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Genomics and gene transcription kinetics in yeast

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As an adaptive response to new conditions, mRNA concentrations in eukaryotes are readjusted after any environmental change. Although mRNA concentrations can be modified by altering synthesis and/or degradation rates, the rapidity of the transition to a new concentration depends on the regulation of mRNA stability. There are several plausible transcriptional strategies following environmental change, reflecting different degrees of compromise between speed of response and cost of synthesis. The recent development of genomic techniques now enables researchers to determine simultaneously (either directly or indirectly) the transcription rates and mRNA half-lifes, together with mRNA concentrations, corresponding to all yeast genes. Such experiments could provide a new picture of the transcriptional response, by enabling us to characterize the kinetic strategies that are used by different genes under given environmental conditions.

Gene expression changes in eukaryotes

Gene expression in eukaryotes is a complex process that involves numerous successive steps, from the binding of transcription factors to their target sequence to the post-translational modification of proteins. After any environmental change (e.g. a temperature shift), the cell adapts to the new circumstances by, among other responses, altering the expression of certain genes. Each step of gene expression can be quantitatively regulated. However, it is not always recognized that the rate at which gene expression changes is as important as the magnitude of that change.

Cells need to cope with the 'time factor' throughout the process of modification of gene expression. For example, the transcription and translation processes take place at a limited speed. RNA polymerase II has been calculated to travel at ~18–42 nucleotides per second on chromatin templates [1–5]. This speed might not be constant across all genes and conditions, but if we take it to be a representative average value, then the time required to 'read' a gene is not negligible: 25–50 seconds for 1 kb (the average length of a yeast gene [6]); 2–3 minutes for a typical mammalian gene [7]; and up to 16 hours for certain long intron-containing human genes [3]. Pausing and termination further delay the release of mRNA molecules from the genes (as discussed in Ref. [4]). Moreover, maturation and transport of the mRNA to the cytoplasm [4,8], and translation and transport of the protein to its correct subcellular location are also time-consuming processes. Therefore, the appearance of a 'functional protein' after a 'transcription order' has been received can take from several minutes in unicellular eukaryotes to several hours for long genes in vertebrates. This limits how fast a cell can react to environmental shifts. Furthermore, an optimal response requires an ordered sequence of gene expression changes. Therefore, the cell must control the timing of these changes in a gene-specific manner.

Here, we focus on the transcription kinetics of the yeast *Saccharomyces cerevisiae*, highlighting recent developments. Current genomic techniques now enable the relevant kinetic parameters to be determined for all genes from a eukaryotic organism. These data can then be used to reveal the different transcriptional strategies that responsive genes follow after an environmental shift.

Kinetics of gene expression

Changes in gene expression can be analysed using chemical kinetics. The synthesis of both mRNA and protein follows zero-order kinetics (see Glossary), whereas their decay follows first-order kinetics [7,9]. Thus, the concentration of either of these macromolecules at a steady state

Glossary

DNA macroarray: a series of gene probes bound to a nylon filter at a low density; this filter is used for genomic DNA or RNA analyses of radioactively labelled samples.

DNA microarray: a series of gene probes bound to a glass slide at a high density; this slide is used for genomic DNA or RNA analyses of fluorescently labelled samples.

First-order kinetics: a reaction rate that is characterized by being proportional to the concentration of a single reactant.

Genomic run on (GRO): a scale-up of the run-on technique, enabling measurement of transcription rates for all genes simultaneously (Figure 2b).

mRNA half-life ($t_{1/2}$): the time needed for a given mRNA population to reduce to half through degradation or turnover. If degradation follows first-order kinetics with rate constant k, then $t_{1/2}$ is inversely related to k with a proportionality constant equal to the natural logarithm of 2 (~0.693) (Equation 2).

Run-on assay: a technique for the *in vivo* labelling of nascent RNAs (Figure 2a). It is also known as transcription run on (TRO) or nuclear run on (NRO).

Serial analysis of gene expression (SAGE): a genomic technique that obtains a series of sequence tags from the 3′ part of the mRNAs of the expressed genes; the tag amounts are proportional to the abundance of the corresponding mRNAs.

Steady state: a stationary situation that is created in a dynamic system by balancing inward and outward fluxes. A given mRNA is said to be in a steady state if the amount does not change because the rate of synthesis equals the rate of degradation.

Zero-order kinetics: a constant reaction rate, which is independent of the concentration of a specific reactant.

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(C_{ss}), when rates of synthesis and degradation are equal, can be expressed as a ratio of the rate constant for synthesis (k_s) to the rate constant for decay (k):

$$C_{ss} = \frac{k_s}{k} \quad \text{[Eqn 1]}$$

Because the synthesis is a zero-order reaction, the rate of synthesis is the same as the rate constant. However, k is commonly expressed as a half-life ($t_{1/2}$), these parameters being related as follows:

$$t_{1/2} = \frac{0.693}{k} \quad \text{[Eqn 2]}$$

Here, we focus on the process of transcription and, consequently, on the concentration of mRNA generated in response to a change in environmental conditions. If a given mRNA is in a steady state at a concentration m_I, and it is compelled to change its concentration to reach a new steady-state level, m_F, by changing its transcription rate (TR) from TR_I to TR_F, then the mRNA concentration varies exponentially with time (t) according to:

$$m = m_F - (m_F - m_I) \times \exp(-kt) \quad \text{[Eqn 3]}$$

(see Box 1 for a description of elementary RNA kinetics).

It can be seen from Equation 3 that the time required for readjustment depends only on k (i.e. on the mRNA half-life) [7,9]. However, m_F depends on TR_F because the steady-state relationship (Equation 1) applies to the new steady state; that is:

$$m_F = \frac{TR_F}{k} \quad \text{[Eqn 4]}$$

Hence, the final transcription rate, TR_F, determines the mRNA concentration of the new steady state, m_F, and the mRNA half-life determines the transition time. This has profound implications for gene regulation. To facilitate a rapid change in the expression of a gene, the corresponding mRNA should have a short $t_{1/2}$. There are important differences, in this respect, between single-celled organisms, such as yeast, and higher eukaryotes. Changes in gene expression are much faster in unicellular organisms that have generation times in the range of hours.

Kinetic strategies for changing mRNA concentrations

As mentioned, mRNA concentration depends on both the synthesis rate and the degradation rate. Therefore, cells can use different strategies to increase or decrease mRNA concentrations, by modifying TR and/or k. In this section, we examine the consequences of these different strategies with regard to transition speed and synthetic cost.

Strategies to increase mRNA concentration

Examples of strategies to increase gene expression are considered in Table 1 (strategies 1–7), using realistic data for yeast. For example, consider an mRNA with one copy per cell; a new steady state is then achieved, in which the mRNA concentration has increased fivefold (a reasonable assumption for stress-responsive genes, see Refs [10] and [11]) as a result of an instantaneous shift in TR and/or k. Because of the exponential nature of Equation 3, the difference in the concentration between the old and the new steady state is reduced by half with each successive $t_{1/2}$. Therefore, 98.4% of the transition will be completed after six half-lifes, and we take this interval ($6 \times t_{1/2}$) as a reasonable estimate of the time needed to reach the new steady state.

The easiest way to increase the mRNA concentration to five copies per cell is to increase TR fivefold. This would take 30 minutes for an unstable mRNA (strategy 1 in Table 1) but more than 2 hours for an average yeast mRNA (strategy 2 in Table 1). The latter time period is too long for an organism with a 90-minute life cycle, such as yeast. Increasing $t_{1/2}$ instead of TR (strategy 3 in Table 1) would take longer. Given that the $t_{1/2}$ of most

Box 1. Elementary mRNA kinetics

If the transcription rate (TR) of a gene is kept constant and its mRNA is degraded following first-order kinetics (with a rate constant k), the mRNA concentration (m) will vary with time (t) according to the following:

$$\frac{dm}{dt} = TR - km \quad \text{[Eqn I]}$$

and the mRNA concentration will reach a steady state (m_{ss}) at the following concentration:

$$m_{ss} = \frac{TR}{k} \quad \text{[Eqn II]}$$

When a steady state (m_I) has been established for a certain transcription rate (TR_I), if at a given time (taken as $t = 0$), the transcription rate switches instantaneously to a new value (TR_F), then it follows (by integrating Equation I) that the mRNA concentration will change according to the following:

$$m = \left(\frac{1}{k}\right)[TR_F - (TR_F - TR_I) \times \exp(-kt)] \quad \text{[Eqn III]}$$

Thus, in due course, the mRNA concentration will reach a new steady state (m_F) defined by the following:

$$m_F = \frac{TR_F}{k} \quad \text{[Eqn IV]}$$

Indeed, Equation III can also be written as:

$$m = m_F - (m_F - m_I) \times \exp(-kt) \quad \text{[Eqn V]}$$

Remarkably, even if the new steady-state value depends on the current transcription rate (TR_F), the transition time between steady states is determined by the vanishing (i.e. decaying to 0) of the exponential term in Equation III or V and, therefore, it is only a function of k.

Equation III is based on the assumption that the transcription rate shifts instantaneously (at $t = 0$) from TR_I to TR_F. However, this change might take considerable time. Transcription rates could be experimentally measured at times t_1 and t_2, yielding values TR_1 and TR_2 respectively. If the time points are close enough, then the change in the transcription rate can be assumed to be linear during the time interval $\Delta t = t_2 - t_1$. Thus:

$$TR = TR_1 + p(t - t_1) \quad \text{[Eqn VI]}$$

where p is the slope:

$$p = \frac{TR_2 - TR_1}{\Delta t} \quad \text{[Eqn VII]}$$

and Equations VI and VII are valid for $t_1 \le t \le t_2$.

Substituting Equation VI into Equation I, and integrating (between t_1 and t_2), yields the following:

$$p - k(TR_2 - km_2) = [p - k(TR_1 - km_1)] \times \exp(-k\Delta t) \quad \text{[Eqn VIII]}$$

where m_1 and m_2 are the mRNA concentrations at t_1 and t_2, respectively.

Equation VIII (together with auxiliary Equation VII) enables calculation of the decay rate constant (k) if m_1, m_2, TR_1, TR_2 and Δt are known (although this calculation involves numerical analysis to solve for k in Equation VIII). It should be noted that k is assumed to remain constant in the above derivation. In the case that k is not constant, k can still be derived, but its value will be an average value along Δt.

Table 1. Strategies for achieving a fivefold increase in the mRNA concentration for a given gene[a]

Strategy	Kinetic variables	Initial steady state	Final steady state	Time required (min)[b]	Turnover cost (molecules/min)[c]
1. Increase *TR* (low $t_{1/2}$)	*m*	1	5		
	TR	0.14	0.69	30	0.69
	$t_{1/2}$	5	5		
2. Increase *TR* (average $t_{1/2}$)	*m*	1	5		
	TR	0.03	0.15	138	0.15
	$t_{1/2}$	23	23		
3. Increase $t_{1/2}$	*m*	1	5		
	TR	0.03	0.03	690	0.03
	$t_{1/2}$	23	115		
4. Increase *TR* and $t_{1/2}$	*m*	1	5		
	TR	0.03	0.07	297	0.07
	$t_{1/2}$	23	49.5		
5. Increase *TR* and decrease $t_{1/2}$	*m*	1	5		
	TR	0.03	1.5	14	1.5
	$t_{1/2}$	23	2.3		
6. Over-increase *TR* (× 2)	*m*	1	5		
	TR	0.03	(0.3)[d] 0.15	19	0.15
	$t_{1/2}$	23	23		
7. Over-increase *TR* (× 20)	*m*	4	20		
	TR	0.03	(3)[d] 0.15	1.4	0.15
	$t_{1/2}$	23	23		

[a]Changes in mRNA half-life ($t_{1/2}$) and/or transcription rate (*TR*) are assumed to be instantaneous for simplicity. The units for the kinetic variables are: *m* (mRNA concentration), molecules/cell; *TR*, molecules per minute; $t_{1/2}$, minutes.
[b]Time needed for completing 98.4% of the required change (calculated using Equation 3).
[c]Synthetic cost of maintaining the new steady state (equals the *TR* of the final steady state).
[d]Transient *TR* between steady states. The *TR* is increased twofold (strategy 6) or 20-fold (strategy 7) compared with the *TR* value of the final steady state.

yeast mRNAs (~90%) is >10 minutes [12], changes in mRNA concentration cannot occur within a reasonable time using these simple strategies (i.e. by increasing either *TR* or $t_{1/2}$); a mixed strategy (strategy 4 in Table 1) is also not suitable for inducing a rapid change. A possible alternative is the seemingly paradoxical strategy of increasing *TR* more than is required while reducing $t_{1/2}$ (strategy 5 in Table 1). The reduction in mRNA stability shortens the response but needs to be balanced by a compensating increase in *TR*. However, this is a costly strategy in terms of turnover rate of mRNA molecules at the new steady state (Table 1). A better strategy would be a transient excessive increase in *TR* without a considerable change in $t_{1/2}$ (strategies 6 and 7 in Table 1). This strategy achieves an effective reduction of the transition time and maintains a reasonable mRNA turnover at the new steady state. Nevertheless, it relies on the feasibility of a significant transient increase (e.g. 20-fold in strategy 7) in the transcription rate compared with the final steady-state value. It is conceivable that there is a limit to the transcription speed that can be achieved in the cell.

Strategies to decrease mRNA concentration

For genes that are downregulated, similar possible strategies are shown in Table 2 (strategies 8–14). Because the transition time depends on the final $t_{1/2}$ of the mRNA, a fast strategy is to reduce $t_{1/2}$ (strategy 10 in Table 2). In fact, this is the only acceptable solution for average or long-lived mRNAs, because strategies in which there is excess reduction of *TR* (strategies 12 and 13 in Table 2) are limited in that *TR* cannot be reduced further than 0. This shows that turning off transcription is not enough to achieve a rapid reduction in the concentration of an mRNA with a $t_{1/2}$>5 minutes. Therefore, because most yeast mRNAs seem to have a $t_{1/2}$>5 minutes in standard growth conditions [10,12,13], we conclude that those mRNAs that need to be downregulated quickly require regulatory mechanisms for mRNA stability. Indeed, extremely short responses can be achieved by a transient excess reduction in $t_{1/2}$ (strategy 14 in Table 2).

The importance of regulating mRNA stability has been stressed by several research groups [10,13–16]. In an extensive study of decay rates of human mRNAs, Yang *et al.* concluded that mRNAs encoding transcription factors have faster decay rates than other transcripts [17]. The short $t_{1/2}$ of these mRNAs contributes to faster production of the transcription factors in response to changing conditions and, consequently, to a better adaptation of the cells. Alternatively, it has been shown in yeast that expression of an RNase involved in mRNA degradation is needed for rapid induction of the genes involved in the iron starvation response [18].

Despite the interesting conclusions that might be drawn from our analysis, it is, evidently, a simplification. For most genes, the *TR* or mRNA $t_{1/2}$ does not change to a new value in a single step, and the change clearly cannot be instantaneous. In this respect, it is now clear that the initiation of transcription requires the gradual recruitment of RNA polymerase and other factors, and that the timing and order of this recruitment is gene specific [19]. It can be assumed, therefore, that changes in *TR* take time and occur progressively. In many cases, mRNA concentrations do not simply switch to a new steady state but oscillate during the time course of the response [10,11]. Sometimes, the intended response is only a transient departure from the permanent steady state. One such case occurs during stress responses. The concentration of mRNA transcribed from most stress-responsive genes increases up to a maximum within several minutes of

Table 2. Strategies for achieving a fivefold decrease in the mRNA concentration for a given gene[a]

Strategy	Kinetic variables	Initial steady state	Final steady state	Time required (min)[b]	Turnover cost (molecules/min)[c]
8. Decrease *TR* (low $t_{1/2}$)	*m*	5	1		
	TR	0.69	0.14	30	0.14
	$t_{1/2}$	5	5		
9. Decrease *TR* (average $t_{1/2}$)	*m*	5	1		
	TR	0.15	0.03	138	0.03
	$t_{1/2}$	23	23		
10. Decrease $t_{1/2}$	*m*	5	1		
	TR	0.15	0.15	28	0.15
	$t_{1/2}$	23	4.62		
11. Decrease $t_{1/2}$ and *TR*	*m*	5	1		
	TR	0.15	0.07	62	0.07
	$t_{1/2}$	23	10.3		
12. Over-decrease *TR* (× 0.5)	*m*	5	1		
	TR	0.15	(0.015)[d] 0.03	69	0.03
	$t_{1/2}$	23	23		
13. Over-decrease *TR* (maximum)	*m*	5	1		
	TR	0.15	(0)[d] 0.03	51	0.03
	$t_{1/2}$	23	23		
14. Over-decrease $t_{1/2}$ (× 0.1)	*m*	5	1		
	TR	0.15	0.15	1	0.15
	$t_{1/2}$	23	(0.46)[d] 4.62		

[a]Changes in mRNA half-life ($t_{1/2}$) and/or transcription rate (*TR*) are assumed to be instantaneous for simplicity. The units for the kinetic variables are: *m* (mRNA concentration), molecules/cell; *TR*, molecules per minute; $t_{1/2}$, minutes.
[b]Time needed for completing 98.4% of the required change (calculated using Equation 3).
[c]Synthetic cost of maintaining the new steady state (equals the *TR* of the final steady state).
[d]Transient *TR* (or $t_{1/2}$) between steady states. The transient *TR*s decrease to 0 (strategy 13) or to a fraction (half in strategy 12 and one-tenth in strategy 14) of the final steady state *TR*.

exposure to stress, then decreases, relaxing to the initial steady state. This peak in mRNA concentration is preceded by a similar (but more pronounced) peak in *TR* (Figure 1a). This characteristic time course can be reproduced theoretically by transiently increasing *TR* and keeping *k* constant (Figure 1b). Remarkably, the mRNA concentration peak is delayed ~10 minutes with respect to the *TR* peak (Figure 1b), as occurs in the experimental case (Figure 1a). Thus, the shift between the *TR* and mRNA concentration peaks arises naturally from the kinetic relationships, a factor that should be considered when comparing *TR* and mRNA concentration values that have been simultaneously sampled. Moreover, to emulate the rapid decrease in mRNA concentration (after reaching its maximum) that is observed experimentally (Figure 1a), it needs to be assumed that mRNA stability decreases after the peak concentration (Figure 1b). Again, the mRNA $t_{1/2}$ controls the persistence of the effect of a transient *TR* increase (Figure 1c).

mRNA kinetics at the genomic level

During the past few years, the development of several techniques has extended the measurement of gene expression parameters to the genomic level. The new findings might also have important consequences for the field of gene expression kinetics. There is some uncertainty associated with measuring *TR*, *m* (mRNA concentration) and *k* for a single gene, and this detracts from the precision of the conclusions that can be drawn from these data. However, when analysing thousands of genes simultaneously, the genes tend to cluster in defined profiles for each of these three parameters [10,20]. Therefore, statistically robust profiles can be obtained from genomic data, and gene expression strategies can be compared.

The best-suited organism for genome-wide expression analyses is the yeast *S. cerevisiae*. Since the development of serial analysis of gene expression (SAGE) [21] and DNA microarray technologies [11,22], it is possible to measure the amount of most of the mRNAs in a cell in any physiological state. However, the raw data supplied by these techniques are in arbitrary units. To obtain absolute values for mRNA amounts, both DNA microarray data and SAGE data should be normalized assuming a fixed value of 15 000 mRNA molecules per yeast cell [23]. Because SAGE data are precise for highly abundant mRNAs, they have been used to correct DNA microarray data, which are inaccurate when a high concentration of a particular mRNA(s) is present [24].

For most genes in yeast, the half-lifes of the corresponding mRNAs have been determined using the classical protocols of transcriptional blockade with RNA polymerase inhibitors or RNA polymerase II thermosensitive mutants [12,13]. These analyses have established that the mRNA half-lifes for yeast range from 3 to 300 minutes, with an average of 23 minutes [12]. However, the use of these procedures is questionable for precise determinations, because mRNA half-lifes are calculated from data obtained during a considerable time interval (up to 60 minutes). Therefore, the measured half-lifes are averages over a wide temporal window, smoothing out the rapid fluctuations in stability that are typical of fast cellular responses. In addition, these methods result in a global perturbation of the cell, because the temperature shift or drug addition needed to block transcription (and stopping transcription itself) creates abnormal conditions that can change the expression of some genes or alter the mRNA degradation mechanisms during the experiment. This problem has been discussed [25], and, after testing transcriptional blockade procedures in genome-wide studies, it was

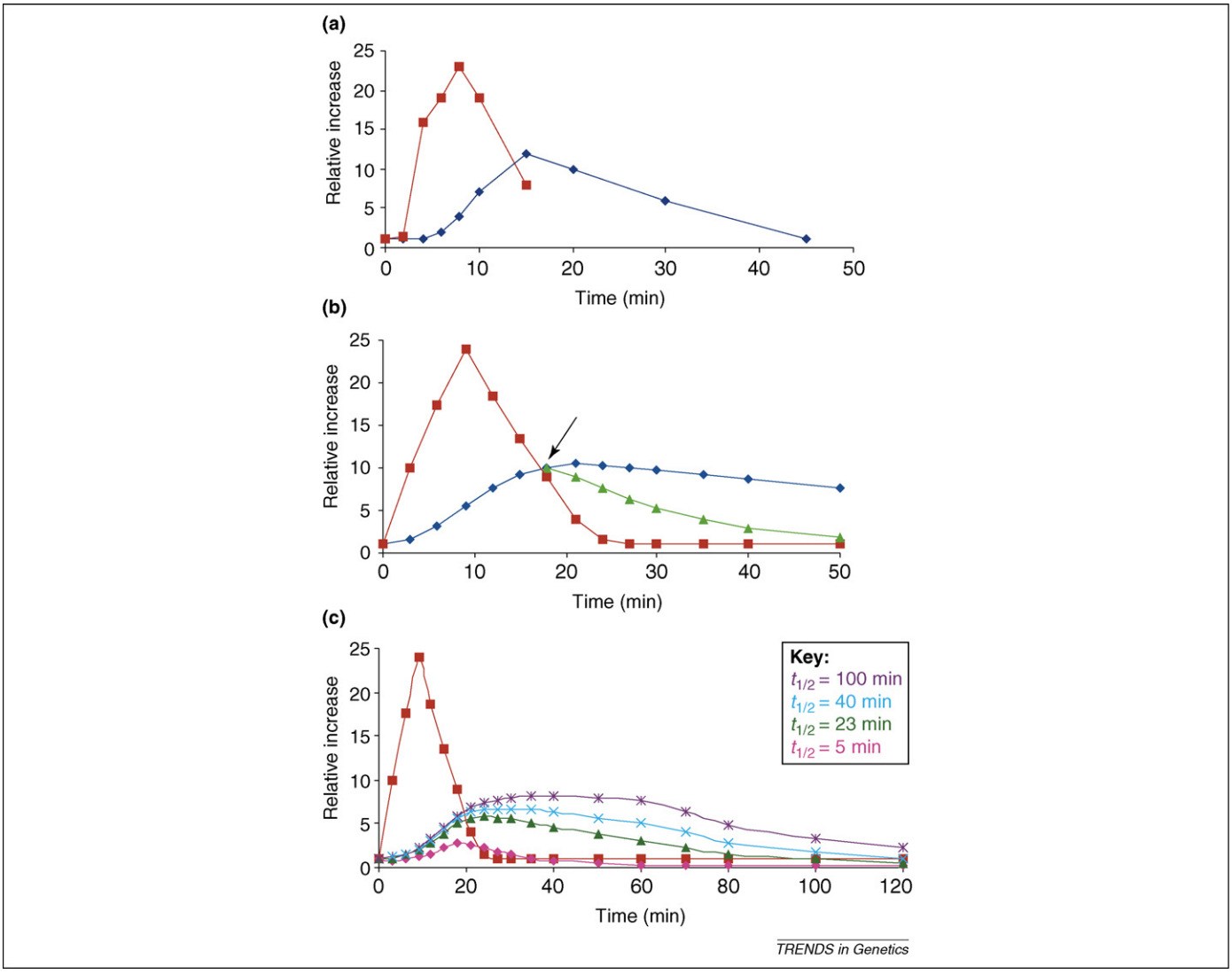

Figure 1. Theoretical kinetic strategies, including changes in mRNA degradation rate can reproduce experimental kinetic data. Transcription rate (*TR*) and mRNA concentration (*m*) are shown over time relative to their initial value, taken arbitrarily as 1. **(a)** Experimental data. The curves show changes in *TR* (red) and *m* (blue) for the yeast gene *STL1* after osmotic stress induced at time 0 by treatment with 0.4 M NaCl (P.M. Alepuz and L. Romero-Santacreu, unpublished). Experimental values were obtained using the GRO technique (Figure 2b). **(b)** Theoretical data. The change in *TR* (red), a transient 24-fold increase (from 0.02 to 0.48 molecules per minute) peaking after 10 minutes of stress, was designed to roughly simulate the experimentally observed time course in (a). The *m* curve (blue) was calculated, from this assumed *TR* data, using Equation VIII in Box 1 for an mRNA with a half-life ($t_{1/2}$) of 45 minutes. The time-zero value for *m* was determined according to Equation II in Box 1, assuming steady-state initial conditions. The green line shows the time course of *m* if the mRNA half-life decreases to 10 minutes after the peak (marked with an arrow). **(c)** Effect of $t_{1/2}$ on *m*. The *m* curves (different colours) were obtained as described in (b), using the same conditions except for $t_{1/2}$, which varied as indicated. It can be appreciated that $t_{1/2}$ modulates the height and breadth of the *m* curve.

concluded that such studies are not appropriate for monitoring stress-induced genes [12,13].

At present, the only technique for determining transcription rates is the transcription run-on assay, which has long been used for measuring the density of elongating RNA polymerases [26,27]. Assuming that RNA polymerases elongate at a constant rate, quantification of their density provides an estimate of *TR* at the moment of RNA labelling (Figure 2a). Because possible variations in elongation rate as a result of DNA sequence or chromatin structure have not been documented, there is an inherent uncertainty in assuming a constant rate. In any case, the hybridization of labelled nascent RNA to a single DNA macroarray containing multiple gene probes enables quantitative comparisons. This procedure has been used on various eukaryotic cell types [20,28–32] to analyse and compare the respective influences of *TR* and mRNA stabilities on the final mRNA steady-state concentration, thereby supporting the concept of 'post-transcriptional operons' [33]. However, this approach was limited because of the small number of genes analysed and because of the absence of rigorous normalization methods and reference data in the systems studied. Recently, run-on assays and DNA macroarrays have been adapted to the genomic level in yeast, taking advantage of the following: (i) the existence of data on absolute mRNA amounts corresponding to all genes in yeast under the most common culture conditions [24]; (ii) the feasibility of accurate normalization methods; and (iii) the ability to carry out run-on assays on whole cells. This method, called genomic run on (GRO) (Figure 2b), is conceptually similar to using run-on

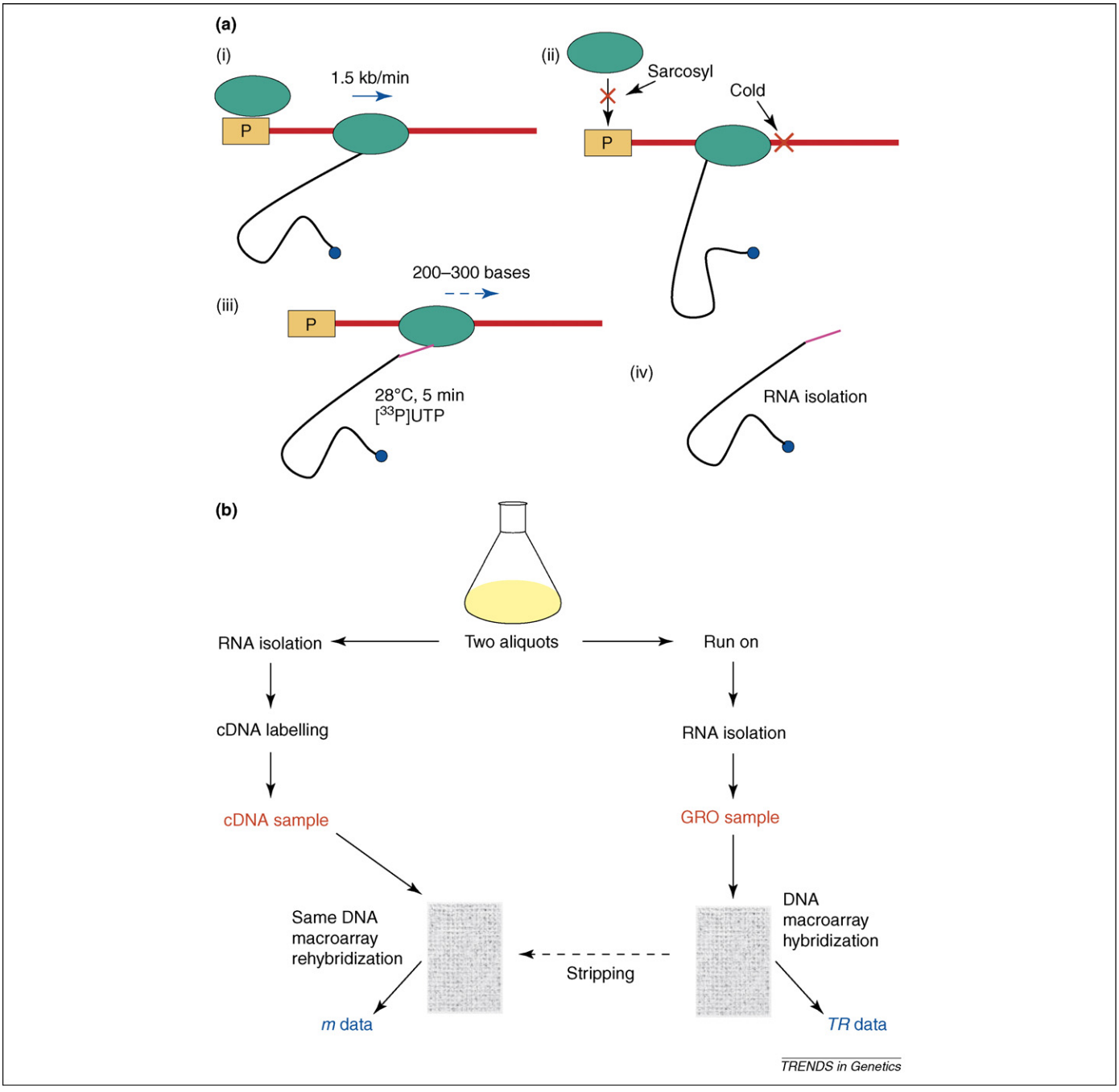

Figure 2. The fundamentals of run-on and GRO techniques. **(a)** Run-on experiments. Elongating RNA polymerases (green) (i) are stopped by the addition of a cold buffer (ii) containing sarcosyl. Re-initiation by new polymerases is also blocked. After changing to a new, warm medium that does not contain sarcosyl, stopped polymerases carry out a nonphysiological elongation of several hundred bases while incorporating radioactive uridine (iii). The radioactive label is proportional to the density of RNA polymerases on a given gene, which is, in turn, proportional to its transcription rate (*TR*). Total RNA isolated after labelling (iv) can be used for DNA macroarray hybridization as described in (b). **(b)** GRO experiments. Two identical cell aliquots from a yeast culture are used for conventional RNA isolation and for a run-on protocol. The hybridization of a DNA macroarray with *in vivo*-labelled nascent RNA provides data for determining the *TR*, and the subsequent hybridization with *in vitro*-labelled cDNA provides data for determining the mRNA concentration (*m*) corresponding to every gene (see Ref. [10] for further details).

assays together with DNA macroarrays [20,28–32], but, because the signals for every probe and every filter are normalized, it enables the absolute values of *TR* and *m* to be determined for every yeast gene [10].

Simultaneous determination of the kinetic variables *TR*, *m* and *k*

A full characterization of the transcriptional response would ideally involve monitoring the time course of *m*, *TR* and *k* after a starting event. Whereas, in principle, these parameters can be measured separately, the simultaneous determination of all three variables can be experimentally impractical under certain circumstances (e.g. during fast responses). This is mainly because measured values for *TR* and *m* cannot be satisfactorily matched with those for *k* because of the disparity of sampling times. Because *m* is always easily determined, it is tempting to measure *m* and either *TR* or *k*, then to deduce the missing parameter (*k* or *TR*) from the other two. This is straightforward (by using Equation II

in Box 1) if steady-state conditions can be experimentally proved (or justifiably expected) to hold, and this approach has been used several times for calculating TR [34,35] or k [10].

Working with prokaryotes, Cocaign-Bousquet's group developed a concept, derived from metabolic control analysis, to evaluate the relative contribution of synthesis and degradation to mRNA concentration variation from simultaneous m and k determinations [36,37]. However, the validity of this procedure is again restricted to steady-state conditions [38]. Therefore, this approach does not apply to many experimental situations involving a fast gene expression response after a signalling event or exposure to stress. As discussed earlier for stress genes (Figure 1), the environmental shift can trigger an abrupt transient oscillation of TR (and/or k) that brings the system far from steady-state conditions. Furthermore, even in stable environmental situations, organisms such as yeast can display a cyclical pattern of gene expression [39], never reaching a true steady state.

We propose an alternative approach that overcomes these difficulties. Taking advantage of the fact that GRO enables fast, repeated sampling, m and TR values under non-steady-state conditions can be determined at short time intervals (Figure 2b). For example, in a typical experimental situation, transcription rate values TR_1, TR_2, TR_3 and so on can be determined at times t_1, t_2, t_3 and so on, sketching the temporal variation. Although the exact time course of TR is unknown, a smooth linear change between experimental points can be assumed. Although this is not necessarily the case, the actual deviation can be negligible if sampling points are close enough (every few minutes). Under these circumstances, the following relationship between parameters at two successive points (e.g. t_1 and t_2) can be derived (Box 1):

$$p - k(TR_2 - km_2) = [p - k(TR_1 - km_1)] \times \exp(-k\Delta t) \quad \text{[Eqn 5]}$$

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

$$p = \frac{TR_2 - TR_1}{\Delta t} \quad \text{[Eqn 6]}$$

Solving Equations 5 and 6 numerically, the value of k can be deduced for each time interval. Thus, a stepwise change in the mRNA half-life for every yeast gene can be deduced from the m and TR values determined by the GRO technique along a time course. This procedure allows a full characterization of the transcriptional response at the genomic level.

Conclusions and perspectives

The recent development of genomic techniques for measuring mRNA amounts, transcription rates and mRNA stabilities will change our understanding of gene regulation in eukaryotes. These techniques enable us to monitor the 'whole' transcriptional response of an organism to any physiological event, thereby offering, for the first time, the possibility of comparing the transcriptional strategies used by different genes. Although genomic methods are, in general, less accurate than conventional techniques, the ability to compare a large number of genes simultaneously will strengthen the reliability of the conclusions. Nevertheless, current genomic techniques still need to be improved, especially by developing unbiased normalization procedures.

When analysing genomic data, it becomes clear that cells have evolved different strategies to cope with the kinetic features of gene expression in eukaryotes. The regulation of mRNA stability is the key mechanism for rapid adaptation of cellular processes to a changing environment. However, in general, a fast response has a high cost, so this solution is most probably limited to necessary situations: for example, the transcription of histone mRNAs, which undergo abrupt concentration changes during the cell cycle [10,12,40]. In this regard, the situation of free-living cells (such as yeast), which must react quickly to most situations, clearly differs from that of tissue cells, which can tolerate a delayed response.

Transcription and mRNA processing, however, are only the first steps in gene expression: mRNA translation and protein turnover are also kinetically constrained in eukaryotes. Recently, the first global data for protein amounts [24,41], mRNA translation rates [42] and protein half-lifes [43] in yeast have been published. The development of techniques for simultaneously determining these parameters is needed for a reliable translation kinetics study. Only then, will it be possible to attempt an integration of both transcriptional processes and translational processes into a single kinetic description [9].

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