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Genomics of mRNA turnover

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Abstract

Most studies on eukaryotic gene regulation have focused on mature mRNA levels. Nevertheless, the steady-state mRNA level is the result of two opposing biological processes: transcription and degradation, both of which can be important points to regulate gene expression. It is now possible to determine the transcription and degradation rates (TR and DR), as well as the mRNA amount, for each gene using DNA chip technologies. In this way, each individual contribution to gene expression can be analysed. This review will deal with the techniques used for the genomic evaluation of TR and DR developed for the yeast *Saccharomyces cerevisiae*. They will be described in detail and their potential drawbacks discussed. I will also discuss the integration of the data obtained to fully analyse the expression strategies used by yeast and other eukaryotic cells.

Keywords: *run-on; macroarray; transcription rate; mRNA stability; ChIP-chip*

INTRODUCTION

Eukaryotic gene expression is a complex process (Figure 1) that is regulated at different steps, such as transcription rate (TR), mRNA processing, mRNA stability (RS) and translation rate. Traditionally, however, gene expression analysis methods mainly focus on the evaluation of mRNA amounts (RA). Nonetheless, the amount (concentration) of an individual mRNA is the result of a balance between its TR and RS. Thus, the study of mRNA synthesis and degradation describes in more detail how the RA is obtained or changed. When the environment does not change, it is logical to assume that most genes have constant RA, and are, therefore, steady-state conditions. In this situation, TR and degradation ~~rate~~ (DR) rates for each mRNA are equal. In other situations where RA varies, TR, DR, or both, change.

Whereas RA has been studied at a genomic scale since the development of SAGE [1] and DNA chip [2] technologies, the genomic study of RS and TR is more recent. Most genomic techniques for transcription studies are based on the use of DNA chips. Their use for RA evaluation has been reviewed many times [3]. For this reason, I only review the protocols and data for RS and TR here. However, it is convenient to state that the RA data obtained from microarray experiments entail the problem that, in most cases, they are provided as a relative increase/decrease to a reference sample, or they are given in arbitrary units and have to be converted to absolute units (mRNA molecules per cell) if they are to be used in mathematical calculations. An additional problem is that mRNA molecules follow a maturation pathway, and total or poly(A) mRNA will be measured depending on the extraction protocol. What RA precisely means in each experiment is, therefore, variable.

A general feature of genomic techniques is the higher uncertainty involved in measuring values for a single gene in comparison with individual techniques (e.g. RT-PCR), which may imply that less accurate conclusions may be drawn from these data. For instance, Wang *et al*. [4] compared their genomic data for yeast mRNA half-lives with those of 34 mRNAs which were previously determined by northern analysis. The Pearson coefficient was 0.74, which indicates a reasonable global correlation, but they showed a variable individual fitting. However, because these techniques analyse thousands of genes simultaneously, it is possible to make use of statistics to obtain robust profiles for the three parameters; RA, TR and RS [5]. The appearance of functionally-related genes within the same cluster demonstrates the use of common regulatory pathways at various levels in eukaryotic transcription. Therefore, gene expression strategies can be compared to determine,

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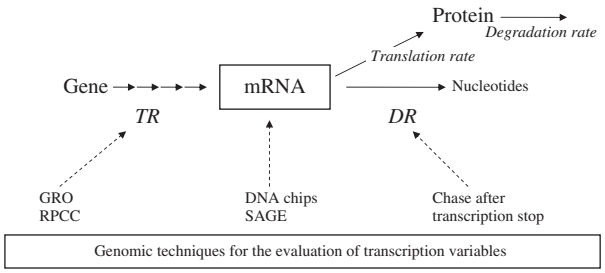

Figure 1: mRNA turnover. mRNA is transcribed, processed and transported from genes to the cytoplasm. The amount (concentration) of mRNA (RA) is dependent on the dynamic equilibrium between its transcription (TR) and degradation (DR) rates. Genomic techniques to evaluate TR, DR and RA are indicated. Current techniques evaluate only nascent TR. Gene regulation can be performed at various levels that include TR, DR and other steps in mRNA maturation and, obviously, at the level of protein turnover and maturation.

for instance, which particular TR and DR a gene uses to produce a given amount of mRNA. Furthermore, the analysis of these data may possibly have important consequences for the field of gene expression kinetics. The best-suited organism for genome-wide expression analyses is the yeast *Saccharomyces cerevisiae*. I will mainly review experiments with this organism as it is the only one for which comprehensive data of TR and RS have been obtained to date [4–6].

GENOMIC EVALUATION OF mRNA STABILITIES

Different techniques have been used for single RS determination in the yeast *S. cerevisiae* [reviewed in 7, 8]; while some are not applicable to genomic studies, other protocols are suitable for genomic scaling. For example, they use transcription stopping by means of either transcription inhibitors (mainly thiolutin or phenantroline) or the temperature sensitive mutant *rpb1-1*, and then chase the remaining RA to calculate the decay over a time interval. In 1998 Holstege *et al.* [9] published a comprehensive study on the effects of several mutations in components of the RNA polymerase II holoenzyme and on other subunits of the transcriptional complexes in the transcriptome. One of the cases studied was the *RPB1* gene encoding the catalytic subunit of the RNA polymerase II, where an *rpb1-1* strain was used, and a temperature shift to 37°C was included to study the changes in the transcriptome. The authors described the use of those data to calculate the 'apparent half-life' of each mRNA species. This does not appear in print but in the supplementary information provided on the authors' web site (http://web.wi.mit.edu/young/expression/halflife.html). Therefore, those data were based on the slopes of the lines calculated only for two experimental time points: 0 and 45 min after heat-shock. Despite the obvious low quality of the data, it was the only dataset available for a genomic estimation of yeast mRNA half-lives for 4 years. In 2002 Pat Brown's lab published a paper [4] in which a similar experiment with *rpb1-1* was done, but where nine time points were used. They obtained high confidence mRNA half-life values for 4687 genes for both total mRNA and poly(A) mRNA populations. These analyses have established that the mRNA half-lives for yeast range from 3 to 300 min, whose average is 23 min [4]. Yeast genes belonging to the same functional category, showed a tendency to have similar RS, especially those belonging to stoichiometric macromolecular complexes. Two years later Grigull *et al.* [6] used RNA pol II inhibitors to analyse mRNA half-lives in wild-type strains and some mutant strains. They discovered that some gene groups, such as ribosomal proteins and ribosome biogenesis factors, are especially controlled at the level of RS.

However, the use of these procedures involves the problem that mRNA half-lives are calculated from data collected over a considerable time interval (up to 90 min) (Table 1). This means that the measured half-lives are calculated over a wide temporal window by averaging any stability fluctuation occurring during the experiment. In addition, these methods lead to a global perturbation of the cell because the temperature shift or drug addition, needed to block transcription, creates abnormal conditions that can either change the expression of some genes or alter the mRNA degradation mechanisms during the experiment. This problem has been discussed [7]. The conclusion drawn after testing transcriptional blockade procedures in genome-wide studies was that such studies were not appropriate for monitoring stress-induced genes [4, 6].

Therefore, alternative strategies are needed to avoid such problems. One such strategy is based on the properties of the steady-state conditions and on chemical kinetics laws. mRNA synthesis follows zero-order kinetics, whereas its decay follows first-order kinetics [10, 11]. Since the synthesis is

Table 1: Main features of the genomic techniques used to evaluate transcription rates and mRNA stabilities

	Advantages		Limitations				Refs.
Transcription rates							
Run-on based methods (GRO)		Direct estimation of elongating RNA pol II densities. Nascent TR	Requires nuclei isolation in higher eukaryotes cells	Assumes a constant elongation rate for RNA pol II			[5] [38] [42]
Chromatin immunoprecipitation-based methods	Different antibodies allow to differentiate the RNA pol II states	Direct estimation of RNA pol II densities. Nascent TR	A fraction of RNA pol II molecules are not elongating	Assumes a constant elongation rate for RNA pol II			[21][a]
Indirect estimation from RA and RS (TRi)	No need for experimental protocol		Error can be increased by mathematical calculations	Relies on calculations from other experimental values		Assumes steady-state conditions for mRNAs	[9]
In vivo labelling with thionucleotides	Uses whole living cells or organs	Fluorescent labelling	Requires a time lapse				[44] [45]
mRNA stabilities							
rpb1-ts	Simple method		Involves a heat shock to cells	Difficult to use under dynamic conditions	Requires a time lapse	Requires a mutant strain	[4] [6]
RNA pol II inhibitors	Simple method		Involves a toxic shock to cells	Difficult to use under dynamic conditions	Requires a time lapse		[6] [47]
Simple Indirect estimation from RA and TR (RSi)	No need for experimental protocol	Instantaneous measurement	Assumes steady-state conditions for mRNAs	Error can be increased by mathematical calculations	Relies on calculations from other experimental values		[5]
Indirect estimation from RA and TR	No need for experimental protocol	Does not assume steady-state conditions for mRNAs		Error possibly more increased by complex mathematical calculations	Relies on calculations from other experimental values	Needs several time points.	[12]

[a]Pelechano and Pérez-Ortín (unpublished data).

a zero-order reaction, the synthesis rate is the same as the rate constant ($TR = k_s$). However, DR, which depends on RA, follows first-order kinetics ($DR = k_d\ RA$). Thus, in steady-state conditions:

$$TR = k_d\ RA \qquad \text{and} \qquad k_d = TR/RA$$

In most papers, RS is given as a half-life (referred to here as RS) instead of k_d, where:

$$RS = In2\ RA/TR \tag{1}$$

This allows to calculate values for either TR or RS whenever the respective values, as well as the RA, are available. Experimentally determined TR values are needed for RS determination (see the next section). This method offers the advantage of not needing a time lapse where circumstances may vary. However, it has two important drawbacks: it is necessary to assume steady-state conditions for each mRNA species (which is not usually easy to demonstrate), and it has the problem of the mathematical amplification of experimental errors may appear (Table 1).

Since steady-state conditions cannot be guaranteed for most genes under many circumstances, a different mathematical approach can be applied. In those cases where Equation (1) is not usable, a differential equation could be used instead [12]. Its integration requires some simplifications. For example, if the TR values TR_1, TR_2, $TR_3 \ldots$ and the mRNA values RA_1, RA_2, $RA_3 \ldots$ can be determined at time points t_1, t_2, $t_3 \ldots$ in an experimental situation, and if a smooth linear change of TR between the experimental points is assumed, then the following relationship between the parameters at two successive points (e.g. t_1 and t_2) can be derived [see ref. 12 for details]:

$$\begin{aligned} &p - k_d\ (TR_2 - k_d\, RA_2) \\ &= [p - k_d\ (TR_1 - k_d\ RA_1)]\ e^{-kd\ \Delta t} \end{aligned} \tag{2}$$

where $\Delta t = t_2 - t_1$ and p is the slope of TR variation between points,

$$p = \frac{(TR_2 - TR_1)}{\Delta t} \tag{3}$$

Thus, Equation (2) can be used to calculate k_d from an experimentally determined time series of the RA and TR values. We have used this approach in two experiments in which yeast cells were subjected to either saline or oxidative stress. We found that changes in RS are very common as most genes undergo changes their RS during the stress response (Romero-Santacreu *et al*. manuscript in preparation; Molina-Navarro *et al*., manuscript in preparation). The problem here is that the experimental error is more prone to be mathematically amplified owing to the complex calculations required. To overcome this problem, we calculated only the k_d average values for genes with common RA and TR profiles. In this way, the common behaviour for RS of a group of genes can be obtained under dynamic conditions in which other techniques fail as transcription stopping techniques do not take into account the changes occurring during the time lapse of the measurements, and also because the indirect calculation from Equation (1) is not applicable.

Table 2: Correlation coefficients between the different data obtained using genomic methods to evaluate mRNA stablities

Methods for mRNA stability*	*rpb1-1*	RS_i	Thiolutin
rpb1-1	0.40[a]	0[b]	0.21[d]
RS_i		0.65[c]	0[e]
Thiolutin			0.42[f]

*Spearman rank coefficients between yeast data sets. All correlations are statistically significant (*t*-test) at the $P < 0.01$ level except for those with 0.
[a]Average of comparisons from ref. [6] and two data sets from ref. [4].
[b]Average of two comparisons between data sets from refs. [6] and [4] and indirect calculations from ref. [5].
[c]Average of indirect calculations from several GRO experiments from our lab.
[d]Average of various datasets from refs. [6] and [48].
[e]Average of two comparisons between data sets from ref. [6] and [48] and indirect calculations from ref. [5].
[f]Single comparison between ref. [6] and [48].

A positive, although not very high, correlation is seen for comparisons between direct techniques (Table 2). This can be due to the requirement of mathematical calculations from experimental data that can amplify errors and also to the existence of specific biases of each technique as demonstrated by the higher correlation observed when comparing data obtained with the same technique. No correlations between indirect calculations and other methods were seen. The reason for this lack of correlation is not clear. It may reflect that direct and indirect techniques require different mathematical calculations using the original data that can bias each data set.

GENOMIC EVALUATION OF TR

TR can be defined as the number of mature mRNA molecules produced in a constant-volume cell in a

Table 3: Correlation coefficients between the different data obtained using genomic methods to evaluate transcription rates

Methods for TR*	GRO	TR_i	RPCC
GRO	0.81[a]	0.36[b]	0.45[d]
TR_i		0.06[c]	0.4[e]
RPCC			0.57[f]

*Spearman rank coefficients between yeast data sets. All correlations are statistically significant (*t*-test) at the $P < 0.01$ level.

[a]Average of several data sets from ref. [5] and other unpublished data from our laboratory.

[b]Single comparison between ref. [5] and indirect data calculation from ref. [4]. Comparison with data from ref. [9] gave no correlation.

[c]Single comparison between indirect data calculations from refs [4] and [9].

[d]Average of various datasets of RPCC (Pelechano and Pérez-Ortín, manuscript unpublished) and GRO (ref. [5] and other unpublished data from our laboratory).

[e]Average of various datasets of RPCC (Pelechano and Pérez-Ortín, manuscript unpublished) and indirect data calculation from ref. [4].

[f]Average between various datasets of RPCC (Pelechano and Pérez-Ortín, manuscript unpublished).

time lapse. They are usually given in molecules/min or molecules/hr. Obviously, the definition of a 'mature mRNA' is flexible because mRNA is subjected to many maturation steps (Figure 1). In biological terms, the 'useful' mRNA molecule is that is ready to be translated. In eukaryotes, however, it is known that several different populations of mRNA exist in the cell: nuclear or cytoplasmatic, polyadenylated or with very short poly(A) tails, bound to ribosomes or stored in processing bodies (p-bodies). The question is which has to be considered to evaluate TR? Basically, the only available techniques for TR estimation do not directly measure the number of mRNA molecules produced in a given time, rather they determine the number of elongating RNA polymerase II molecules present in a gene. Therefore, they calculate the 'nascent TR' [13]. The techniques that measureRS, however, mostly evaluate the 'mature cytoplasmatic mRNA'. Thus, the actual TR in Equation (1) would include the steps of mRNA processing after the nascent TR. This difference between experimental TR and real TR is likely to be one of the reasons of the discrepancies between direct and indirect techniques to evaluate TR and RS. If we consider that polymerase speed is constant for all genes and physiological situations, then the density of elongating polymerases will be proportional to TR. In that case, the density data can be converted into TR using a factor, which relates them both. This factor can be obtained from either individually calculated TR [14], or the normalization of the indirect TR data calculated from RS and RA (see below).

For a long time, the transcription run-on assay (TRO) has been used to directly quantify the density of elongating RNA polymerases [15], providing a measure of TR at the time of RNA labelling [15]. The ability of the run-on method to detect elongating polymerases has also been used to detect the regulation at the level of elongation [16]. The capability to hybridize labelled nascent RNA to a single filter containing multiple gene probes may allow rigorous quantitative comparisons. Although DIG-labelled nucleotides have been shown to be incorporated into nascent RNA [17], the use of this possibility for fluorescence-based microarrays has not yet been published. Radioactive labelling of nascent RNA was first used in human cells in culture and then in other organisms (see below). In such cases however, no conclusions were obtained as to the absolute TR, or even to the relative TR between genes because of the relatively low number of genes analysed, and of the lack of rigorous normalization methods and reference data in those organisms. The yeast *S. cerevisiae*, however, offers the possibility of overcoming all these limitations because of: (i) the existence of whole genome studies that have provided absolute mRNA levels, (ii) the possibility of using accurate normalization methods and, (iii) the ability to perform TRO assays on whole cells. Yeast cells are treated with the detergent sarkosyl that made them permeable, causing an instantaneous loss of nucleotide triphosphates (NTPs) and a complete chromatin disruption [15]. Both effects cause a sudden stop of transcription but leave the elongating RNA polymerases bound together with the RNA that they were transcribing onto the genes that they were. Cells are dead and remain permeable. A short incubation at 30°C with NTPs, including ^{33}P-UTP, allows stopped polymerases to perform a non-physiological elongation for a short run of about 300 nucleotides [15]. This means that those RNA polymerases stopped without mRNA are 'invisible' for this technique, and that polymerases can elongate the transcribed region of a downstream gene. We have developed a method called Genomic Run-On (GRO) [5], which calculates TR for all yeast genes. The method is conceptually similar to those previously referred to. However, because we normalized the signals for each probe and each

filter for both cDNA and the TRO, we can calculate the TR and RA for each yeast gene.

We applied the GRO method to those cells that were shifted from glucose to galactose. We showed that even though global steady-state mRNA levels are only slightly affected, TRs decrease to about 10% of the initial values, and that they are much more variable throughout the experiment, thus revealing an important contribution of RS as a regulatory mechanism. The clustering of the mRNA and TR profiles allows us to find novel functional relationships between genes and new regulatory pathways [5].

Another method that is thoroughly used for detecting proteins onto DNA sequences is the chromatin immunoprecipitation (ChIP) protocol [18]. This method relies on the *in vivo* instantaneous cross-linking of proteins to the DNA sequence that they are bound to with a bi-functional reactive, usually formaldehyde. This method was extended to the genomic level (ChIP-chip) by amplifying and labelling the immunoprecipitated DNA, and by hybridizing it to either microarrays [19] or macro-arrays [20]. When using an antibody against RNA pol II, ChIP-chip provides a measurement of RNA pol II density, which can be converted to TR. Similarly to GRO, this method assumes a constant RNA-pol II elongation rate, and since it is well established that the elongation rate may be subjected to regulation [16], it is, once more, a limitation of the technique (Table 1). Nonetheless, this method allows the use of different antibodies that can add selectivity to the type of RNA pol II molecules. For instance, an antibody against the Sen1p subunit of RNA pol II [21] or the Myc-tagged version of the Rpb1p subunit will capture all the molecules on the genes. The use of antibodies against phosphorylated forms of the CTD tail of Rpb1p [22] enables a selection for elongating molecules. We have implemented this technique, which we call RPCC (RNA pol ChIP-chip), and have compared the TR results obtained with a different antibody and with GRO. The general tendency of TR data is similar between RPCC and GRO, although the correlation coefficient is relatively poor (Table 3), and may be a consequence of a specific bias associated with either technique. This bias, however, can provide an insight into the biology of transcription. We have used the comparison between RPCC and GRO using an antibody (8WG16) against all forms of Rpb1p to demonstrate that the presence of non-elongating forms of RNA pol II differs between functional categories of yeast genes (Pelechano *et al.*, manuscript in preparation). TR data calculated from RPCCs are, however, noisier and the dynamic range is lower than those obtained from GRO.

On the other hand, the TR can be calculated from the knowledge of not only the steady-state level of each mRNA species (the transcriptome), but also of the half-life of that species (TRi in Tables 1 and 3). This approach is similar to that described for the indirect estimation of mRNA half-lives (see above), but the advantage is that it does not need a method to measure the RNA pol II density. However, it does have drawbacks as it relies on the hypothetical assumption of steady-state conditions and on the mathematical increase of experimental errors (Table 1). Despite these problems, the use of indirect calculations for TR is widely generalized in the literature [23, 24]. As previously stated, the approximate half-lives calculated and the RA for the genes at zero time, before a temperature shift, were used to give 'apparent transcriptional frequency', as seen in the supplementary information by Holstege *et al.* [9]. Despite the uncertainty associated with indirect calculations from 'apparent half-life' data, these data are still the most widely used in the literature as a source of yeast TR because they positively correlate with the expected enrichment of the transcribed genes in some histone modifications or in active genes-associated proteins [21, 23–25]. Correlations between different data sets obtained with direct techniques are higher than they are for RS (Table 3), especially for those obtained using the same technique. However, the correlation between the indirect TR data of [9] and direct ones, or even with the indirect TR data of [4], is almost zero (Table 3).

GENE EXPRESSION STRATEGIES: THE ROLE OF KINETICS

For the first time in any organism, the existence of genomic data for all three variables involved in transcription (RA, TR and RS) allows for a detailed study of the strategies followed by yeast genes to cope with the functions they perform. As mRNA is only the messenger between the gene and the protein, it is not the final goal of gene expression (Figure 1). Therefore, transcription should be considered an intermediate step on the way to the protein. mRNA should be translated into protein that can also be controlled at the stability level.

A comprehensive study of gene regulation would use all six variables in the gene expression; TR, RA, RS, translation rate, protein amount and protein stability; throughout a changing physiological situation to evaluate the respective contributions of each one to the expression of every gene. Today, this goal is far from being fulfilled because the three protein variables are quite difficult to measure. They are only known for a single condition: exponential growth in a complete medium. We have used these data together with those of the mRNA variables to evaluate the general strategies of yeast genes under that physiological condition. We have found that functionally related genes follow similar strategies. We have also concluded that regulation at the transcription level is quantitatively more important than at the translation level, and that RS has a distinctive role for gene expression: to modulate the speed of the response [26]. This marks the importance of the kinetics in gene expression strategies. In fact, cells need to deal with the 'time factor' throughout the whole gene expression process. This factor involves the successive steps from transcription initiation to the appearance of a mature protein [12]. The appearance of a mature protein after synthesis of its mRNA can be delayed from a few minutes in unicellular eukaryotes, to several hours in some vertebrate genes. This limits how fast a cell can react to environmental shifts. The amount of protein and the optimal delay for its appearance vary for different genes. Therefore, the cell must control the timing of these changes in a gene-specific manner. Once more, no genomic data are available for kinetic studies at the protein level. However for the first time in a eukaryote, the techniques, which previously accounted for the genomic evaluation of RA, TR and RS allow to study the kinetics of the gene response to a changing environment in yeast.

By following chemical kinetics laws, if a given mRNA is in a steady-state at concentration RA_I, and it is compelled to reach a new steady-state level, RA_F, then RA varies exponentially with time (t) by changing its TR from TR_I to TR_F, as follows:

$$RA = RA_F - (RA_F - RA_I)\, e^{-k\, dt} \qquad (4)$$

As Equation (4) depicts, the time required for readjustment depends only on k_d (i.e. on the RS) [10, 11]. However, RA_F depends on TR_F because the steady-state relation, Equation (1), applies to the new steady-state (i.e. $RA_F = TR_F/k_d$). Hence, the final TR, TR_F, gradates RA_F and the RS (k_d) gradates the transition time. This has profound implications for gene regulation. By way of example, in order to facilitate a rapid change in the expression of a gene, the corresponding mRNA should have a low RS. In this sense, there are important differences between single-celled organisms, such as yeast and higher eukaryotes. Changes in the gene expression have to be, and in fact are, much faster in unicellular organisms whose generation times fall in the range of hours.

Equation (2) can also be used to study a stress response in yeast. We first used this equation to model the responses of yeast genes that positively react to a stress by using realistic data for RA, TR and RS [12]. There are several different kinetic strategies that can be followed, but RS should change to permit a beneficial response within a few minutes for the vast majority of yeast mRNAs with medium or high stabilities. We have also experimentally studied responses to different stresses (osmotic, oxidative, heat) using GRO to simultaneously determine TR, RA and k_d (RS). In general, our results show that stress-response genes mainly change their RA through a change in TR, but most of them also use changes in RS to refine the timing and size of the RA peak. The use of RS is variable between gene functional groups, and it probably depends on specific factors controlling the decay regulons [4, 27, 28]. Interestingly, a sudden decrease in RS is used to lower the RA of genes repressed after stress (Romero-Santacreu *et al.*, manuscript in preparation; Molina-Navarro *et al.* manuscript in preparation).

STUDIES IN OTHER ORGANISMS

When analysing genomic data, it becomes clear that different strategies of gene expression have evolved at the same time as the other cells features. All of them have to cope with the kinetic limitations of that particular process. It is clear that the regulation of RS is a key instrument for the rapid adaptation of cellular processes to a changing environment. In general terms however, a fast response means a high mRNA turnover and, therefore, a high cost. This solution is most probably limited to the necessary situations. In that respect, the situation of free-living cells (such as yeasts and prokaryotes), which must react quickly to most situations, clearly differs from that of

tissue cells, which can tolerate a deferred response in most situations.

Although techniques for genomic evaluation of RA are widely developed, the state of the art for TR and RS determinations in organisms other than *S. cerevisiae* is much less advanced. Transcription stopping with antibiotics has been used to measure at the genomic level RS for some prokaryotes such as *Escherichia coli* [29, 30], *Bacillus subtilis* [31], the archaeon *Sulfolobus* [32]; lower eukaryotes such as fission yeast [33], *Plasmodium falciparum* [34]; and higher eukaryotes, such as human cells in culture [35, 36]. mRNA half-lives are short for free-living organisms (a few minutes), and are much longer for complex eukaryotes (up to many hours). This correlates with the slow responses expected in multicellular organisms. In some cases, a role for RS in gene regulation has been predicted or demonstrated. Interestingly, a negative correlation between RS and TR has been observed for *E. coli* [29, 30], *Sulfolobus* [31] and *S. cerevisiae* [26]. The low RS of highly transcribed genes in these organisms has been partially interpreted as a feature for expression noise minimization and also as a way for rapid adaptation to environmental changes.

For TR determination, run-on techniques are only possible in eukaryotes. In prokaryotes, genomic TR has been calculated indirectly from RS and RA [37], and in higher eukaryotes, the TRO should be performed on isolated nuclei, which require a protocol to purify them. This causes a lag between the actual physiological state of the cell and the capture of nascent RNA (Table 1). Moreover, studies in which a comparison between RAs and TRs has been made, offer only qualitative results [38–42]. Nevertheless, the authors could conclude that many genes were regulated at the level of transcription initiation. For instance, *Myc* proto-oncogen acts at the TR level, and posttranscriptional mechanisms operate to uncouple the TR and the amount of poly(A) mRNA [39]. The most comprehensive studies have been published by the groups of Myriam Gorospe [13, 38] and Jack D. Keene [42]. In the former, the stress responses of human cells at the TR level were studied. The authors developed a protocol to analyse and compare the respective influences of TR and mRNA stabilities on the final mRNA steady-state level. Genes were categorized accordingly after a stress shock. On the other hand, Tennebaum *et al.* [42] developed the protocol en masse run-on assay in which the run-on profiling is combined with ribonomic profiling. Ribonomics is a term that defines the use of immunoprecipitated mRNP complexes to analyse the representation of individual mRNA species associated with a particular RNA-binding protein. The analysis of both kinds of data has led to the proposal that 'post-transcriptional operons' work in the control of the eukaryotic gene expression [28, 42]. Plants have also been studied. Tobacco cells were used to isolate plastids for TRO [43]. The authors discriminate the RNA synthesis made by the different RNA polymerases by using specific antibiotics and TRO. The comparison of mRNA amount and TRO data indicates that post-transcriptional regulation of mRNA is acting. A new method to evaluate TR has been developed in human cells: pulsing with thiouracil [44] or thiouridine [45]. Thiolated RNA is then purified by affinity chromatography and used for microarray analysis. This method is suitable for *in vivo* applications, and may overcome some of the disadvantages o.f the run-on and ChIP assays in higher eukaryotes in determining TR. As the measured TR needs a long (1–2 h) pulse with the precursor, however, it is not instantaneous, but corresponds to an average in the time-labelling period (Table 1).

The ChIP-chip technique has also been used to evaluate TR in single genes [46] and to map the RNA pol II distribution in human cells [47]. In this case, a different antibody has also been used which allowed locating hypophosphorylated RNA polymerase II almost exclusively to the 5′ ends of genes. On the other hand, the localization of total RNA polymerase II revealed a variety of distinct landscapes across the genes with 74% of the enriched locations being observed at exons. No TR values have been obtained from those data despite them being highly informative of the eukaryotic transcription.

The limitations of the current methods for the stability determinations of both TR and mRNA at the single gene level are the main cause of the drawbacks they present when used at the genomic level (Table 1). Therefore improved methods are required to determine TR and DR experimentally. To this end, more research on the molecular events occurring during the run-on, ChIP and the transcription stop methods is needed. Additionally, the mathematical analysis of the results obtained with all the available methods will enable us to know, and to correct, the biases that each one has.

Key Points

- Both transcription and mRNA degradation are important points to regulate gene expression.
- Current DNA chip technologies allow to evaluate mRNA amounts, transcription rates and mRNA stabilities at the genomic level in the yeast *S. cerevisiae*.
- The genomic techniques could either directly measure transcription rates and mRNA stabilities or they could be calculated mathematically using chemical kinetics laws.
- Each technique has some particular drawbacks and biases that could be filtered out by detailed comparative analysis of their results.

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