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INTERPLAY OF NONCODING RNAs, mRNAs, AND PROTEINS DURING THE GROWTH OF EUKARYOTIC CELLS

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Numerous biological functions of noncoding RNAs (ncRNAs) in eukaryotic cells are based primarily on their ability to pair with target mRNAs and then either to prevent translation or to result in rapid degradation of the mRNA–ncRNA complex. Using a general model describing this scenario, we show that ncRNAs may help to maintain constant mRNA and protein concentrations during the growth of cells. The possibility of observation of this effect on the global scale is briefly discussed.

1. INTRODUCTION

The genomes of eukaryotic cells often (e.g., in humans) contain relatively rare protein-coding sequences. The rest of the genome includes numerons transcript units representing ncRNAs. During the past decade, it has become obvious that such RNAs form a cornerstone of the regulatory network of signalling that operates in concert with the protein network (see recent reviews focused on long ncRNAs obtained directly after gene transcription [1–4], small ncRNAs (from 20 to 200 nucleotides) obtained by cleavage of long ncRNAs [5–11], and experiments [12–14]). The important role of ncRNAs has been identified in a wide variety of cellular processes including differentiation, proliferation, death, and metabolism, both in the normal state and during deceases (e.g., cancer). The numerous biological functions of ncRNAs in general and small ncRNAs in particular are based primarily on their ability to pair with target mRNAs and then either to prevent translation or to result in rapid degradation of the mRNA–ncRNA complex (the former channel seems to dominate in animals [8]). One of the most important and abundant subclasses of small ncRNAs includes microRNAs (miRNAs), which are 20–22 nucleotides long. Each miRNA is known to have hundreds or even thousands of targets [6, 12, 13]. The abilities of long ncRNAs are actually much more diverse [1–4]. In fact, such ncRNAs can participate in almost every step of gene expression.

Despite the current boom in studies of ncRNAs, the understanding of the effect of ncRNAs on genetic networks is now limited. Many aspects in this area can be rationalized, illustrated, and/or clarified by using kinetic models. Presently, the kinetic models focused on the mRNA-protein interplay are numerous (see, e.g., reviews focused on stochastic effects [15–18], oscillations [19, 20], and complex networks [21–23]). The models describing the mRNA–ncRNA interplay (without [24–27] or with protein-mediated feedbacks [28–30]) are not abundant, however, and were focused exclusively on the situations where the cell volume is constant. Complementing the already available studies, we analyze here the interplay of mRNA, protein, and ncRNA during the growth of eukaryotic cells.

In prokaryotes, the growth of cells is well known to be exponential and the average mRNA and protein concentrations are nearly constant during the growth [31]. The interpretation of these features appears to be straightforward if we take into account that in this case, the cell cycle is relatively short, the DNA replication occurs during the whole cycle, the dependence of the DNA amount on time is nearly exponential, and the rate of the mRNA synthesis is proportional to the DNA amount (see Ref. [32] and the references therein for the corresponding models).

Although the cell growth in eukaryotes is often con-

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sidered to be exponential [31, 33], the linear and bilinear growth models have also been proposed [34]. In yeasts, for example, deviations from the exponential growth are well visible in the end of the cycle [34]. On the global scale, the experiments [35, 36] indicate that the concentration of the majority of proteins during the growth of eukaryotic cells is nearly constant as in prokaryotes.

Weak dependence of the concentration of most proteins on the cell volume seems to be beneficial for the cell function, because it may facilitate the control of various intracellular processes. But the interpretation of this feature in eukaryotic cells is not straightforward. In such cells, the cycle is traditionally divided into four sequential phases: G_1 (gap phase), S (DNA replication), G_2 (gap phase), and M (division) [37]. The cycle duration is typically about one day (it may be shorter in simple organisms, e.g., in yeasts, or longer, e.g., in mammals). The duration of the S phase is often relatively short, and one could expect that the growth would be different before and after this phase. In reality, this does not seem to be the case. The understanding of why the growth is apparently insensitive to the S phase is still limited. Our goal is to clarify one of the likely related factors.

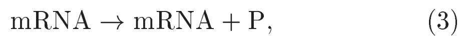
2. MODEL

In our treatment, we use a general scheme including transcription of two genes to mRNA and ncRNA,

$$\mathrm{Gene}_1 \to \mathrm{Gene}_1 + \mathrm{mRNA}, \qquad (1)$$

$$\mathrm{Gene}_2 \to \mathrm{Gene}_2 + \mathrm{ncRNA}, \qquad (2)$$

translation of mRNA to protein (P),



$$\mathrm{mRNA} \to \mathrm{mRNA} + \mathrm{P}, \qquad (3)$$

conventional enzyme-mediated degradation of these species,

$$\mathrm{mRNA} \to \oslash, \qquad (4)$$

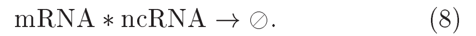
$$\mathrm{ncRNA} \to \oslash, \qquad (5)$$

$$\mathrm{P} \to \oslash \qquad (6)$$

reversible association of mRNA and ncRNA,

$$\mathrm{mRNA} + \mathrm{ncRNA} \rightleftharpoons \mathrm{mRNA} * \mathrm{ncRNA}, \qquad (7)$$

and degradation of the mRNA–ncRNA complex,



$$\mathrm{mRNA} * \mathrm{ncRNA} \to \oslash. \qquad (8)$$

As already noted, ncRNAs often pair with target mRNAs and then either prevent translation or result in rapid degradation of the mRNA–ncRNA complex. Our model (steps (7) and (8)) takes both these channels into account.

To analyze steps (1)–(8), we neglect diffusion-related concentration gradients in a cell (this widely used approximation is valid if the mRNA and ncRNA populations are not too large [26]). In this case, the kinetic equations for the mRNA, ncRNA, protein, and mRNA–ncRNA-complex populations in a cell, N_m, N_n, N_p, and N_c, are

$$\frac{dN_m}{dt} = w_m - k_m N_m - r_a N_m N_n + r_d N_c, \qquad (9)$$

$$\frac{dN_n}{dt} = w_n - k_n N_n - r_a N_m N_n + r_d N_c, \qquad (10)$$

$$\frac{dN_c}{dt} = r_a N_m N_n - r_d N_c - k_c N_c, \qquad (11)$$

$$\frac{dN_p}{dt} = v N_m - k_p N_p, \qquad (12)$$

where w_m, w_n, v, k_m, k_n, k_c, and k_p are the rates and rate constants of the reactant synthesis and degradation, and r_a and r_d are the rate constants of the mRNA and ncRNA reversible association.

During the cell cycle, all the parameters in Eqs. (9)–(12) may depend on time, and we take the key factors behind this dependence into account (this is a novel ingredient of our work). One of the factors might be the regulation of the mRNA, ncRNA, and protein synthesis by proteins. Concerning this aspect, we note that the number of cycle-related mRNAs and proteins in cells is large, but their relative abundance in the global mRNA and protein pool is modest [38, 39] and they can hardly control the population of the majority of proteins. This population, as already noted in the Introduction, is approximately proportional to the cell volume and their concentration is nearly constant [35, 36]. We therefore ignore the protein-mediated feedbacks in our model. In particular, v is considered constant.

On the other hand, the DNA replication occurring during the S phase of the cell cycle results in a twofold increase in the number of genes and the corresponding increase in the gene transcription rate resulting in the mRNA and ncRNA synthesis. Taking into account that

the time scale of the S phase is often relatively short compared to the duration of the cell cycle, we mimic the DNA replication by a stepwise increase in w_m and w_n (this approximation used earlier to simulate the effect of the cell growth on the bistable kinetics of gene expression [40] can be traced back to Ref. [41]). In particular, the rate of the mRNA synthesis is represented as

$$w_m(t) = \begin{cases} w_m^0 & \text{for } V_0 \le V(t) < V_r, \\ 2w_m^0 & \text{for } V_r < V(t) \le 2V_0, \end{cases} \quad (13)$$

where $V_0 \equiv V(0)$ is the initial cell volume and V_r is the cell volume corresponding to the DNA replication. The rate of the ncRNA synthesis is described in analogy with Eq. (13).

The rate constants of conventional degradation of mRNAs, ncRNAs and proteins are proportional to the concentrations of the corresponding enzymes. Basically, enzymes are proteins, and in our coarse-grained model, accordingly, these rate constants are expected to be proportional to the protein concentration. In analogy with the majority of proteins [35, 36], the concentration of the enzymes under consideration can be considered constant or, at least, weakly dependent on time so that this dependence can be neglected, and hence k_m, k_n, k_p, and k_c can be assumed constant. This approximation is used in the kinetic models of the mammalian cell cycle [33], and we accept it as well. (Our validation of this approximation is obviously not rigorous, and it is generally desirable to take the time dependence of the degradation rate constants into account. At present, the corresponding models are lacking however.)

Dissociation of the mRNA–ncRNA complex is an elementary monomolecular step, and therefore r_d is constant. Although association of mRNA and ncRNA is also an elementary step, its rate constant r_a depends on the cell volume because we operate with the mRNA and ncRNA populations. In particular, the association rate per unit volume can be represented as

$$W = \kappa c_m c_n,$$

where κ is the volume- and time-independent rate constant, and c_m and c_n are the mRNA and ncRNA concentrations. Taking into account that

$$c_m = \frac{N_m}{V(t)}, \quad c_n = \frac{N_n}{V(t)},$$

we have

$$W = \frac{\kappa N_m N_n}{V^2(t)}.$$

The total rate of the mRNA and ncRNA association is

$$W_{tot} \equiv WV(t) = \frac{\kappa N_m N_n}{V(t)}.$$

On the other hand, the total rate is defined by Eqs. (9) and (10) as

$$W_{tot} = r_a N_m N_n.$$

Comparing these expressions, we conclude that the dependence of r_a on volume (or time) can be represented as

$$r_a(t) = \frac{r_a^0 V_0}{V(t)}, \quad (14)$$

where $r_a^0 = \kappa/V_0$.

To complete the validation of the model, we note again that the number of distinct potential ncRNA-targets is often high (up to 1000 [6, 12, 13]). The number of distinct ncRNAs is also high. Under these conditions, the full set of equations describing the interplay between distinct mRNAs, ncRNAs, and protein is often large. In such situations, Eqs. (9)–(13) can nevertheless be used by assuming that N_m, N_n, and N_p represent the average numbers of large groups of mRNAs, ncRNAs, and proteins.

As already noted in the introduction, the time scale of the cell cycle is one day. In contrast, steps (1)–(8) usually occur on the time scale of a few minutes. Taking this difference into account, we can use a steady-state approximation in order to solve Eqs. (9)–(11) and to illustrate the dependence of the mRNA, ncRNA, and protein populations on the cell volume. In this approximation, Eqs. (11) and (12) yield

$$N_p = \frac{\upsilon N_m}{k_p}, \quad (15)$$

$$N_c = \frac{r_a N_m N_n}{r_d + k_c}. \quad (16)$$

Using the last expression, Eqs. (9) and (10) can be rewritten as

$$w_m - k_m N_m - r N_m N_n = 0, \quad (17)$$

$$w_n - k_n N_n - r N_m N_n = 0, \quad (18)$$

where

$$r = \frac{r_a k_c}{r_d + k_c} \quad (19)$$

is the effective rate constant of the mRNA and ncRNA association and degradation. Because r_a depends on V

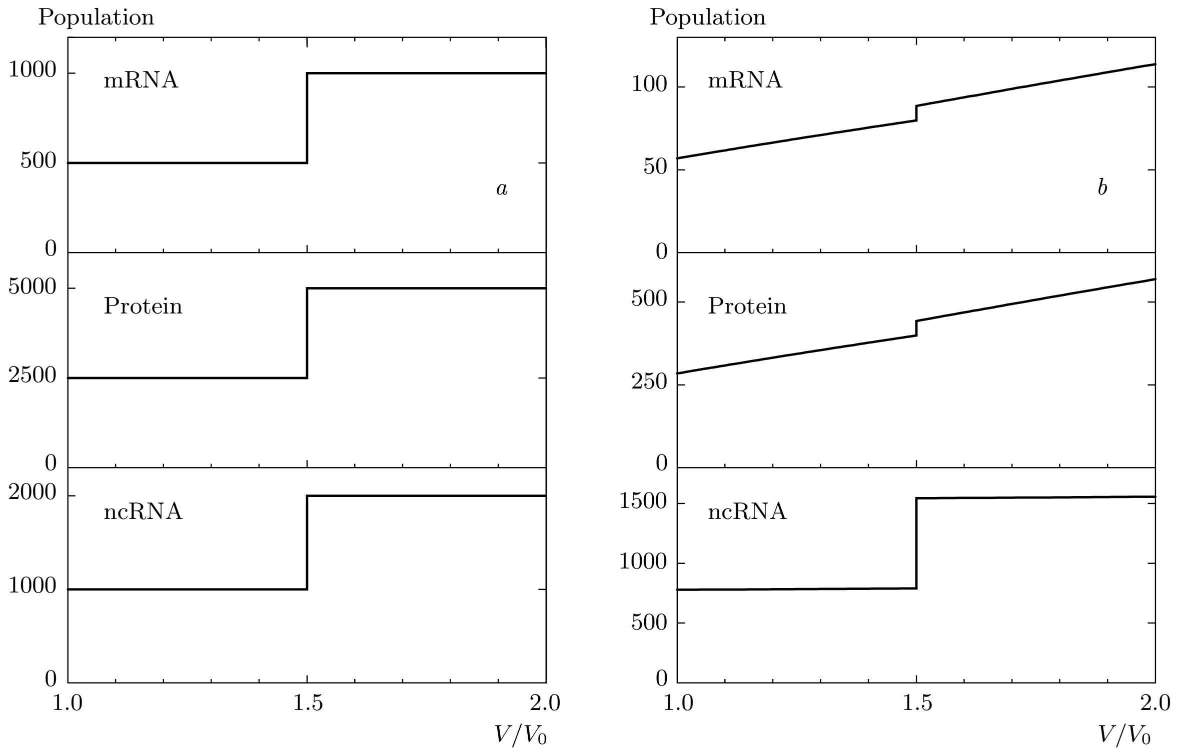


Fig. 1. mRNA, protein, and ncRNA populations as functions of the cell volume during the cell cycle (a) in the absence of the mRNA-ncRNA interaction ($r_0 = 0$) and (b) with this interaction ($r_0 = 10^{-3}$ min^{-1}). The stepwise increase in the populations corresponds to the DNA replication

(see Eq. (14)), it follows that r also depends on V and can be represented as

$$r = r_0 \frac{V_0}{V}, \qquad (20)$$

where

$$r_0 \equiv \frac{r_a^0 k_c}{r_d + k_c}$$

is the rate constant corresponding to $V = V_0$.

Equations (17) and (18) were previously used in the other contexts in [24, 25], and their solution is well known to be

$$N_m = \frac{rw_m - rw_n - k_m k_n}{2rk_m} + \left[\left(\frac{rw_m - rw_n - k_m k_n}{2rk_m}\right)^2 + \frac{k_n w_m}{rk_m}\right]^{1/2},$$

$$N_n = \frac{rw_n - rw_m - k_m k_n}{2rk_n} + \left[\left(\frac{rw_n - rw_m - k_m k_n}{2rk_n}\right)^2 + \frac{k_m w_n}{rk_n}\right]^{1/2}.$$

Using the equations presented above, we can easily calculate the reactant populations as a function of V.

3. PARAMETERS

To perform calculations, we need biologically reasonable model parameters. Because the conventional degradation of RNAs, ncRNAs, and proteins in eukaryotic cells often occurs on the time scales from a few minutes to one hour [15, 19, 42], we use $k_m = 0.1$ min^{-1}, $k_n = 0.2$ min^{-1}, and $k_p = 0.1$ min^{-1}. To describe the mRNA, ncRNA, and protein synthesis, we set $w_m^0 = 50$ min^{-1}, $w_n^0 = 200$ min^{-1}, and $\upsilon = 0.5$ min^{-1}. With these parameters, our model predicts biologically reasonable mRNA, ncRNA, and protein populations. For example, in the absence of the mRNA–ncRNA association at $V_0 \le V < V_r$, we have

$$N_m = \frac{w_m^0}{k_m} = 500, \quad N_n = \frac{w_n^0}{k_n} = 1000,$$

$$N_p = \frac{\upsilon N_m}{k_p} = 2500.$$

The rate of association of mRNA and ncRNA is limited by diffusion of these species, and the corresponding rate constant κ should be lower than $4\pi D\rho$ [43], i.e., r_0 should be lower than

$$\frac{4\pi D\rho}{V_0} \approx 3 \cdot 10^{-3} \text{ min}^{-1}$$

(see [25, 28]), where D is the RNA diffusion coefficient, and ρ is the RNA dimension. In our calculations, we use $r_0 = 10^{-3}$ min^{-1}.

The DNA replication is considered to occur at

$$V = V_r = 1.5V_0.$$

The parameters above allow us to calculate the reactant populations as a function of V in the steady-state approximation. The choice of the parameters is obviously not unique. If necessary, all the parameters can be changed. For example, the mRNA, ncRNA, and protein degradation rates can be reduced. To keep biologically reasonable populations of these species, the rates of their synthesis should also be somewhat reduced in this case. Our predictions below are fairly insensitive to such variation of the parameters.

In principle, Eqs. (9)–(12) can easily be integrated explicitly. In this case, the dependence of the cell volume on time should be introduced. As noted in the introduction, one of the reasonable options here is the exponential growth,

$$V(t) = V_0 \exp(k_g t),$$

where

$$k_g \equiv \frac{\ln 2}{t_c}$$

is the growth rate constant and t_c is the cell-cycle duration. In practice, however, there is no need in time-dependent integration of Eqs. (9)–(12) because steps (1)–(8) are very fast on the time scale of the cell growth, and hence the steady-state approximation is fairly accurate. All the results shown below were therefore obtained in this approximation.

4. RESULTS OF CALCULATIONS

Using the parameters above, we have first calculated the mRNA, ncRNA, and protein populations in the absence of the mRNA–ncRNA association (for $r_0 = 0$). In this case, the dependence of the population of these species on V is of the stepwise type (Fig. 1*a*). In particular, these populations become two times larger after the DNA replication. This is explained by the twofold increase in the gene-transcription rate due to an increase in the number of genes. This increase results in the twofold increase of the mRNA and ncRNA populations. The increase in the mRNA population results in an increase in the mRNA translation rate and in the corresponding increase in the protein population.

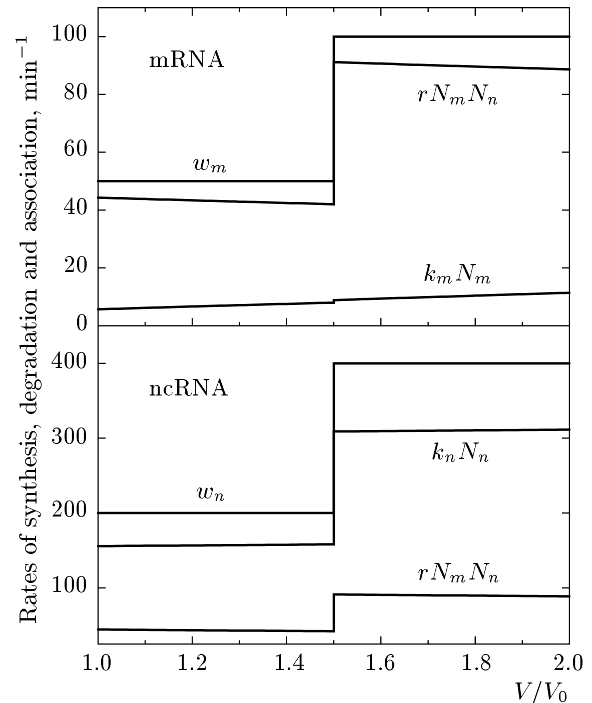


Fig. 2. Rates of mRNA and ncRNA synthesis, degradation, and association during the cell cycle shown in Fig. 1*b*

With the mRNA–ncRNA association ($r_0 = 10^{-3}$ min^{-1}), the dependence of the ncRNA population on V is qualitatively the same (Fig. 1*b*). In contrast, the dependence of the mRNA and protein populations on V is nearly linear, i.e., the concentration of these species is nearly constant. The stepwise dependence of N_n on V in the presence of the mRNA–ncRNA association is explained by a relatively small contribution of this process to the degradation of ncRNA (Fig. 2). The nearly linear dependence of N_m and N_p on V is related to appreciable suppression of the mRNA population due to the mRNA–ncRNA association and degradation (Fig. 2). In particular, the rate of the mRNA synthesis becomes two times higher after the DNA replication. However, this effect is nearly compensated by an appreciable increase in the rates of steps (7) and (8) due to the increase in the ncRNA population. Thus, the DNA replication results in minor changes in the mRNA population. The protein population is proportional to the mRNA

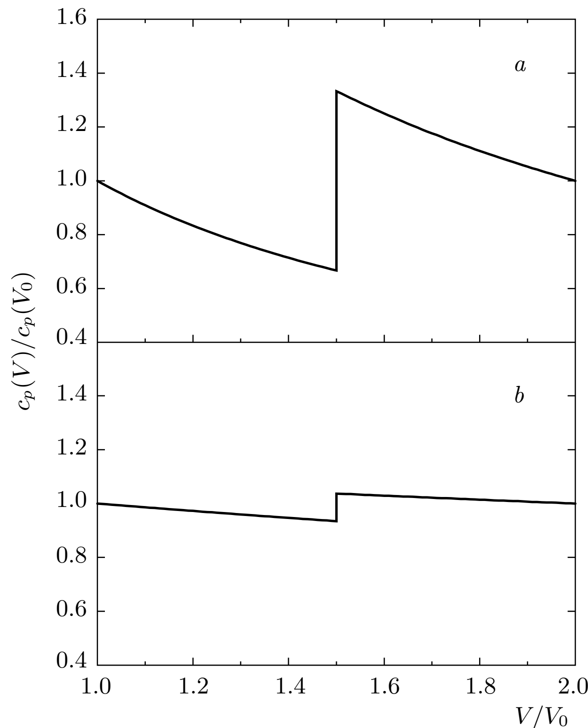


Fig. 3. Normalized protein (or mRNA) concentration as a function of the cell volume during the cell cycle (a) in the absence of the mRNA–ncRNA interaction ($r_0 = 0$; cf. Fig. 1a) and (b) with this interaction ($r_0 = 10^{-3}$ min^{-1}; cf. Fig. 1b)

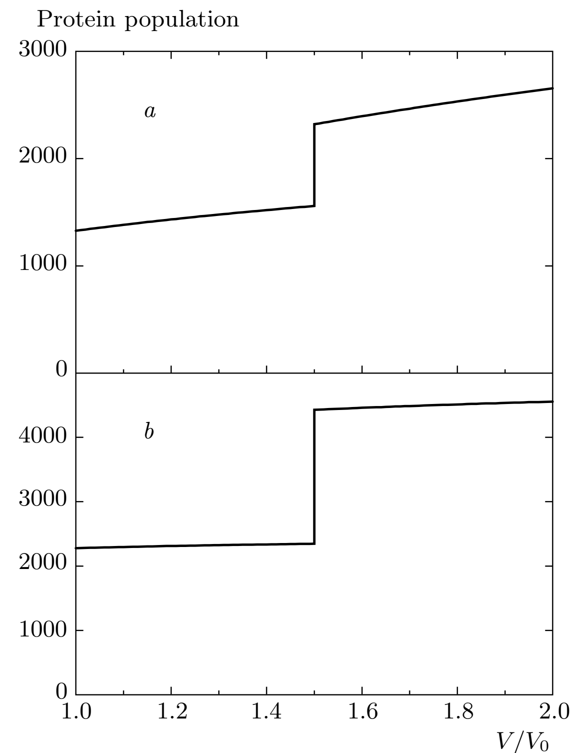


Fig. 4. Protein population as a function of the cell volume for $r_0 = 10^{-4}$ min^{-1} (a) and 10^{-5} min^{-1} (b)

population, and hence the changes in the protein population are minor as well.

Figure 3 exhibits the corresponding dependence of the protein (or mRNA) concentration on V. The changes in the concentration are appreciable in the absence of the mRNA–ncRNA association (Fig. 3a) and nearly negligible with the mRNA–ncRNA association (Fig. 3b).

The kinetics shown in Figs. 1 and 3 were calculated for $r_0 = 0$ and 10^{-3} min^{-1}. Figure 4 illustrates what happens between these values. As r_0 decreases from 10^{-3} min^{-1} (Fig. 1b) to 10^{-4} min^{-1} (Fig. 4a), the stepwise feature at $V = 1.5V_0$ becomes more appreciable, but nevertheless remains much weaker than that at $r_0 = 0$ (Fig. 1b). The kinetics calculated for $r_0 = 10^{-5}$ min^{-1} (Fig. 4b) are close to those at $r_0 = 0$ (Fig. 1a).

The results presented in Figs. 1–4 were obtained using a steady-state solution of Eqs. (9)–(12). Direct integration of Eqs. (9)–(12), performed by complementing the parameters above, e.g., by $r_d = k_c = 0.1$ min^{-1}, yields the same results. The inclusion of fluctuations (by the standard Gillespie Monte Carlo algorithm) does not change the results either. Concerning Fig. 1b, we note that the behavior shown there is insensitive to the choice of the parameters and can be observed in a wide range of parameters. The two key necessary conditions of its realization are a relatively high population of ncRNAs compared to mRNAs and a relatively strong mRNA–ncRNA interaction (steps (7) and (8)). Our calculations performed with biologically reasonable parameters indicate that these conditions can be met in specific mRNA and ncRNA pairs.

5. CONCLUSION

In summary, our analysis, based on the available information about the mRNA, protein, and ncRNA synthesis and degradation, shows that ncRNAs may help to maintain constant mRNA and protein concentration during the growth of eukaryotic cells. The experiments indicate that the concentration of the majority of proteins during the cell growth is nearly constant (both in prokaryotes and eukaryotes). In this context, it is interesting to speculate on whether ncRNAs or, more specifically, miRNAs can contribute to the global con-

trol of the the mRNA and protein population. Concerning this point, we note that the number of confidently identified miRNA genes in humans has presently surpassed 400 [9]. The number of protein-coding genes, approximately $3 \cdot 10^4$, is much larger, and the first impression might be that the global control by miRNAs is unlikely. But there are at least three factors indicating that the reality is more subtle.

(i) The number of miRNAs will undoubtedly increase as high-throughput sequencing continues to be applied [9].

(ii) The transcriptional activity of many protein-coding genes is very low, while the miRNA studies, e.g., using the ncRNA microarray technique [44], tend to be focused on miRNAs with relatively high populations. Therefore, the identified miRNA genes are on average more active that the protein-coding genes.

(iii) miRNAs are transcribed as long ncRNA and then generated via a two-step processing pathway including the formation of a few different approximately 65 nt pre-miRNAs followed by conversion of each of them into the corresponding miRNA [45]. This is an additional reason why the efficiency of the genes generating miRNAs may be a few times higher than that of the protein-coding genes.

Taking all these points into account, we believe that the possibility of an miRNA contribution to the global control of the mRNA and protein population cannot be excluded. For this, many miRNAs must be appreciably expressed in different tissues. Interestingly, this is the case in normal human tissues [46].

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