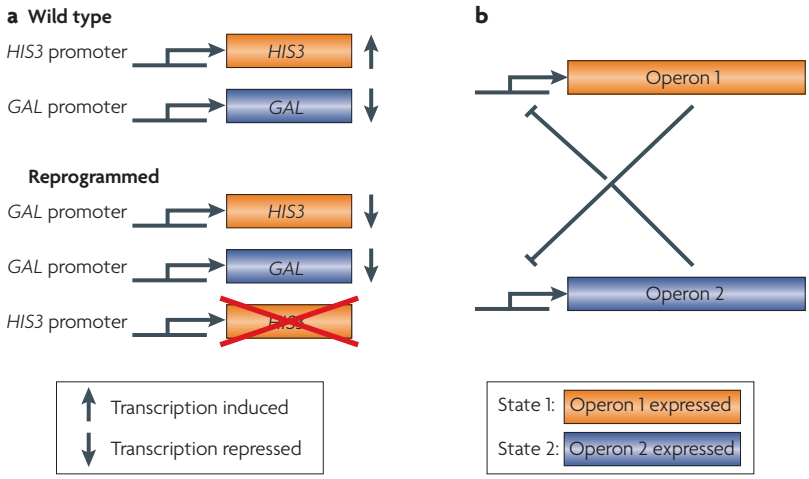


Figure 3 | **Synthetic regulatory circuits to study responses to unknown challenges. a** | Schematic representation of relevant expression profiles in the reprogrammed yeast strain that was used to study novel challenges[80]. Wild-type cells express *HIS3* under its normal promoter, and expression is independent of glucose but activated by the absence of histidine. This simple circuit has been re-wired in the reprogrammed yeast cells, which express *HIS3* under the control of the *GAL* promoter, leading to *HIS3* repression in the presence of glucose. Thus, when the reprogrammed cells are grown in the presence of glucose and absence of histidine, they encounter a serious challenge, because *HIS3* expression is downregulated under conditions in which its expression is essential. **b** | An illustration of a simple genetic switch, which was used in *Escherichia coli* to study attractor selection, consisting of two operons that mutually inhibit each other[84]. Operon 1 codes for a transcriptional repressor of operon 2, whereas operon 2 codes for a transcriptional repressor of operon 1. If operon 1 is expressed above a threshold, it will repress the expression of operon 2 and vice versa. High expression of operon 1 and 2 are mutually exclusive, thus defining two stable states. Both operons also encode condition-specific selectable markers and different fluorescent proteins for gene expression monitoring at the single-cell level, enabling the study of adaptation to different cellular states in the absence of dedicated signalling pathways.

Conclusions

"Adversity has the effect of eliciting talents, which in prosperous circumstances would have lain dormant". Horace's quote very much applies to cells exposed to stress, which use impressive strategies to ensure that global gene expression meets environmental challenges. To stay competitive, yeasts and other free-living microorganisms balance maximal cell growth with stress protection, depending on a multitude of environmental factors. This balance requires the interplay between regulatory mechanisms controlling growth- and stress-related gene expression programmes. Variable gene expression, either driven by hard-wired signalling pathways or by stochastic transcriptional plasticity, helps to cope with variable environments in the short term but might also trigger longer-term adaptations during evolution by enhancing phenotypic variability and robustness. Consistent with this idea, intriguing parallels exist between cellular stress-response and differentiation programmes: different stresses trigger yeast cells to differentiate into different stress-resistant states; cellular stress responses and differentiation are both regulated by conserved signalling pathways in all eukaryotes; and the differential use of the core transcriptional machinery for gene expression reprogramming during metazoan differentiation is reminiscent of the transcriptional mechanisms used during the yeast stress response. These parallels raise the possibility that stress responses are primordial processes for the evolution of cellular differentiation.

Many of these concepts have first been developed in yeast and other simple model organisms, but the available studies in multicellular organisms indicate that they reflect fundamental principles with broad implications and relevance for more complex processes. The recent findings reviewed here therefore provide a valuable basis to better understand gene expression programmes in human cells, including the varied reprogramming of gene expression accompanying ageing, cancer and other diseases.

Compared with transcriptional mechanisms, post-transcriptional mechanisms for gene expression tuning are relatively poorly understood, although they clearly have important roles[86]. Issues that are yet to be resolved are the relative contribution of post-transcriptional control in tuning gene expression to environmental change, and how transcriptional and post-transcriptional layers of control are integrated for coordinated cellular responses. Finding the answers will require multi-dimensional approaches to sample different regulatory levels[87]. Future work will deepen both our understanding of the impact that variable environments have on evolution and our insight into the contribution of gene expression to short- and long-term adaptation to environmental factors, for which cells need to juggle regulatory plasticity with robust transcriptional responses.

The findings reviewed here provide the foundation for a systems-level understanding of gene expression tuning to changing environments. This ambitious goal will require the integration of diverse and comprehensive data sets, and the application of interdisciplinary approaches to model regulatory networks that are triggered by environmental factors[88,89] and, eventually, to predict quantitative cellular responses to different environments[90]. Such deep understanding will ultimately enable us to re-engineer regulatory circuits and design the cellular responses we want, thus having gene expression play to our tunes.

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DATABASES
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
HIS3
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Bdf1 | Mot1 | TAF3 | TBP | TRF3

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