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REVIEW

# The importance of controlling mRNA turnover during cell proliferation

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**Abstract** Microbial gene expression depends not only on specific regulatory mechanisms, but also on cellular growth because important global parameters, such as abundance of mRNAs and ribosomes, could be growth rate dependent. Understanding these global effects is necessary to quantitatively judge gene regulation. In the last few years, transcriptomic works in budding yeast have shown that a large fraction of its genes is coordinately regulated with growth rate. As mRNA levels depend simultaneously on synthesis and degradation rates, those studies were unable to discriminate the respective roles of both arms of the equilibrium process. We recently analyzed 80 different genomic experiments and found a positive and parallel correlation between both RNA polymerase II transcription and mRNA degradation with growth rates. Thus, the total mRNA concentration remains roughly constant. Some gene groups, however, regulate their mRNA concentration by uncoupling mRNA stability from the transcription rate. Ribosome-related genes modulate their transcription rates to increase mRNA levels under fast growth. In contrast, mitochondria-related and stress-induced genes lower mRNA levels by reducing mRNA stability or the transcription rate, respectively. We critically review here these results and analyze them in relation to their possible extrapolation to other organisms and in relation to the new questions they open.

**Keywords** Growth rate · Gene expression · mRNA turnover · Yeast · Transcription · mRNA half-life

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## What expression changes are needed to change the growth rate?

Free-living microorganisms grow at a rate that depends on environmental conditions: availability of nutrients, temperature, presence of toxics and genetic mutations condition the cellular growth rate (GR). Metazoan cells that have lost their developmental restrictions to proliferate, like cancer cells, also grow at a GR that depends on environmental conditions, as suggested by mathematical modeling (Schuster et al. 2015).

For a given microbial strain, the maximum GR is obtained when optimal environmental conditions offer no physiological constraint. During non-optimal growth conditions, the GR is usually below the maximum possible because proliferation and stress defense compete for limited resources in the cell (Ho and Gasch 2015). It is commonly assumed that free-living microorganisms have evolved to grow as fast as possible because this is the simplest behavior that fits Darwinian natural selection for single cells (Bosdriesz et al. 2015). The physiology behind the optimal GR may differ for each microorganism because of their different life styles. The study of model organisms,

however, may cast light on the common physiological principles on which the GR is based on. Most studies in this field have been performed in the gram-negative eubacterium *Escherichia coli* and in the eukaryote yeast *Saccharomyces cerevisiae*.

The macromolecular composition (DNA, RNA, proteins) in exponentially growing microbial cells depends on the GR that environmental conditions allow (Klump et al. 2009; Scott et al. 2010). To adapt the metabolism to obtain the optimal GR, a microorganism must change its metabolite concentrations by altering the activities of selected enzymes post-translationally and/or by changing the expression of their coding genes. As the adjustment of a GR to a defined condition is usually a long-term goal, it is logical that gene expression changes account for an important part of the total change. Gene expression is a process that is divided into multiple steps. Although the final protein level is the goal of gene expression, it is well known that transcriptional regulation plays the most important part in it (Csárdi et al. 2015; Li and Biggin 2015). In a cell population global amounts of mRNAs and proteins increase gradually during growth because cells accumulate mass (mainly proteins). At the single-cell level, in the cell cycle cells grow in volume and mass and both decrease sharply upon cell division when molecules are mostly distributed randomly between two cells (Shahrezaei and Marguerat 2015; Huh and Paulsson 2011). Usually, most experiments have only studied cell populations in cultures in which the GR, gene expression and mass changes represent population averages. Cell-to-cell variability is usually referred to as noise (Newman et al. 2006) and has been discussed in several reviews (Paulsson 2004; Shahrezaei and Marguerat 2015). In addition to noise, epigenetic mechanisms can also introduce variation into microbial cell populations (see later).

During a constant GR, the concentration of macromolecules remains at the steady state and expression changes are not required. However, changes in the GR will need physiological changes to accommodate new macromolecule synthesis rates. As previously stated, it seems reasonable that they should be correlated with changes in gene expression which provoke (and respond to) changes in both mRNA and protein levels, at least for some genes. Since most of the cell mass are proteins it is commonly assumed that the protein synthesis rate parallels the GR in microorganisms because the cost of protein production is the limiting factor (Kaczanowska and Rydén-Aulin 2007; Klumpp et al. 2009; Scott et al. 2014; Bosdriesz et al. 2015). This suggests that translation capability is the energy-limiting step in gene expression (Ho and Gasch 2015), although this established view has been recently challenged (Kafri et al. 2016). Proteins are synthesized by ribosomes, which are made of ribosomal proteins (RP) and rRNAs. Thus RP are usually very abundant (about 15 % of the total protein mass in *E. coli* and 13 % in *S. cerevisiae,* see Bremer and Dennis 1996 and García-Martínez et al. 2007) and the majority of protein synthesis is dedicated to RP themselves (see Ho and Gasch 2015 for discussion). In eukaryotes, rRNA is transcribed by RNA polymerase (RNA pol) I and should be equimolecular to RPs. tRNAs and 5S rRNA are transcribed by RNA pol III. The cost of making proteins and the machineries to make it (ribosomes and tRNAs) entails an enormous proportion of the energy expenditure of a cell, and is both exquisitely regulated and tightly coordinated with GR (Warner 1999; Scott et al. 2014). Prokaryotes usually coordinate the synthesis of all ribosomal components by organizing rRNA, tRNA and RP genes (RPG) into polycistronic operons (Klumpp et al. 2009; Kaczanowska and Rydén-Aulin 2007), whereas eukaryotes should coordinate the transcription of RNA pol I, III and the RNA pol II genes involved in protein synthesis, especially RPG and Ribosome Biogenesis (RiBi) genes (Warner 1999; Jorgensen et al. 2004). The impairment of this coordination between RPG and RNA pol I and III products provokes nucleolar stress and cell cycle arrest (Gómez-Herreros et al. 2013).

Therefore, it seems reasonable a priori that the levels of rRNA, tRNA and ribosome-related mRNAs should be coordinated to the GR. However, it is not obvious if the rest of mRNAs are correlated, or not, with the GR. In fact in *E. coli,* it has been shown that only rRNA, but not mRNA, synthesis is correlated with the GR (revised in Kaczanowska and Rydén-Aulin 2007). Some groups have performed studies on the transcriptomes of model microorganisms to find which proteins (genes) the GR adjustment is based on. The most comprehensive and carefully conducted study is that by D. Botstein's group in the yeast *S. cerevisiae* (Brauer et al. 2008; Airoldi et al. 2009; Slavov et al. 2011, 2012; Slavov and Botstein 2011, 2013), although other studies have been performed in bacteria, especially in *E. coli* (Pedersen et al. 1978; Klumpp et al. 2009; Scott et al. 2010). Brauer et al. (2008) performed a systematic study of mRNA levels at the transcriptomic level for yeast cells that grow in a chemostat at different GRs. They found that 27 % of all yeast genes (≈1500) are expressed in a way that is closely correlated (either negatively or positively) with the GR of the culture. Among them, 337 genes had significant negative slopes (more than 1.5 standard deviations less than the average) and were enriched, among others, in the functions related to energy and oxidative metabolism, while 291 genes had positive slopes with functions, among others, related to translation, ribosome biogenesis, and rRNA metabolism, including RPG and the RiBi regulon, as expected (see above). They also found that the *ESR-induced cluster*, as defined by Gasch et al. (2000), was overrepresented in the genes with negative slopes with the GR and, similarly, the

*ESR-repressed cluster* was overrepresented in the positive slopes set, as expected because this last group is mainly composed of RPG and RiBi. These results raised the possibility that ESR-defined genes were not directly related to stress, but instead responded to a reduction in the GR secondary to stress (Castrillo et al. 2007; Brauer et al. 2008). There is a balance between growth and stress defense (Ho and Gasch 2015). In fact, O'Duibhir et al. (2014) recently showed that the ESR-induced gene expression pattern is similar to what those authors called *slow growth signature,* which is typical of mutants with a GR lower than their wild type. Although the experiments conducted by Brauer et al. (2008) used wild-type cells in a chemostat subjected to variable limiting nutrients, the authors derived a *growth rate calibration signature* of 72 genes to successfully predict the GR from transcriptomes in other culture conditions, strains or, even, other Crabtree+ (see below) yeast species (Airoldi et al. 2009).

These results confirm the previous proposal that higher GRs need (or are correlated with) higher protein synthesis rates because protein synthesis is directly dependent on the cell's ribosome content (Kaczanowska and Rydén-Aulin 2007; Scott et al. 2010; Bosdriesz et al. 2015), and also because the synthesis of ribosome components and biogenesis factors are mainly controlled at the transcriptional level in yeast (Warner 1999; Jorgensen et al. 2004). This explains the positively sloped mRNAs. The conclusions drawn from the negatively sloped mRNAs are not so straightforward: it can be concluded that lower GRs are correlated in *S. cerevisiae* with higher oxidative metabolism (respiration) and increased levels of typical stress-induced genes. Interestingly, these three gene groups are well defined in terms of transcriptional mechanisms. RPGs have a characteristic chromatin promoter structure with fragile nucleosomes that has been proposed to have evolved for the regulation of highly expressed, growth-related genes (Kubik et al. 2015). RPGs also have distinctive transcription elongation with a high proportion of backtracked RNA polymerases (Pelechano et al. 2009). RiBi genes possess some similar regulatory features to RPGs, although they are less transcribed and have less-marked chromatin structures (Pelechano et al. 2009). Respiratory and mitochondria-related genes in *S. cerevisiae* usually behave contrarily to RP and RiBi genes in transcriptional regulation (Ihmels et al. 2005a). For instance, their behavior in transcription elongation during the physiological change from glucose to galactose-containing medium is contrary to that of RPGs and there is less than average backtracked RNA pol II in glucose, so they increase over the average in galactose (Pelechano et al. 2009). This behavior, opposite to that of RPGs, seems an adaptation of this yeast to the respiro/fermentative metabolism that has evolved to compete in high-sugar fruit juice environments (Whiteway et al. 2015; Hagman and Piškur 2015). Accordingly, this high GR corresponds to lower respiration, and vice versa (Brauer et al. 2008). Finally, stress-induced genes are driven by canonical TATA promoters (Basehoar et al. 2004; Rhee and Pugh 2012). TATA genes comprise only about 20 % of the yeast genome, have the characteristic chromatin architecture at their promoters and depend on the SAGA complex, instead of on TFIID, for initiation (Basehoar et al. 2004; Huisinga and Pugh 2004). They have higher (Jordán-Pla et al. 2015) and much broader transcription rates (TR, from almost zero to very high values) and noisier mRNA levels than TATA-like counterparts, such as RPGs, RiBi and respiratory genes (Newman et al. 2006).

The coordination between gene expression and the GR has also been studied at the systems biology level by N. Barkai's group (Levy et al. 2007; Levy and Barkai 2009). These authors discussed that coordination could be obtained by either a mechanism in which the environment affects the GR and that this, in turn, feedbacks to gene expression; or by a feed-forward mechanism in which the environment affects the GR both directly (as in the other mechanism) and indirectly thorough gene expression, which also affects the GR. Based on their own experiments on expression responses to external perturbations, and on the comparison of an *adh1* mutant with its wild type, they concluded that the acting mechanism is a feed-forward one (Levy and Barkai 2009).

## The respective influence of transcription and degradation rates on mRNA levels

As stated above, chromatin-controlled transcription initiation and elongation play a central role in the regulation of the three abovementioned gene groups, which provokes major differences among them. It is possible to think that the mRNA levels for those genes are determined only by transcription. However, mRNA levels are not only the result of their TR in the nucleus, but also of their degradation rate (DR) in the cytoplasm (Pérez-Ortín et al. 2013). Therefore, [mRNA] regulation is not only transcriptional, but also post-transcriptional. The TR usually plays the main role in determining the mRNA level, but sometimes the DR also plays an important role (e.g., see Canadell et al. 2015) and is the key parameter that influences the speed of transient responses (Pérez-Ortín et al. 2007). Thus, both the TR and DR define mRNA turnover. In the commonest situation (steady state) [mRNA] is maintained constant because TR = DR, but each gene with constant [mRNA] has a particular turnover rate. Low turnover saves energy and resources, but involves slow transcriptional response. High turnover is more expensive, but allows for faster responses (Pérez-Ortín et al. 2007). Interestingly, the

three aforementioned gene groups have different [mRNA] and turnover rates in the steady state: RP and RiBi are highly expressed and have a relatively low turnover; stress-induced genes are very lowly expressed with a high turnover during the stress response (Canadell et al. 2015), and respiratory-related genes have an intermediate expression level and are tightly regulated at the mRNA stability level (García-Martínez et al. 2004; Miller et al. 2014; Olivas and Parker 2000).

Recently, we and others (Goler-Baron et al. 2008; Harel-Sharvit et al. 2010; Haimovich et al. 2013; Sun et al. 2013) have demonstrated that, in yeast, TR and DR are coupled by cross-talk mechanisms that imprint mRNAs during their transcription in the nucleus so as to determine their fate in the cytoplasm. Conversely, some proteins that belong to decay machineries, such as 5′–3′ exonuclease Xrn1 (Haimovich et al. 2013) and the 3′-deadenylase complex Ccr4-Not (Kruk et al. 2011), shuttle from the cytoplasm to the nucleus and activate transcription at both the initiation and elongation levels. This cross-talk apparently serves as a way to compensate global changes in one of the two taps that fill or empty the total [mRNA] pool (Pérez-Ortín et al. 2013) to keep *ribostasis* (defined by Ramaswami et al. 2013 as the homeostasis of [RNA]).

Given these considerations, it is clear that the study of transcriptomes (mRNA levels, [mRNA]) does not provide a full picture of the GR dependence of the gene expression because the molecular mechanisms that connect the GR and gene expression could act at either of the two arms of the equilibrium. It is necessary to analyze how the GR affects each step of the mRNA cycle. Is transcription the main determinant of the GR-gene expression coupling for genes with positive or negative correlations? For this purpose, we developed a study based on genomic data from our own laboratory and from the data published by another group (Sun et al. 2013). In our study (García-Martínez et al. 2016), we found that the GR influences gene expression by acting in both synthesis and mRNA degradation. Globally, there is a linear correlation between the synthesis of mRNA and the GR. Nevertheless, as fast as cells grow, the DR increases and maintains an approximate constant [mRNA]. This fact makes steady-state global [mRNA] independent of the GR (see later).

Although these global tendencies are robust, some gene function categories have trends that deviate from average behavior. As expected from the Botstein's group results previously described, translation-related genes (such as RP, ribosome biogenesis, translation factors, etc.), showed a positive correlation between GR and [mRNA], whereas ESR-induced and respiration and mitochondria-related genes lowered [mRNA] at higher GRs. However, the way to achieve this change is not the same. Whereas translation-related genes raise the mRNA level by increasing its synthesis (TR), the drop in respiration and mitochondria-related mRNAs is not due to a change in the synthesis rate, but to an increase in the DR (mRNA destabilization). This observation agree with Ihmels et al. (2005a) who found that the expression of the genes that encoded the mitochondrial ribosomal protein (MRPs) was correlated with the expression of the stress genes induced during slower respiratory growth in non-fermentable carbon sources, although that study did not address the contributions of the TR and mRNA stability to such changes. Other gene groups, like that formed by stress-induced genes (ESR-up), follow a completely different strategy to lower mRNA levels, mainly due to a lowering TR, with no remarkable changes in their stability. On the other hand, Lu et al. (2009) showed that a low GR induces stress resistance and that the transcriptional stress response is lower in respiratory-deficient yeast cells, which suggests a role for greater respiratory activity during slow growth and the stress response that also characterizes it. Thus, there is a connection between the two main groups of genes that are negatively correlated with the GR.

A slow GR has been associated with a mild stress response under chemostat conditions (Lu et al. 2009). We found that the down-regulation of stress-induced genes in fast-growing populations is due exclusively to reduced transcription. Thus, control at an mRNA synthesis rate is the main determinant of the GR dependence of both ESR-up and stress-repressed genes (mostly the RP and RiBi genes). This is in contrast to the participation of mRNA degradation, which we (Molina-Navarro et al. 2008; Romero-Santacreu et al. 2009) and others (Molin et al. 2009; Shalem et al. 2011; Sun et al. 2013) have found in the fast transcriptomic response to environmental changes (revised in Solé et al. 2015). Therefore, transcriptomic changes in the initial shock phase, with slower or stopped growth, heavily depend on the stabilization or destabilization of already existing transcripts, which leads to rapid changes in the proteome, whereas transcriptomic changes after resuming growth rely more exclusively on changes in the TR.

Interestingly these three gene groups are also periodically expressed in the so-called yeast metabolic cycle (YMC). Several decades ago, it was observed that *S. cerevisiae* follows a metabolic cycle. Under slow growth conditions, yeast cells are subjected to a metabolic cycle in which respiratory and fermentative metabolism phases alternate. These phases are also characterized by high and low oxygen consumption (HOC and LOC, respectively) (Slavov and Botstein 2011; Slavov et al. 2011). More recently, YMC transcriptome has been characterized (Tu et al. 2005; Klevecz et al. 2004). The YMC has been linked to the cell division cycle (Tu et al. 2005), although this connection remains unclear since metabolic cycling does not necessarily require cell division cycling (Slavov et al. 2011). The proportion of cells in each YMC phase

may underlie the GR response, the ESR, and the cross protection among different stress factors. In this cycle, mitochondria and respiration genes have RA peaks in the high oxygen consumption phase (Tu et al. 2005; Slavov and Botstein 2011; Slavov et al. 2011). It is noteworthy that the yeast metabolic cycle seems to lower, or is even absent, at a high GR (Slavov et al. 2012). Thus, the growing importance of respiration (and the genes related to it) at a low GR may be a direct result of the ever-increasing importance of metabolic cycling, which has also been suggested for the distantly related fission yeast *Schizosaccharomyces pombe,* and even in humans, and could explain the gene expression and respiratory patterns in these eukaryotes (Lemons et al. 2010; Slavov and Botstein 2011; Slavov et al. 2011; Peng et al. 2005; Rustici et al. 2004).

Our study on TR and mRNA degradation rates used data from mainly mutant strains grown in flasks in several liquid media (see García-Martínez et al. 2016 for further details). As each mutant has its particular metabolic and gene expression defect, it was necessary to compare a large set of these noisy data to obtain robust correlations. To cross-check our results we used a totally different experimental strategy: an analysis on the proliferative heterogeneity of a wild-type cell population. Alginate-encapsulated microcolonies grown under the same environmental conditions can be separated according to their size, which depend on their GR. This phenomenon reflects the existence of distinct proliferative lineages, likely sustained by epigenetic mechanisms, in an isogenic population. Differential gene expressions between big and small microcolonies can be analyzed by RNAseq methodology. We found differential mRNA levels in the genes related to cell respiration and ribosome, which were overexpressed in slow- and fast-growing cells, respectively (García-Martínez et al. 2016). A similar result has been found using a different method for microcolony isolation (Van Dijk et al. 2015). These results confirm that GR itself, and not the stress caused by mutations or environmental conditions that limit growth, dictates the gene expression patterns that characterize slow or fast growth. In addition, the analysis of the 3′-end mRNA isoforms and the differential presence of binding sites for mRNA stability factors support the regulation of mRNA decay as an important ingredient of GR-dependent gene expression (García-Martínez et al. 2016).

## What happens to global gene expression when growth rate changes?

So far we have reviewed how gene expression changes support growth rate variation and how these changes are restricted to some specific functional groups of genes. However, as we described above, we found a direct relationship of total RNA pol II TR and the cell GR, and an inverse correlation of global mRNA stability with the GR. The TR and [mRNA] changed in parallel, so global mRNA levels remained constant across the analyzed range of growth rates. Similar results have been found by other groups in yeast (Gresham et al. 2015) and in *E. coli* (Klumpp et al. 2009), which reinforces our finding. In prokaryotes, however, as there is only one RNA pol that transcribes all RNA, including rRNA and mRNA, the meaning is not clear. In fact, it has been published that the synthesis rates of mRNAs remain approximately constant, while the synthesis of rRNA is growth rate dependent (revised in Kaczanowska and Rydén-Aulin 2007), which precludes further discussion. Our result also means that mRNA turnover accelerates with the GR (García-Martínez et al. 2016). Interestingly, the change in mRNA turnover equals the change in the GR, which suggests that the ratio between the GR and the average mRNA degradation constant ($k_d$) must remain invariable under a wide variety of physiological (and growth) conditions. These striking results were not due to any experimental bias introduced by transcriptomic techniques since the data obtained with two different methodologies (genomic run on and RNA metabolic labeling) gave consistent conclusions (García-Martínez et al. 2016). Therefore, the cells that grow slowly have similar total mRNA levels to fast-growing cells, but the mRNA turnover of the latter is higher than slow-growing cells.

A practical consequence of the general dependence of mRNA turnover on the GR is that genes cannot be annotated by their characteristic mRNA half-lives since these absolute values depend on the GR. This fact explains, at least in part, the major discrepancies between mRNA stability databases (for a review see Pérez-Ortín et al. 2011). Genome-wide methods for the direct measurement of mRNA stability usually involve transcription inhibition by drugs or RNA pol II inactivation (Pérez-Ortín et al. 2013). These protocols have a strong negative impact on cell growth. In contrast, mRNA stability measurement using metabolic labeling, or indirect methods that combine the measurement of TRs and mRNA levels, cause milder perturbation of cell growth (Pérez-Ortín et al. 2013). In general, the first kind of methods produces longer half-lives than the latter ones, which is consistent with the sharp drop in the GR that transcription inhibition provokes (Pérez-Ortín et al. 2011).

## Consequences of coupling mRNA turnover to the growth rate for cell identity

Variation in the global mRNA turnover across the GR range and the constant GR/$k_d$ ratio introduces an important new concept for cell identity. We are used to considering the set of mRNA levels that conforms a transcriptomic

profile as the characteristic signature of a cell. This view is based on the assumption that mRNA levels reflect specific cell composition, and that this combination of cell components determines the physiological capacities of the cell and, by extension, its interaction with the environment. However, cell identity does not only depend on the molecular composition of the cell when it receives stimuli, but also on its capability to modify this composition in response to these inputs. This plasticity is an important ingredient of cell responsiveness and strongly depends on the turnover of cell components, including mRNAs. In order to be effective, responsiveness should be fast enough to allow cells to change their molecular characteristics before the next round of cell division, otherwise adaptation to the environment would be uncoupled of proliferation and would jeopardize cell viability. Therefore, cell components half-lives should be short enough to allow their degradation in a short fraction of the cell division length. This involves close coordination between cell growth and mRNA turnover. In agreement with this prediction, we found a constant ratio between mRNA turnover and cell growth across the range of GRs that we explored (García-Martínez et al. 2016). The GR/$k_d$ ratio value is 0.11. So we can state that, on average, the mRNAs molecules in *S. cerevisiae* last for one ninth of the cell cycle. This balance is the result of the parallel change of transcription and degradation rates with GRs, with [mRNA] remaining constant. The mechanistic coupling between transcription and mRNA degradation machineries (see above) likely facilitates this process. A single regulatory mechanism, sensing the GR and acting on single machinery, either the transcriptional one or the set of factors involved in mRNA degradation, would modulate mRNA turnover without modifying [mRNA]. In contrast, uncoupling this parallel change in transcription and degradation would cause impaired responsiveness and a deficient change in cell phenotypes in response to stimuli (Fig. 1a).

In addition to responsiveness, cell cycle regulation is a second process that likely benefits from the coupling of mRNA turnover and GRs. For instance, the genes that up-regulate after mitosis and down-regulate before entering the DNA synthesis phase (i.e., the genes expressed during G1) need to accommodate their mRNA half-life to G1-length, which is directly linked to the GR (Ferrezuelo et al. 2012). In this case, uncoupling mRNA turnover from the GR would cause the interference of these genes with the mitotic cycle (Fig. 1b). This prediction is supported by the close association of mRNA synthesis and degradation across the cell cycle for cell cycle-regulated genes (Eser et al. 2014). Both highly responsive and cell cycl-regulated genes are characterized by canonical TATA-boxes in their core promoters (Rhee and Pugh 2012; Eser et al. 2014), which suggests a close connection of TATA promoters to the coupling between mRNA turnover and the GR.

## Remaining questions

The above-described results provide some answers to previously formulated questions on the ways that eukaryotic cells use to control their mRNA levels in variable environments, but also raise new questions that are currently left unanswered. The first one is related with the potential generalization of the *S. cerevisiae* results to other eukaryotes. Given the very specific adaptation of this yeast to high-sugar environments (Hagman and Piškur 2015; Whiteway et al. 2015), is the negative correlation seen between the GR and respiration specific for Crabtree+ microorganisms? This question could be answered by using other model organisms, such as Crabtree− yeasts (e.g., *Candida albicans*). In fact an evolutionary study by N. Barkai's group has already shown that mitochondria-related genes in *C. albicans* have expression patterns and gene promoter architectures like their RPG, and not the opposite to them as in *S. cerevisiae* (Ihmels et al. 2005b). In any case, it is interesting to note that in *S. cerevisiae* mitochondrial-related mRNAs use mRNA stability as a regulatory mechanism apart from their transcriptional regulation. Why do these genes need this additional regulation to a greater extent than other genes? Moreover, the correct balance between GR and stress response or apoptosis is fundamental for proper cell function. An improper stress response can lead to unchecked growth, which is a critical driver of cancer (discussed in Ho and Gasch 2015). From this point of view, the results obtained with model organisms are useful and inspiring to understand the molecular basis of several diseases (Schell et al. 2014).

Perhaps, however, the most surprising question to arise from these results is why do total RNA pol II transcription rate increase with the GR? Is this caused by an increase in the RNA pol II total level? In *E. coli* it has been shown that the abundance of RNA pol molecules with the GR explains the increase in the TR (see Klumpp et al. 2009). In *S. cerevisiae* our previous calculations have indicated a vast excess of total RNA pol II molecules compared to elongating ones (Pérez-Ortín et al. 2007). However, the total RNA pol II levels varied with growth temperature (Miguel et al. 2013), which suggests some control at the RNA pol II level. It would be necessary to evaluate RNA pol II total and elongating levels to answer the question. Another related topic is why do transcription and mRNA degradation increase in parallel between them and in parallel to the GR, and make the GR/$k_d$ ratio almost constant? We have previously discussed the logic behind the mRNA turnover balance to the GR. However, now we have to consider that the important objective for the cell to cope with variable situations and to survive is to have the right protein levels, which are the phenotypically relevant players, and not the mRNA levels, which are mere expression intermediates.

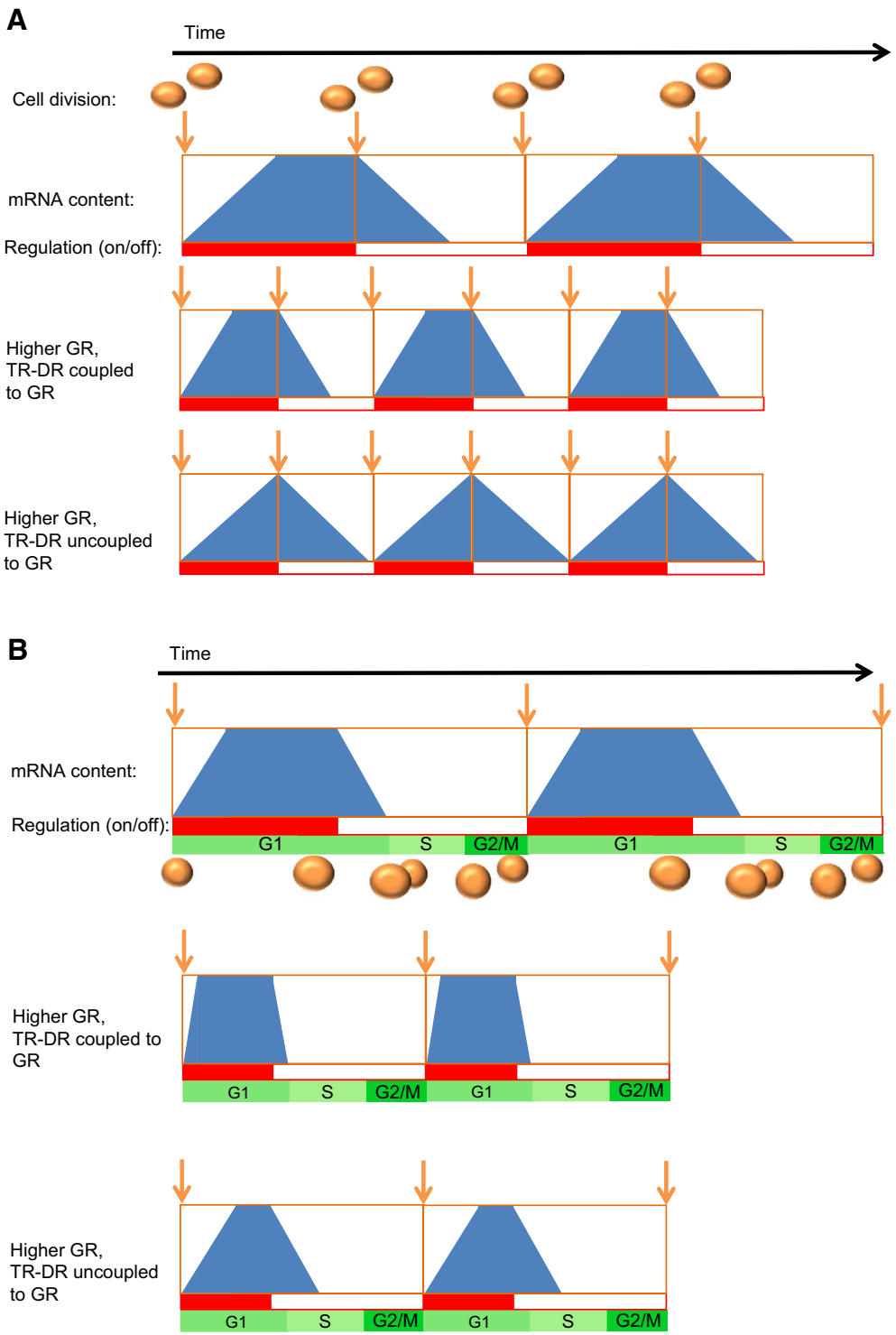


**Fig. 1** Consequences of coupling between transcription and mRNA degradation to the growth rate for cell phenotypes and cell cycle. **a** The phenotypic response of a daughter cell to a regulatory stimulus can be conditioned by the expression of the corresponding genes in the mother cell. If the daughter cell needs to down-regulate a gene, its mRNA should decay at a rate that allows the cell phenotype to change. Likewise, when the daughter cell needs to up-regulate a gene, its mRNA should accumulate fast enough. Proper induction and extinction kinetics depends on the adjustment of the TR and DR to cell division. In a hypothetical uncoupled situation, an increase in the GR would cause impaired up- or down-regulation due to suboptimal kinetics. **b** Similarly in a cell cycle, the expression of cycling genes, particularly those that are expressed during G1, need to follow optimal up- and down-regulation kinetics to avoid invading other cell cycle phases. The coupling between transcription and mRNA degradation to the GR should avoid these potentially harmful situations

Perhaps the reason stems from the fact that most gene regulation occurs at the transcription level, and translation acts as a post-transcriptional nonlinear amplification of transcriptional regulation for most genes (Csárdi et al. 2015; Li and Biggin 2015).

Finally, an open question is what are the cell elements that mediate the communication between the GR and mRNA turnover? It has been proposed that yeast cells regulate mRNA levels of growth-related genes by following a feed-forward mechanism that anticipates growth necessities by sensing environmental conditions (Levy and Barkai 2009). A simpler feedback mechanism has been proposed for *E. coli* (Klumpp et al. 2009). Our results favor a feedback model also for *S. cerevisiae* because the use of a large battery of mutants that grow under several media and conditions show a clear tendency with the GR. In fact, the results of Levy et al. (2007) with the single *adh1* mutant can be interpreted differently with regard to that these authors proposed (Levy and Barkai 2009) because they did not take into account the larger cell volume of yeasts in glycerol. Whatever the true model, for those genes that regulate mRNA levels in a GR-dependent manner, we do not know if the global regulation of mRNA turnover by the GR follows a similar mechanism. If not, do cells utilize the same elements that link the GR with cell cycle regulation for regulating mRNA turnover (Ferrezuelo et al. 2012)? What would the primary target be in gene transcription machinery? Alternatively, would this be the primary target located in mRNA degradation machinery, which would subsequently transmit the signal to the transcriptional machinery via transcription/mRNA degradation coupling? It is necessary to answer these mechanistic questions to fully comprehend this phenomenon.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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