Think again

What do you mean by transcription rate?

The conceptual difference between nascent transcription rate and mRNA synthesis rate is essential for the proper understanding of transcriptomic analyses

JoséE. Pérez-Ortín[1)], Daniel A. Medina[1)], Sebastián Chávez[2)] and Joaquín Moreno[1)]*

mRNA synthesis in all organisms is performed by RNA polymerases, which work as nanomachines on DNA templates. The rate at which their product is made is an important parameter in gene expression. Transcription rate encompasses two related, yet different, concepts: the nascent transcription rate, which measures the in situ mRNA production by RNA polymerase, and the rate of synthesis of mature mRNA, which measures the contribution of transcription to the mRNA concentration. Both parameters are useful for molecular biologists, but they are not interchangeable and they are expressed in different units. It is important to distinguish when and where each one should be used. We propose that for functional genomics the use of nascent transcription rates should be restricted to the evaluation of the transcriptional process itself, whereas mature mRNA synthesis rates should be employed to address the transcriptional input to mRNA concentration balance leading to variation of gene expression.

Keywords:
eukaryotes; mRNA synthesis; nascent transcription; transcription rate; yeast

Introduction: What is transcription rate?

The rate at which the genes are transcribed is the first and probably one of the most important regulated steps along the flux of genetic information. In eukaryotes, synthesized mRNA undergoes processing and transport to become a mature form that can be translated by the ribosomes. Besides, a mature mRNA molecule is translated only a number of times as regulated by mRNA degradation processes.

The appearance of genomic techniques to measure the concentration, as well as the transcription and degradation rates, of the mRNAs of all genes in an organism has vastly improved our knowledge of gene expression by revealing the relative weight of synthesis and decay on mRNA changes during global transcriptional responses [1, 2]. RNA polymerase (RNA pol) density can easily be determined genome-wide [2, 3] (Fig. 1) and converted into a transcription rate by assuming uniform RNA pol speed during transcription. In this way, the number of mRNA molecules being synthesized per unit time and gene copy can be calculated. We will call this measure a "nascent" transcription rate (nTR) to distinguish it from other uses of the term (Box 1). Accordingly, nTR is an estimation of transcriptional activity at the gene level.

However, a different aspect of understanding the role of transcription in gene expression is achieved by evaluating the change in the mature mRNA concentration ([mRNA]). The [mRNA] is determined by both the synthesis (SR) and the degradation (DR) rates. mRNA synthesis is considered to be independent of its concentration, whereas decay is assumed to follow first-order kinetics with rate constant k_d [1, 4]. Thus, the rate of [mRNA] change can be written in its simplest form as:

$$\frac{d[\mathrm{mRNA}]}{dt} = \mathrm{SR} - k_d \times [\mathrm{mRNA}] \qquad (1)$$

Although SR and k_d may vary with time, [mRNA] is bound to reach a steady state whenever both SR and k_d remain unchanged for a time interval which is longer than $(1/k_d)$ [1]. This condition may never be achieved by genes of cyclic expression, but it is usually fulfilled for most genes under fixed

DOI 10.1002/bies.201300057

[1)] Departamento de Bioquímica y Biología Molecular, Universitat de València, Burjassot, Spain
[2)] Departamento de Genética, Universidad de Sevilla, Sevilla, Spain

***Corresponding author:** José E. Pérez-Ortín
E-mail: jose.e.perez@uv.es

Abbreviations:
DR, degradation (decay) rate; **dsDNA,** double stranded DNA; **HL,** mRNA half-life; k_d, degradation constant; **[mRNA],** cytoplasmatic mRNA concentration; **nTR,** nascent transcription rate; **RNA pol,** RNA polymerase; **SR,** mRNA synthesis rate.

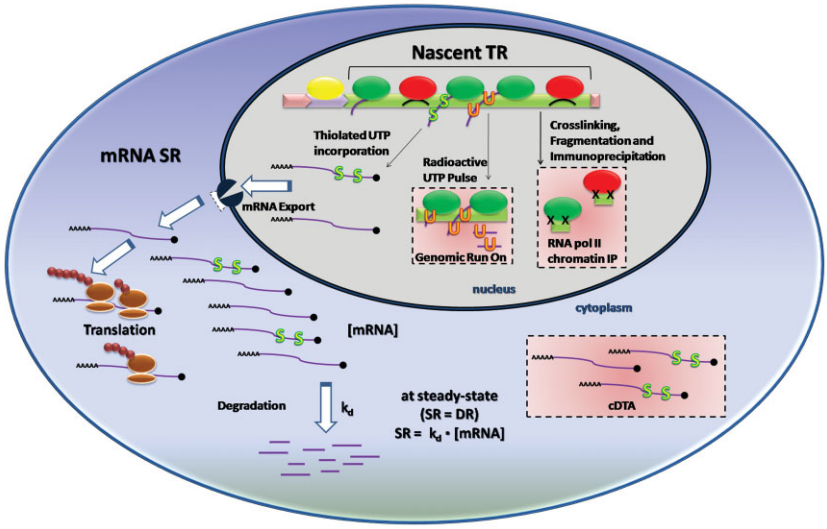

Figure 1. Scheme of the gene expression process in eukaryotes showing the different techniques that measure the nTR and mature mRNA SR. The nascent TR is determined from the RNA pol (colored ovals) density on chromatin templates. Some of the RNA pol molecules are elongating (green), others are initiating (yellow) and some are backtracked (red). They can all be detected by chromatin immunoprecipitation, but only those that are actually elongating are measured by Genomic run-on. If cells are incubated for a pulse with thiolated UTP precursors, the newborn mature mRNA can be purified and quantified together with total non-labeled mRNA. The current techniques cannot distinguish between the mature mRNA transiently in nucleus (supposed to be a minor part) and the cytoplasmic one. By assuming steady-state conditions, this protocol, called comparative Dynamic Transcriptome Analysis (cDTA [6]), allows the determination of the SR. See [27] for further details on the techniques depicted herein.

environmental conditions leading to a steady state in which the synthesis and degradation rates are equal:

$$SR = DR = k_d \times [mRNA] \quad (2)$$

In that instance, [mRNA] can be expressed as a ratio between the synthesis rate and k_d, which is inversely related to the mRNA half-life (HL):

$$HL = \frac{\ln 2}{k_d} \quad (3)$$

The appearance of mature mRNA can also be determined genome-wide in a direct or indirect manner (Fig. 1) [5, 6]. Moreover, because this appearance is related to [mRNA] and its DR through Equation (1) (or particular embodiments of it), functional genomics techniques are currently used to infer one of the parameters from the experimental determination of the other two. For example, the steady-state condition described above (Equation 2) can be used to determine the SR if the concentration of the mature mRNA and the HL (k_d) are known [2]. SR is also a measure of transcriptional activity and is frequently referred to as "transcription rate" [1, 3, 7–10]. We use here "synthesis rate" instead (as used in references [5, 6]) to avoid confusion. Indeed, SR represents another aspect of the mRNA biosynthetic process different from nTR (Box 1). As mentioned above, SR relates to the rate of appearance of mature (in contrast to nascent) mRNA. Furthermore, because [mRNA] balance in the cell results from mass action law, SR in Equations (1) and (2) should express the change in concentration, and not the change in absolute amount (as does nTR). Therefore, SR is a measure of the transcriptional input to mature mRNA concentration at the cellular level.

In this paper, we compare nTR and SR (both of which are frequently alluded in the bibliography as "transcription rate" in an undistinguishable way) and discuss their proper use when studying gene expression in eukaryotes. This leads to a re-appraisal of some published results, and also to a better understanding of mRNA metabolism and expression strategies.

Nascent transcription rate can be measured from in vivo RNA pol density

RNA polymerases are highly processive enzymes, which work on dsDNA. Processive nanomachines have been described as "cars on a single-lane highway" [11, 12]. Thus, RNA pol travels at a variable speed and gets into traffic jams, crashes with other RNA pol, stops and backtracks. In kinetic terms, the two most important features of transcribing RNA pol are its average speed and the density of the molecules on the track. The movement of RNA pol is characterized by a given speed or elongation rate (in kb/min) that might be variable [13, 14]. The product of elongation speed and RNA pol density is a measure of "transcriptional productivity" (in nascent RNA molecules/min). We propose the acronym nTR to design the number of full nascent mRNA molecules per unit time produced by the RNA pol molecules acting on a single gene copy. nTR is determined by the number of transcription initiation events at the promoter, by RNA pol speed changes, and by elongation failure (premature drop-off) [15], all three being regulated processes [16].

At a given average speed, the transcriptional activity on a gene is directly proportional to the total number of elongating RNA pol molecules on the track. Nevertheless, as nTR computes the production of full-length mRNAs and genes have different lengths, the relevant parameter for estimating nTR is not the number of RNA pol per gene, but its density (in molecules/kb) [17]. Genome-wide nTR can be quantified by measuring the density of the polymerases acting on each gene by either genomic run-on [3] or chromatin immunoprecipitation of RNA pol II [7, 18] (Fig. 1). A caveat of these techniques is that not all detected RNA pol molecules would finish transcription because of a stochastic elongation failure that may cause polymerase drop-off [6, 15]. Thus, because longer genes have higher number of drop-off events, the

Box 1

"Concept and units of nTR and SR"

The nascent transcription rate (nTR) measures the work done by the biological machine RNA polymerase (RNA pol) and can be evaluated in terms of the product (RNA) made per unit time. If nucleotides and RNA pol molecules are not limiting, the number of nascent RNA molecules for a given gene should be proportional to its copy number. To keep the original significance of nTR as a standard measure of transcriptional activity, we propose dividing the rate of mRNA synthesis by the average number of gene copies to obtain a normalized "per gene copy" nTR. Thus, the nTR units might be:

mRNA molecules/min/gene copy.

The synthesis rate (SR) represents the transcriptional input to mature mRNA concentration. It reflects the maximum hypothetical rate of change in the mature mRNA concentration due exclusively to its synthesis, i.e. the concentration change that would occur if there were no decay. Therefore, to calculate SR, the number of mature mRNA molecules released in the cytoplasm per unit time has to be divided by the cytoplasmic volume. Thus, the units of SR might be:

mRNA molecules/L/min

From the above definitions, it can be concluded that nTR and SR are different in two significant ways. First, nTR refers to nascent mRNAs (i.e. full copies of the gene message as released by the RNA pol) while SR counts only mature mRNAs (i.e. the fraction of nascent mRNAs that undergo correct processing and transport as to become translatable). Second, nTR is a rate of production of *amount* of mRNA while SR is a rate of change of mRNA *concentration*.

To illustrate the different biological meaning of nTR and SR one may consider a cell growing in size. Even if nTR is maintained constant for a particular gene, SR would monotonically decrease because of the fixed synthetic input being divided by an ever increasing volume. In this case, a constant nTR will reflect a steady work done by the RNA polymerase, while the reduction in SR will reveal the decreasing impact on mRNA concentration (hence, on mRNA translation) of a fixed transcriptional input on a growing volume. This example highlights the functional difference between these two parameters addressing distinct aspects of transcription.

calculated nTR is overestimated in a gene-length-dependent way. This bias has to be corrected from an estimate of the drop-off rate. Besides, in contrast to single-cell techniques that can measure mRNA production in absolute units (i.e. mRNA molecules/min) [19–22], common genome-wide techniques (averaging millions of cells) deliver only relative values in arbitrary units. However, because these values are internally proportional, different genes can be compared [2, 3] and arbitrary units can be converted into absolute ones through an external reference. For instance, the SR of the yeast *HIS3* gene previously determined by other authors in the same experimental conditions [23] was used to convert all yeast genes nTRs into absolute values [3].

Once correctly expressed as the number of mRNA molecules synthesized per unit time and gene copy, nTR is a valuable indicator of transcriptional activity, and it is directly impacted by all transcriptional regulatory mechanisms acting on the polymerase or the template. As nTR measures productivity per gene copy, cell volume does not enter its calculation. But the experimentally determined number of nascent mRNA molecules is to be divided by the number of gene copies to get the actual nTR (Box 1). This corrects for the case of multiple copies of the same gene or when comparing cells with different levels of ploidy (see later).

mRNA synthesis rate is the relevant measure in kinetic equilibria

The scenario is different when studying changes in the level of translatable mRNA. In this case, the transcriptional input should rather be measured as the rate of appearance of mature mRNA in the cytoplasm. Eukaryotic mature mRNA (usually determined as polyA-RNA) results from nascent mRNA after a number of steps, which it might fail to pass. For instance, problems during splicing and further processing may preclude maturation of nascent mRNA. As a result, only a fraction of nascent mRNA reaches the cytoplasm. At this point, the impact on further gene expression depends on cytoplasmic mRNA concentration change. Although the [mRNA] balance assumed in Equations (1) and (2) is undoubtedly a gross oversimplification of a complex process that takes place in a spatially compartmentalized environment, it is still a valid assumption that the DR depends on [mRNA] rather than on mRNA amount. Therefore, to fit dimensionally in Equation (1), SR should be expressed as the mature mRNA molecules released per unit time and unit volume. This magnitude has been called "transcription rate" in many publications [1, 9, 10] but we propose to call this magnitude mRNA synthesis rate (SR) to distinguish from nTR (Box 1). Because mRNA translation events are also kinetically controlled, protein production is expected to depend on [mRNA], too. Therefore, by expressing SR in terms of concentration and time, it also reflects how efficiently transcriptional changes (as measured by nTR) are transmitted into the downstream steps of gene expression. As such, SR is a biologically relevant parameter in its own right, and is related (but is not equivalent) to nTR.

Values for SR can be obtained indirectly by applying Equation (2). In fact, the first genome-wide calculation

of SR was carried out in yeast using experimentally determined [mRNAs] and HLs, and assuming steady-state conditions [24]. These indirect SR values obtained at the steady state can be plotted against nTR values (in arbitrary units), which have been experimentally determined under the same environmental conditions, to find a conversion factor [2, 3, 25]. This is a more robust alternative to get real units for nTR than using a single gene value (see above). This factor might be later applied to transform nTR data under non-steady state conditions into SR. This procedure assumes that the efficiency of nuclear mRNA degradation and export remains constant for every gene. That is obviously a simplification, but it is a valid conversion from nTR into SR because the use of [mRNA] in calculating standard SRs (those used for normalization) introduces the correct volume factor.

Recently, genome-wide techniques for directly measuring the rate of apparition of mature mRNA have been implemented in yeast [5, 6] and mammalian cells [9, 10] (Fig. 1). They are based on the capture of newborn mature mRNA, which is labeled during a short pulse with thiolated uracil or uridine analogs. The subsequent isolation and quantification of labeled mRNA can be used to evaluate SR. Because no subcellular fractionation is done, the mature mRNA measured includes both nuclear and cytoplasmic fractions, although the latter is considered to be much more abundant (Fig. 1). Quantification of non-labeled or total mRNA facilitates the determination of [mRNA] in the same experiment and the subsequent estimation of HLs by assuming a steady-state situation [26–28]. By using these protocols, SR can be obtained directly in real absolute units after dividing by the cell volume. Furthermore, it is a common practice to divide directly the amount of synthesized mRNA by the number of cells, leading to SR data expressed as a rate of [mRNA] change per cell. This procedure is acceptable provided that the cell volume does not vary between the samples being compared. However, if it is not possible to verify that cell volume remains constant, it might be preferable to normalize the data to total cell mass or total protein amount (see Box 3) because these parameters are expected to maintain a better proportionality with cell (or cytoplasmic) volume than the number of cells.

From all the above, it seems clear that nTR and SR are conceptually, dimensionally, and numerically different magnitudes which should be used in different contexts. They might be proportional if cell volume, DNA content and mRNA maturation efficiency remain constant (a common, but not universal, situation that is usually assumed), but will diverge otherwise. Despite all this, and because most available techniques determine primarily nTR, it is a regular practice to use the latter in calculations in which, according to Equation (2), SR should be used instead. The incorrect use of nTR values disregarding the volume factor operating on SR may lead to misleading conclusions drawn from valuable experimental data (Boxes 2 and 3).

Box 2

"mRNA concentration, not quantity, is pertinent for transcriptomics"

The relevant factor in chemical reaction kinetics is the concentration, not the amount, of molecules present. Thus, comparisons made between different samples in transcriptomics studies should be raised at the [mRNA] level.

For instance, a new protocol for performing global expression analyses from samples that may differ as far as the amount of RNA/cell is concerned was recently proposed [41]. The authors concluded that the common assumption that samples of the same number of cells under different treatments have equivalent total amounts of mRNAs is not always true. They used data from two other reports [42, 43] in which an elevated induced expression of human transcription factor c-Myc brought about a general increase in the levels of thousands of mRNAs, which in turn, increased the total amount of mRNA per cell. The authors warn about the usual normalization practice of processing the same amount of mRNA from each sample, which has been used in most experiments for many years. However, we wish to stress that the comparison made between samples should not be based on mRNA/cell content, but on [mRNA]. This does not imply a major difference in most cases in which cells of different samples are similar in size. However in these reports [42, 43], the cell types used in the experiments were of different sizes. This means that the actual mRNA concentrations in the cells overexpressing c-Myc come closer to those in the control cells than suspected since the control cells were smaller. The actual sizes of cells were not directly provided in the papers, but from the plots in Fig. S6A of [42], it is stated that cell volume increases about 1.3 times between 1.5 and 6 h after c-Myc induction. This means that the observed rise in mRNA per cell (1.5 times between 0 and 8 h) is probably compensated. Therefore, what the cited papers actually reveal is that c-Myc overexpression causes a general increase in nTR but, because of the parallel increase in cell volume, it does not produce a parallel rise in [mRNA] or in SR.

What is the biological relationship between nTR and SR?

nTR and SR are related biologically relevant parameters, but involve distinct constraints. nTR measures the productivity of the transcriptional machinery on a particular gene. This machinery is ruled by physicochemical laws. Its speed and performance depend on temperature, DNA sequence, nucleosome positioning, and so on. With a gene transcribed at a given speed, any increase in nTR requires a proportional increase in RNA pol density. This involves a higher frequency of initiation events by free polymerases, which are probably in excess [2]. RNA pol density is also limited by physical constrains, such as the size of the transcriptional apparatus. The "Christmas trees"

Think again

Box 3

"SR and the transcriptional strategy to cope with cell size"

SR redefinition offers new views to some published data. The first case is the comparative analysis of HLs, SRs, and [mRNA] for *Saccharomyces cerevisiae* (Sc) and *Schizosaccharomyces pombe* (Sp) [6]. The authors discovered that the [mRNA] in both yeasts are quite similar because the mRNA molecules in Sp are 3.1 times more abundant than in Sc, while its volume is also 2.7 times larger. The median HLs in Sp are 5 times longer (0.2 factor difference in k_d; see Equation 3), rendering a ratio of 0.23 between the DRs in Sp and Sc. At the steady state, this ratio should match the equivalent ratio for SRs (see Equation 2). In spite of using a method (cDTA in Fig. 1) that experimentally measures production of mature mRNAs, values for SR are given in nTR units (44 mRNAs/cell-cycle in Sp vs. 53 in Sc), which suggests a much higher ratio (0.83), thus casting doubts on the steady-state assumption. However, the 3.6-fold difference (0.83 vs. 0.23) results from using the incorrect units. Since Sp is almost three times larger, the published data fit quite well after having corrected the calculated nTR by the volume (i.e. converted back to SR). Thus, the data indeed support a steady state around a similar [mRNA].

Another example is the analysis of Sp mutants of variable size [7, 8, 44, 45]. A main conclusion drawn was that "global transcription rates scale with size" [45]. The transcription rates were calculated after RNA pol ChIP and pulse labeling of nascent mRNA [7, 44]. Thus, they determined nTR. However, the SR (the relevant parameter for mRNA homeostasis) remained constant because of the parallel increase of nTR and cell size. The authors noted that "the transcription rate per protein" remained constant. Since the protein amount increases linearly with cell size, that parameter is the equivalent of SR. Because the authors also reported similar mRNA decay rates and [mRNA] values, it can be concluded that the k_d were maintained across mutants with different size. This supports the idea that the same steady-state conditions apply in the wild type and the mutants with no change in SR. Interestingly, it was also found that nTR increases with growth only until cells reached twice the wild-type volume. Thereafter, nTR did not increase, probably because of physicochemical constraints. Thus, SR decreased steadily above this volume threshold and cells could no longer support the required increases in protein synthesis [7].

Both examples suggest that cells establish a similar steady-state value for mature [mRNA] by adapting nTR and/or k_d to cell size up to the performance limits of the transcriptional machinery.

formed by RNA pol I when transcribing rDNA (7.3 RNA pol/kb on average and up to 24 molecules/kb) [29] probably represents the absolute maximum [30, 31]. The highest density measured for RNA pol II in yeast is, at least, threefold lower [2]. In higher eukaryotes, the average RNA pol II density is probably lower than in yeast [27], although high RNA pol II densities, up to 10 RNA pol II/kb, have been observed in HIV or rat-kidney [22, 32]. The scarcity of RNA pol II elongating molecules and their physically limited elongation speed may have forced cells to increase the number of gene copies when a higher nTR is required, as in the common case for rDNA (150 repeats of the 35S gene in *Saccharomyces cerevisiae*, and 350 copies per haploid genome in human cells) [33]. There are other examples of highly transcribed genes that obtain high global TR by adding new copies to a single genome. For instance, in the haploid genome of *S. cerevisiae* most ribosomal protein genes are duplicated, and the genes encoding methalotionein *CUP1* get amplified in response to the presence of heavy metals in the medium [34]. Conversely, pathological gene duplication may be detrimental due to imbalanced expression, as in cancer and some neurodevelopmental disorders [31]. In those cases in which a small number of genes shift to an altered copy number, the observed RNA pol II density per gene copy (and the nTR) is expected to remain unchanged, as far as each copy behaves independently from the others and RNA pol II is in excess, as is usually assumed (discussed in [2]). However, SR will increase proportionally to copy number amplification. This example further illustrates the physiological difference between nTR and SR.

In the special case of whole genome duplications (e.g. from diploid to tetraploid), a parallel increase in all the components of the transcriptional machinery (and subsequently in the components of other metabolic and gene expression pathways) also occurs. This may lead to an increased nuclear volume, which in turn, brings about increased cell volume, resulting in near constant concentrations for almost all molecules [8]. In fact, the ratio between nuclear and cell volumes remains constant with ploidy (reviewed in [35]). Thus, in the case of genome duplication, nTR remains constant because the effect of twice as many transcribing RNA pol is corrected by the presence of two gene copies (according to our definition; see Box 1). Similarly, SR does not vary because the doubled input of mature mRNA is compensated in concentration terms by the doubling of the cell volume.

Conversely, for those cells with the same genome, but different cell volumes, SR ought to be adapted. As observed in yeast, slight changes in volume can be buffered by proportional changes in nTR in order to keep SR and [mRNA] constant (see Box 3). However for human cells, which can differ up to 1,000 times in volume, it is difficult to imagine how nTR can adapt to such huge changes. Since the relevant volume for the equilibrium in Equation (2) is that of the cytoplasm, the "effective" cytoplasmic volume can be reduced by increasing the volume of some organelles (such as the vacuole in yeast). Alternatively, mRNAs can be actively recruited to specialized sites (where they rise to higher concentration) as in the axons of neurons or the mRNA gradients in the *Drosophila* syncytium blastoderm [36]. Another possibility to

buffer the effect of cell volume increase is to lower the DR by decreasing k_d. In fact, mRNA stability is higher in cells with a larger volume, such as *Schizosaccharomyces pombe* relative to *S. cerevisiae* [6]. However, this strategy has a cost in terms of the reduced speed at which the cell can adapt to new environments by changing gene expression [1]. Although cells of multicellular organism may tolerate substantially longer adaptation times, there must be a limiting size beyond which this strategy cannot be further exploited and the cell has to settle for a reduced [mRNA] level. Accordingly, [mRNA] appears to be at least 2.5 times lower in human cells than in yeast cells, even if the HLs of mammalian mRNAs are on average 15 to 20 times longer [28].

It should be finally remarked that an independent determination of nTR and SR for a single organism should be a suitable approach for detecting differential patterns in mRNA processing efficiency (i.e. in RNA splicing or in mRNA transport) between genes. To date, this particular study has not yet been carried out on a genomic scale.

Gene expression is a circular process: Cross-talk between nTR and mRNA stability

[mRNA] homeostasis requires cross-talk between the synthesis and degradation rates [28, 37]. According to the discussion above, the parameter that should balance the DR in order to reach a given steady [mRNA] is SR, which can be changed by affecting nTR (i.e. the recruitment and activity of the RNA pol on the chromatin template) or by altering the cell volume. However, as volume changes affect the steady [mRNA], and subsequently the DR, in a counteracting manner, this is not a feasible alternative. The control over the DR cannot operate on [mRNA] for the same reason, but should act on k_d. Therefore for the coordinated regulation of mRNA synthesis and degradation, the critical parameters are those acting at the molecular level: nTR and the mRNA HL. However, mRNA degradation does not seem to play an important role in the size-dependent control of [mRNA] in yeast (see [7], and discussion in [8]). Thus, it seems that nTR is the key regulatory point. nTR can be controlled at either the initiation or the elongation level. It has been shown that some proteins, such as Rpb4/7 or Ccr4-Not, acting during transcription initiation or elongation are charged onto nascent mRNAs and determine their cytoplasmic fate [27, 37–39]. Contrariwise, some cytoplasmic decay factors affect transcription initiation and elongation after being imported to the nucleus [28, 40]. For instance, impairing mRNA degradation by deleting deadenylase subunits of the Ccr4-Not complex causes decreased decay rates as expected, but also decreased synthesis rates [6]. Thus, gene expression in eukaryotes seems to have evolved to keep total [mRNA] within certain limits by a permanent cross-talk between mRNA synthesis and degradation acting on nTR as the main target.

Conclusions

The wealth of transcription data provided by the current genomic techniques is contributing to illuminate the underlying complexity of the phenomenon. In order to understand the complex and variable transcriptional machinery and to make sense of its regulatory value, it is first necessary to define the pertinent parameters and to clarify the nomenclature. In particular, it must be realized that the common term "transcription rate" has been used by different authors to refer to different parameters, such as rate of nascent RNAs [3, 7, 19, 20], the production of mature mRNAs [9, 10] or even the RNA pol speed [11, 21, 22] in different experimental contexts. Our proposal here is a reasoned plea to clarify nomenclature and also to distinguish between nascent transcription rates and mature synthesis rates, two physiologically relevant but different aspects of RNA synthesis, thereby avoiding confusion in the interpretation and discussion of the data.

Acknowledgments

The authors are grateful to the members of the laboratories in Valencia and Seville for discussion and support and to V. Pelechano for critically reading the manuscript. J.E.P-O. is supported by grants from the Spanish MCINN and the European Union (FEDER) (BFU2010-21975-C03-01), and from the Generalitat Valenciana (PROMETEO 2011/088 and ACOMP/2012/001). J.M. is supported by a grant from the Spanish MCINN (BFU2009-11965). S.C. is supported by grants from the Spanish MCINN (BFU2010-21975-C03-02) the Regional Andalusian Government (P07-CVI-02623 and P08-CVI-03508), and the European Union (FEDER). D.A.M. was a recipient of a Santiago Grisolía fellowship from the Generalitat Valenciana.

References

1. **Perez-Ortin JE**, **Alepuz PM**, **Moreno J.** 2007. Genomics and gene transcription kinetics in yeast. *Trends Genet* **23**: 250–7.
2. **Pelechano V**, **Chavez S**, **Perez-Ortin JE.** 2010. A complete set of nascent transcription rates for yeast genes. *PloS One* **5**: e15442.
3. **Garcia-Martinez J**, **Aranda A**, **Perez-Ortin JE.** 2004. Genomic run-on evaluates transcription rates for all yeast genes and identifies gene regulatory mechanisms. *Mol Cell* **15**: 303–13.
4. **Steiger MA**, **Parker R.** 2002. Analyzing mRNA decay in *Saccharomyces cerevisiae*. *Methods Enzymol* **351**: 648–60.
5. **Miller C**, **Schwalb B**, **Maier K**, **Schulz D**, et al. 2011. Dynamic transcriptome analysis measures rates of mRNA synthesis and decay in yeast. *Mol Syst Biol* **7**: 458.
6. **Sun M**, **Schwalb B**, **Schulz D**, **Pirkl N**, et al. 2012. Comparative dynamic transcriptome analysis (cDTA) reveals mutual feedback between mRNA synthesis and degradation. *Genome Res* **22**: 1350–9.
7. **Zhurinsky J**, **Leonhard K**, **Watt S**, **Marguerat S**, et al. 2010. A coordinated global control over cellular transcription. *Curr Biol* **20**: 2010–5.
8. **Marguerat S**, **Bahler J.** 2012. Coordinating genome expression with cell size. *Trends Genet* **28**: 560–5.
9. **Rabani M**, **Levin JZ**, **Fan L**, **Adiconis X**, et al. 2011. Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nat Biotechnol* **29**: 436–42.
10. **Schwanhausser B**, **Busse D**, **Li N**, **Dittmar G**, et al. 2011. Global quantification of mammalian gene expression control. *Nature* **473**: 337–42.
11. **Dennis PP**, **Ehrenberg M**, **Fange D**, **Bremer H.** 2009. Varying rate of RNA chain elongation during rrn transcription in *Escherichia coli*. *J Bacteriol* **191**: 3740–6.
12. **Tuller T**, **Carmi A**, **Vestsigian K**, **Navon S**, et al. 2010. An evolutionarily conserved mechanism for controlling the efficiency of protein translation. *Cell* **141**: 344–54.
13. **Danko CG**, **Hah N**, **Luo X**, **Martins AL**, et al. 2013. Signaling pathways differentially affect RNA polymerase II initiation, pausing, and elongation rate in cells. *Mol Cell* **50**: 212–22.

14. **Marcello A.** 2012. RNA polymerase II transcription on the fast lane. *Transcription* **3**: 29–34.
15. **Mason PB**, **Struhl K.** 2005. Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. *Mol Cell* **17**: 831–40.
16. **Cheung AC**, **Cramer P.** 2012. A movie of RNA polymerase II transcription. *Cell* **149**: 1431–7.
17. **Hirayoshi K**, **Lis JT.** 1999. Nuclear run-on assays: assessing transcription by measuring density of engaged RNA polymerases. *Methods Enzymol* **304**: 351–62.
18. **Mayer A**, **Lidschreiber M**, **Siebert M**, **Leike K**, et al**.** 2010. Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol* **17**: 1272–8.
19. **Zenklusen D**, **Larson DR**, **Singer RH.** 2008. Single-RNA counting reveals alternative modes of gene expression in yeast. *Nat Struct Mol Biol* **15**: 1263–71.
20. **Larson DR**, **Zenklusen D**, **Wu B**, **Chao JA**, et al**.** 2011. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science* **332**: 475–8.
21. **Maiuri P**, **Knezevich A**, **De Marco A**, **Mazza D**, et al**.** 2011. Fast transcription rates of RNA polymerase II in human cells. *EMBO Rep* **12**: 1280–5.
22. **Femino AM**, **Fay FS**, **Fogarty K**, **Singer RH.** 1998. Visualization of single RNA transcripts in situ. *Science* **280**: 585–90.
23. **Iyer V**, **Struhl K.** 1996. Absolute mRNA levels and transcriptional initiation rates in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **93**: 5208–12.
24. **Holstege FC**, **Jennings EG**, **Wyrick JJ**, **Lee TI**, et al**.** 1998. Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**: 717–28.
25. **Pelechano V**, **Perez-Ortin JE.** 2010. There is a steady-state transcriptome in exponentially growing yeast cells. *Yeast* **27**: 413–22.
26. **Friedel CC**, **Dolken L.** 2009. Metabolic tagging and purification of nascent RNA: implications for transcriptomics. *Mol Biosyst* **5**: 1271–8.
27. **Perez-Ortin JE**, **de Miguel-Jimenez L**, **Chavez S.** 2012. Genome-wide studies of mRNA synthesis and degradation in eukaryotes. *Biochim Biophys Acta* **1819**: 604–15.
28. **Perez-Ortin JE**, **Alepuz P**, **Chavez S**, **Choder M.** 2013. Eukaryotic mRNA decay: methodologies, pathways, and links to other stages of gene expression. *J Mol Biol*, in press, DOI: 10.1016/j.jmb.2013.02.029
29. **French SL**, **Osheim YN**, **Cioci F**, **Nomura M**, et al**.** 2003. In exponentially growing *Saccharomyces cerevisiae* cells, rRNA synthesis is determined by the summed RNA polymerase I loading rate rather than by the number of active genes. *Mol Cell Biol* **23**: 1558–68.
30. **Lucchini R**, **Sogo JM.** 1998. The dynamic structure of ribosomal RNA gene chromatin. In Paule MR, ed; *Transcription of Ribosomal RNA Genes by Eukaryotic RNA Polymerase I*. Austin TX: Landes Bioscience. p. 255–76.
31. **Tang YC**, **Amon A.** 2013. Gene copy-number alterations: a cost-benefit analysis. *Cell* **152**: 394–405.
32. **Maiuri P**, **Knezevich A**, **Bertrand E**, **Marcello A.** 2011. Real-time imaging of the HIV-1 transcription cycle in single living cells. *Methods* **53**: 62–7.
33. **Sakai K**, **Ohta T**, **Minoshima S**, **Kudoh J**, et al**.** 1995. Human ribosomal RNA gene cluster: identification of the proximal end containing a novel tandem repeat sequence. *Genomics* **26**: 521–6.
34. **Chang SL**, **Lai HY**, **Tung SY**, **Leu JY.** 2013. Dynamic large-scale chromosomal rearrangements fuel rapid adaptation in yeast populations. *PLoS Genet* **9**: e1003232.
35. **Huber MD**, **Gerace L.** 2007. The size-wise nucleus: nuclear volume control in eukaryotes. *J Cell Biol* **179**: 583–4.
36. **Holt CE**, **Bullock SL.** 2009. Subcellular mRNA localization in animal cells and why it matters. *Science* **326**: 1212–6.
37. **Goler-Baron V**, **Selitrennik M**, **Barkai O**, **Haimovich G**, et al**.** 2008. Transcription in the nucleus and mRNA decay in the cytoplasm are coupled processes. *Genes Dev* **22**: 2022–7.
38. **Harel-Sharvit L**, **Eldad N**, **Haimovich G**, **Barkai O**, et al**.** 2010. RNA polymerase II subunits link transcription and mRNA decay to translation. *Cell* **143**: 552–63.
39. **Reese JC.** 2013. The control of elongation by the yeast Ccr4-not complex. *Biochim Biophys Acta* **1829**: 127–33.
40. **Haimovich G**, **Medina DA**, **Causse SZ**, **Garber M**, et al**.** 2013. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell* **153**: 1000–11.
41. **Loven J**, **Orlando DA**, **Sigova AA**, **Lin CY**, et al**.** 2012. Revisiting global gene expression analysis. *Cell* **151**: 476–82.
42. **Lin CY**, **Loven J**, **Rahl PB**, **Paranal RM**, et al**.** 2012. Transcriptional amplification in tumor cells with elevated c-Myc. *Cell* **151**: 56–67.
43. **Nie Z**, **Hu G**, **Wei G**, **Cui K**, et al**.** 2012. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell* **151**: 68–79.
44. **Marguerat S**, **Schmidt A**, **Codlin S**, **Chen W**, et al**.** 2012. Quantitative analysis of fission yeast transcriptomes and proteomes in proliferating and quiescent cells. *Cell* **151**: 671–83.
45. **Dungrawala H**, **Manukyan A**, **Schneider BL.** 2010. Gene regulation: global transcription rates scale with size. *Curr Biol* **20**: R979–81.