ЖЭТФ, 2007, том 131, вып. 1, стр. 180–188

STEM CELL PROLIFERATION AND DIFFERENTIATION AND STOCHASTIC BISTABILITY IN GENE EXPRESSION

V. P. Zhdanov[*,a,b]

[a] *Department of Applied Physics, Chalmers University of Technology S-41296, Göteborg, Sweden*

[b] *Boreskov Institute of Catalysis, Russian Academy of Sciences 630090, Novosibirsk, Russia*

Received 23 August 2006

The process of proliferation and differentiation of stem cells is inherently stochastic in the sense that the outcome of cell division is characterized by probabilities that depend on the intracellular properties, extracellular medium, and cell–cell communication. Despite four decades of intensive studies, the understanding of the physics behind this stochasticity is still limited both in details and conceptually. Here, we suggest a simple scheme showing that the stochastic behavior of a single stem cell may be related to (i) the existence of a short stage of decision whether it will proliferate or differentiate and (ii) control of this stage by stochastic bistability in gene expression or, more specifically, by transcriptional «bursts». Our Monte Carlo simulations indicate that this scheme may operate if the number of mRNA (or protein) generated during the high-reactive periods of gene expression is below or about 50. The stochastic-burst window in the space of kinetic parameters is found to increase with decreasing the mRNA and/or regulatory-protein numbers and increasing the number of regulatory sites. For mRNA production with three regulatory sites, for example, the mRNA degradation rate constant may change in the range ±10 %.

PACS: 87.16.-b, 05.40.-a, 05.65.+b

1. INTRODUCTION

Adult stem cells, possessing the ability for self-renewal and generation of more specialized cells, were first identified in the hematopoietic (blood-forming) system in the early 1960s [1]. Later on, stem cell niches were found to exist in the skin [2], gut [3] and brain [4] (for general readership, see Ref. [5]). Despite four decades of intensive studies and high current interest in potential applications in treatment of numerous severe diseases, tissue engineering, diagnostic purposes, drug testing, etc. [6], the understanding of the mechanism(s) of proliferation and differentiation of stem cells is still limited both in details and conceptually [7].

One of the reasons of conceptual difficulties in this area is that the process of proliferation and differentiation of stem cells is inherently stochastic in the sense that the outcome of cell division (whether it results in two stem cell or a stem cell and a differentiated cell) is characterized by probabilities that depend on the intracellular properties, extracellular medium, and cell–cell communication. Whether these probabilities are determined by complex (e.g., chaotic) kinetics, which can be described by deterministic equations, or by stochastic kinetics due to a small number of reactants participating in some of the steps is still not clear.

To illustrate the last point more explicitly, it is instructive to briefly discuss the models used in describing the kinetics of proliferation and differentiation of stem cells.

(i) The simplest approach is based on the use of fixed probabilities for stem-cell self-renewal, differentiation, and death. The corresponding stochastic models have been widely used since the mid-60s (see the earliest models [8] and recent reviews [9] containing numerous relevant references). The advantage of this approach is that it allows one to easily perform analytical and numerical calculations or Monte Carlo (MC) simulations with the various factors (e.g., cell–cell communication or aggregation of cells due to adhesion [10])

*E-mail: zhdanov@catalysis.ru

complicating the stem-cell kinetics taken into account. But such models do not describe explicitly what happens inside cells.

(ii) To clarify general principles of proliferation and differentiation, one can represent a cell by a set of nonlinear chemical reactions without specifying their biochemical function. This strategy was realized in a series of papers by Kaneko and co-workers [11]. In their models, the internal dynamics of a single cell typically exhibits oscillations, chaos, and/or coexistence of multiple attractors, with only one attractor for randomly chosen initial conditions. The emphasis is made on the behavior of an ensemble of cells interacting via exchange of some of the reactants. With such interactions, the models predict differentiation from a «stem» cell to other cell types. Thus, differentiation is essentially considered a collective feature of stem cells having internal oscillatory dynamics.

(iii) Differentiation of stem cells was interpreted in terms of a Boolean network model of genetic regulatory networks [12]. With prescribed rules of switches of the gene states, this model predicts state circles or attractors. Differentiation is viewed as resulting from a transient or persistent perturbation that causes a cell to «jump» from one attractor to another attractor. Although the terminology and mathematics are here quite different from those in item (ii), the general concept is basically the same.

(iv) Recent kinetic models tend to utilize cell-specific data (see reviews [9, 13]). For example, the mean-field kinetic models [14, 15] are focused on signal networks, based on the known components of epidermal growth factor receptor signal pathways (this receptor is often considered to play an important role in proliferation and differentiation). In such models, the proliferation or differentiation events are usually assumed to occur if the concentration of some of the reactants is higher or lower than the critical concentration (see, e.g., Ref. [15]). Practically, this means that under fixed external conditions, the fate of a cell is deterministic. Under transient external conditions, e.g., due to cell–cell communication, the models are able to describe both proliferation and differentiation in an ensemble of cells. (See Refs. [16, 17] for a discussion of various aspects of bistability in cell signaling.)

In general, the proliferation and differentiation of stem cells is usually believed to be related to gene expression. During the past decade, this process was theoretically analyzed in numerous papers. The corresponding models can be divided into three overlapping groups, focused respectively on (i) general principles of gene expression including stochastic effects [18–21] (see Ref. [22] for direct observation of stochastic bursts in protein production during gene expression), (ii) expression of specific genes [19, 23, 24], and (iii) complex genetic networks [20, 21, 23, 25]. Although stem cells are often mentioned in these studies, the details of how differentiation is governed by genes are not discussed there.

Stochastic effects in gene expression are common because most genes exist in single or low copy numbers in a cell. The potential importance of such effects for proliferation and differentiation of stem cells is often articulated in general discussions of these two processes [21, 26]. But the mechanistic details of the interplay of stochasticity of gene expression and differentiation and the quantitative criteria allowing one to understand when this interplay is possible remain vague.

To illustrate the points above explicitly, it is instructive to briefly present typical examples showing the state of the art in studying the mechanisms of differentiation of specific cells. We first mention the comprehensive proliferation- and differentiation-related studies of the gene-expression map in *Arabidipsis* [27]. Despite the analysis of the performance of 22000 genes (90 % of the genome), the mechanisms of proliferation and differentiation remain hidden in this case.

Another example is adult rat neural stem cells or, more specifically, adult hippocampal progenitor cells growing in culture [28]. Under appropriate conditions, these multipotent cells are able to proliferate and/or generate neurons and glial cells (astrocytes and oligodendrocytes) daily for at least the first month of culture. The relative rates of these pathways are known to depend on the growth factors (highly specific proteins mostly required in low concentrations (10^{-9}–10^{-11} M)) [29][1)]. A detailed analysis [30] of the changes of gene expression during differentiation of these cells is impressive. A clear mechanistic interpretation of the results obtained is lacking, however.

To complement the theoretical works described above and to guide experiments, we suggest (Sec. 2) a simple conceptual scheme showing how stochastic bistability in gene expression may result in stochastic proliferation and differentiation of a single cell. In addition, we present (Sec. 3) MC simulations of stochastic bistability in gene expression in order to quantify some of the aspects of our general discussion or, more specifically, to obtain criteria for clarifying the conditions of

[1)] Differentiation can be readily observed by immunocytochemistry, i.e., by detection of expression of proteins, specific to each type of differentiated cell.

realization of the scheme suggested. Taken together, the results in Secs. 2 and 3 extend the conceptual basis for the understanding of the likely role of stochastic intracellular processes in proliferation and differentiation of stem cells and may promote further steps in the direction under consideration.

2. PROLIFERATION AND DIFFERENTIATION

The fate of stem cells is now believed (see the preceding section) to be determined by kinetic switches related to gene expression and/or other biochemical reactions. This general scheme admits various realizations. The scenario discussed here is based on two key assumptions.

(i) The division of a stem cell results in the appearance of two stem cells or a stem cell and a differentiated cell. This means that the cell must come to a decision whether it will proliferate or differentiate. In our scheme, the decision stage is assumed to be narrow compared with the duration of the cell cycle. Physically, it is clear that the decision can hardly be made just after the cell birth, because the cell should grow after the birth, with the conditions inside the cell rapidly changing during this phase. Therefore, the internal and external control of the cell fate cannot be robust. The decision can also hardly be made just before the cell division, because the cell needs time in order to develop the machinery corresponding to the birth of either two stem cells or a stem cell and a differentiated cell. Thus, the decision is expected to be made somewhere in the middle of the cell cycle[2)].

(ii) The decision whether to proliferate or differentiate is assumed to be related to the stochastic expression of one of the genes. In particular, the protein synthesized due to the activity of this gene is considered to govern the performance of a few other genes controlling the cell fate. Specifically, the gene is assumed to operate in the stochastic bistable regime and to exhibit sequential periods of high and low expression (transcriptional bursts) due to positive feedback between the messenger ribonucleic acid (mRNA) and protein production and a small number of mRNA and/or protein. These periods are assumed to be comparable to or somewhat longer than the duration of the decision stage and accordingly much shorter compared to the duration of the cell cycle. The fate of a cell depends on whether the level of the gene expression during the decision stage is high or low.

If assumptions (i) and (ii) are fulfilled, the differentiation rate constant is given by

$$k_{dif} = k_{div} P_1, \tag{1}$$

where k_{div} is the division rate constant, and

$$P_1 = \frac{\tau_1}{\tau_1 + \tau_2}$$

is the probability that the gene is in state 1 corresponding to differentiation (τ_1 and τ_2 are the respective average durations of the gene activity periods corresponding to differentiation and proliferation). For the proliferation rate constant, we have

$$k_{pr} = k_{div} P_2, \tag{2}$$

where

$$P_2 = 1 - P_1 = \frac{\tau_2}{\tau_1 + \tau_2}$$

is the probability that the gene is in state 2 corresponding to proliferation.

We note that Eqs. (1) and (2) do not imply that a cell should somehow measure probabilities P_1 and P_2. Instead, for each given cell, the corresponding stochastic process of gene expression occurs, and the cell fate depends on realization of this process. The probabilities P_1 and P_2 and the rate constants k_{dif} and k_{pr} are introduced for an ensemble of stem cells. In reality, these probabilities and rate constants may depend on the cell concentration if the gene expression and/or other related intracellular processes are influenced by communication between cells.

In general, a stem cell may generate specialized cells of two or more types (e.g., a neural stem cell may generate neurons and glial cells). In such cases, a stem cell is expected to make two or more decisions. The first decision, e.g., should discriminate between proliferation and differentiation, and if differentiation is the choice, the second decision has to discriminate between two types of the differentiated cell.

The special feature of the scenario outlined above is that the average rate of expression of the gene(s) controlling proliferation and differentiation of a stem cell may be the same as that in differentiated cells. In addition, the stochastic bursts in gene expression may be generated only during a part of the cell cycle including the decision stage. This may hinder identification of the mechanism of differentiation and simultaneously explain why the identification of the genes responsible for differentiation is often difficult.

[2)] We note that the proliferation, e.g., of differentiated mammalian cells is controlled by regulating the progression through the G_1 phase and entry into the S phase [31]. There are also indications that this is an early period in differentiation of stem cells [31].

Although the scenario suggested is simple, to our knowledge, it was not explicitly discussed in detail in the literature. In this context, it is of interest to clarify how low the number of mRNA or protein should be in order to realize the scenario above and whether this number depends on the details of the regulation of the gene activity. The answers to these questions are given in the next section.

3. STOCHASTIC BISTABILITY IN GENE EXPRESSION

Expression of the information encoded in DNA is known [32] to occur via a templated polymerization called transcription, in which the genes (segments of the DNA sequence) are used as templates to guide the synthesis of shorter molecules of RNA. Later on, many of these molecules (or, more specifically, messenger RNA) serve to direct the synthesis of proteins on ribosomes. The whole process of gene expression can be regulated at all steps. In particular, the gene transcription, performed by RNA polymerase, is often controlled by master regulatory proteins. In the case of positive feedback between the mRNA and protein production, the gene expression may exhibit bistability [19]. (For the general discussion of various aspects of bistability in cellular systems with emphasis on cell signaling, see Ref. [16].)

In our treatment, we analyze the situation where the gene has a few regulatory sites. The mRNA (R) production rate is considered to be high if all the regulatory sites are occupied by the protein (P). In this case, the generic mean-field equations for the R and P numbers are given by (cf., e.g., Ref. [19])

$$\frac{dN_R}{dt} = k_0 + k_1 \left(\frac{N_P}{K_P + N_P}\right)^n - k_R N_R, \qquad (3)$$

$$\frac{dN_P}{dt} = k_s N_R - k_P N_P, \qquad (4)$$

where k_0 and k_1 are the rate constants of the basal and protein-regulated gene transcription (n is the number of regulatory sites, K_P is the protein association–dissociation constant, and $(N_P/(K_P + N_P))^n$ is the probability that all the regulatory sites are occupied by P), k_s is the rate constant of protein synthesis, and k_R and k_P are the respective rate constants of the mRNA and protein degradation.

Equations (3) and (4) predict bistability if $n \geq 2$. Our calculations below are performed for $n = 2$ or 3. These lowest values of n are most natural. In addition, there are indications that the cell differentiation does occur with participation of autoactivating transcription factors like GATA-3 with $n = 2$ [19].

To illustrate the stochastic kinetics exhibiting transcriptional bursts, we focus our attention on the case where N_R is relatively small. To keep the analysis as simple as possible, (i) N_P is considered to be large, (ii) the protein attachment to and detachment from mRNA are assumed to be rapid, and (iii) the protein formation and degradation are assumed to be rapid as well. Conditions (i) and (ii) guarantee that the effect of the protein on the gene transcription can be described in the mean-field approximation even if N_R is small. Conditions (i) and (iii) guarantee in turn that N_P is close to a steady state, i.e.,

$$N_R \approx \frac{k_P}{k_s} N_P,$$

both in the mean-field and stochastic regimes (the validity of this statement was verified and confirmed by independent MC simulations). Substituting this relation between N_R and N_P in Eq. (4) yields

$$\frac{dN_R}{dt} = k_0 + k_1 \left(\frac{N_R}{\mathcal{K}_P + N_R}\right)^n - k_R N_R, \qquad (5)$$

where

$$\mathcal{K}_P = \frac{K_P k_P}{k_s}.$$

To study fluctuations, we perform MC simulations of the kinetics corresponding to Eq. (5). Specifically, we use the standard MC algorithm [33] based on calculation of the total reaction rate. In our case (Eq. (5)), there are two parallel processes, the mRNA production and degradation, running with the rates

$$W_1 = k_0 + k_1 \left(\frac{N_R}{\mathcal{K}_P + N_R}\right)^n$$

and

$$W_2 = k_R N_R.$$

The total rate of these processes is

$$W_t = W_1 + W_2.$$

For a given number of mRNA, we generate a random number ρ ($0 < \rho \leq 1$) and execute one of the possible processes (i.e., increase or decrease N_R by one) if $\rho < W_1/W_t$ and $\rho > W_1/W_t$, respectively. After each MC trial, time is increased by $|\ln \chi|/W_t$, where χ ($0 < \chi \leq 1$) is another random number.

The time scales characterizing elementary biochemical processes inside cells are about a minute or shorter.

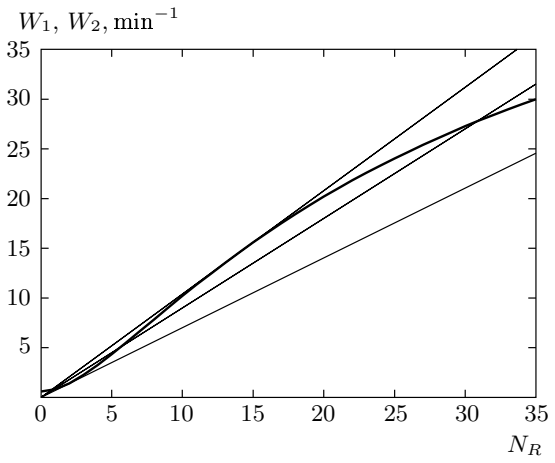

Fig. 1. Rates of the mRNA production W_1 (thick line) and degradation W_2 (thin lines), as functions of N_R for $n = 2$, $k_1 = 60$ min^{-1}, $\mathcal{K}_P = 15$, and $k_R = 0.702$, 0.9, and 1.04 min^{-1}

In contrast, the time scale of division of stem cells is typically about one day. Taking these restrictions on the time scales into account, we use $k_R \approx 1$ min^{-1} in our simulations (k_R is considered to be the governing parameter). The constants k_1 and $\mathcal{K}_P$ are chosen in order to ensure bistability with a relatively small number of mRNA. Typically, k_1 is selected to be appreciably larger than unity. The dependence of the results of simulations on k_0 is weak. To be specific, we set $k_0 = 0.01k_1$ in all the examples. The duration of the MC runs is 2000 min (this value is comparable to or somewhat longer than the time scale of the division of stem cells). We note that although the parameter values indicated above and chosen below are biologically reasonable, the corresponding values are in reality distributed in a wide range (due to the diversity of cells) and accordingly may of course be both smaller and/or larger.

Figure 1 shows the mRNA production and degradation rates as a function of N_R in the case of two regulatory site ($n = 2$). The production rate is calculated for $k_1 = 60$ min^{-1} and $\mathcal{K}_P = 15$. The degradation rate is shown for $k_R = 0.702$, 0.9, and 1.04 min^{-1}. The values $k_R = 0.702$ and 1.04 min^{-1} correspond to the boundaries of the bistability window. The value $k_R = 0.9$ min^{-1} is nearly at the middle of the bistability window. With these parameters, the steady-state numbers of mRNA are low. Typically, N_R is about 30 for the high-active gene-expression regime and N_R is about 5 for the low-active regime. Although the bistability window is relatively

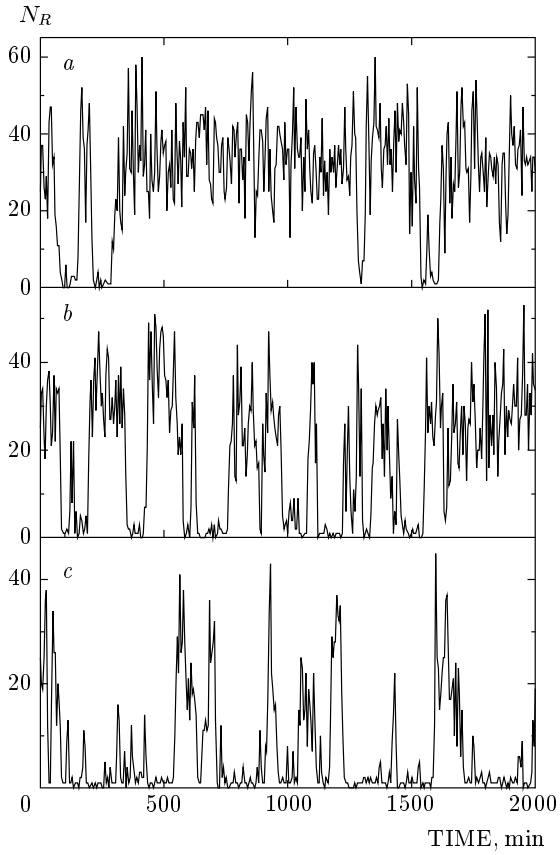

Fig. 2. The number of mRNA as a function of time for $k_R = 0.85$ (a), 0.90 (b), and 0.95 (c) min^{-1}. The other parameters are as in Fig. 1. The initial mRNA number is 25

wide ($0.702 \le k_R \le 1.04$ min^{-1}), the stochastic oscillations with transitions between the high- and low-active regimes can be observed at $t \le 2000$ min only for $0.85 \le k_R \le 0.95$ min^{-1}, as shown in Fig. 2. Outside the last window, the model predicts either high- or low-active regime at $t \le 2000$.

To illustrate what happens for higher values of N_R during the high-active regime, we keep $n = 2$ and use $k_1 = 180$ min^{-1} and $\mathcal{K}_P = 45$. For these parameters, the dependence of the mRNA production and degradation rates on N_R is similar to that shown in Fig. 1 except that the range of the values on the horizontal axis is to be extended to 105. The N_R number for the high-active regime is about 90. The bistability window ($0.702 \le k_R \le 0.104$ min^{-1}) is the same as in the previous case. But the stochastic oscillations with transitions between the high- and low-active regimes can now be observed at $t \le 2000$ min in a very narrow range of the k_R values (from 0.93 to 0.95 min^{-1}). Typical kinetics generated inside and outside this range

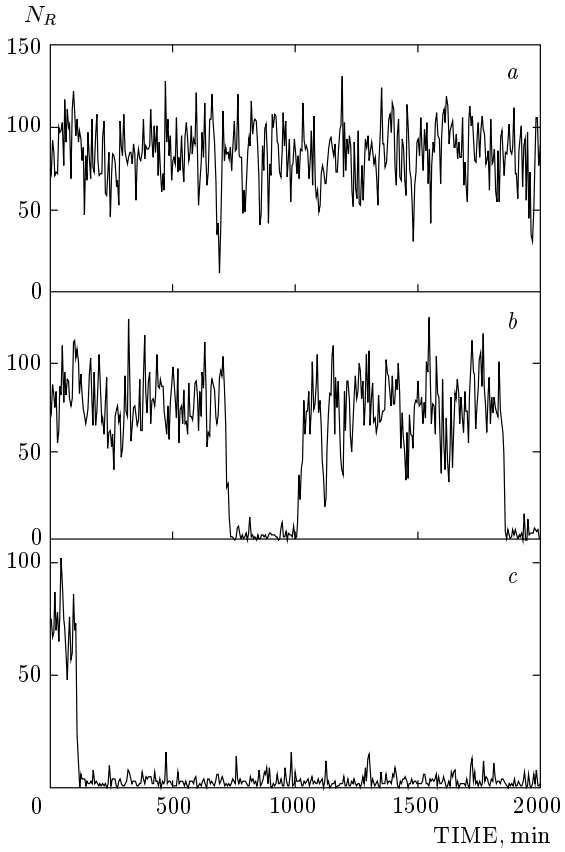

Fig. 3. The number of mRNA as a function of time for $n = 2$, $k_1 = 180$ min^{-1}, $\mathcal{K}_P = 45$, $k_R = 0.92$ (a), 0.94 (b), and 0.98 (c) min^{-1}. The initial mRNA number is 75

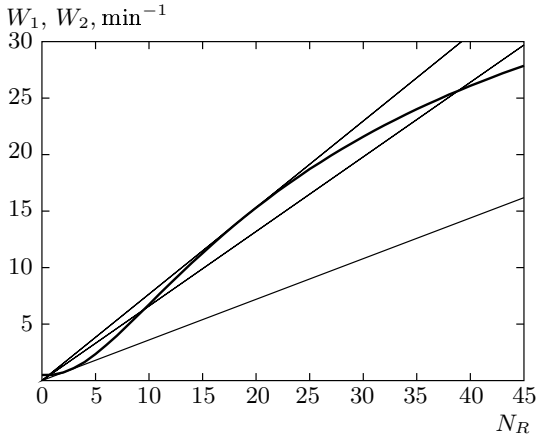

Fig. 4. Rates of the mRNA production W_1 (thick line) and degradation W_2 (thin lines), as functions of N_R for $n = 3$, $k_1 = 50$ min^{-1}, $\mathcal{K}_P = 10$, and $k_R = 0.360$, 0.660, and 0.766 min^{-1}

of k_R are presented in Fig. 3.

Figure 4 shows the dependence of the mRNA production and degradation rates on N_R in the case of three regulatory sites ($n = 3$). The production rate is calculated for $k_1 = 50$ min^{-1} and $\mathcal{K}_P = 10$. The degradation rate is shown for $k_R = 0.360$, 0.660, and 0.766 min^{-1}. With these parameters, N_R is about 40 for the high-active gene-expression regime. The stochastic oscillations with transitions between the high- and low-active regimes are observed (Fig. 5a–c) at $t \leq 2000$ min for $0.58 \leq k_R \leq 0.72$ min^{-1} (this means that inside the stochastic-burst window, the mRNA degradation rate constant is changed in the range $\pm 10\,\%$). The probability of the high-active regime as a function of k_R is exhibited in Fig. 5d.

If we keep $n = 3$ and use $k_1 = 150$ min^{-1} and $\mathcal{K}_P = 30$, the dependence of the mRNA production and degradation rates on N_R is similar to that in Fig. 4 except that the range of the values on the horizontal axis must to be extended to 135. With these parameters, the stochastic oscillations with transitions between the high- and low-active regimes are not observed at $t \leq 2000$ min (see Fig. 6).

Comparing the results in Figs. 4 and 5 with those in Figs. 1 and 2, we conclude that with increasing n from 2 to 3, the bistability and stochastic-burst windows become appreciably wider. With a further increase in n (e.g., up to 5), the bistability window can easily be increased. The stochastic-burst window can also be increased, but only slightly. For the mRNA production with five regulatory sites, for example, the mRNA degradation rate constant can be changed in the range $\pm 13\,\%$ [34]. For the conventional Hill expression for the protein-regulated gene-transcription rate (this model implies cooperative association of P with regulatory sites), the results are similar [34]. In both cases (for the expression in Eq. (5) and for the Hill expression), further increase of the stochastic-burst window is possible with decreasing the R and/or P numbers. If, e.g., $n = 5$, the maximum R number is about 20 and the mRNA degradation rate constant can be changed in the range $\pm 15\,\%$ [34].

Finally, it is appropriate to note that the stochastic bistable kinetics can be scrutinized by calculating the distribution of the numbers of reactants (see, e.g., recent simulations in [35] and the references therein). For the kinetics exhibiting bursts, the distribution is well known to be bimodal. Using such distributions allows compactifying the presentation of results. However, from the standpoint of the understanding of the likely effects of the transcriptional bursts on differentiation of stem cells, it is much more instructive (es-

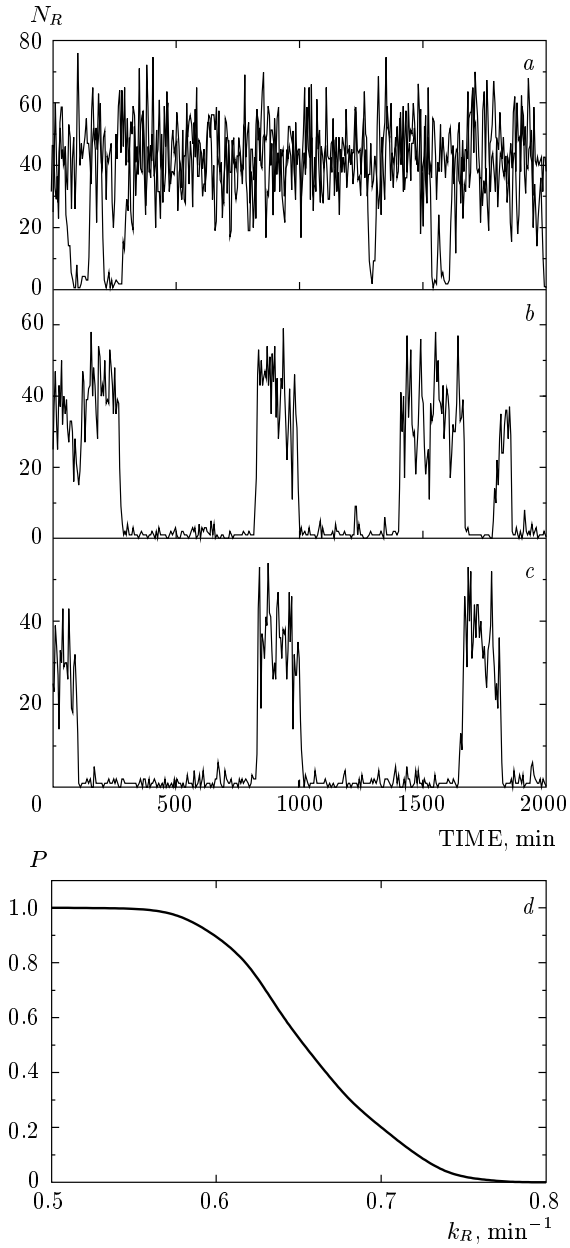

Fig. 5. The number of mRNA as a function of time for $k_R = 0.62$ (a), 0.66 (b), and 0.70 (c) min^{-1}). The other parameters are as in Fig. 4 (the initial mRNA number is 25). Panel (d) shows the probability of the high-active gene-expression regime as a function of k_R for these parameters (each data point used to construct the curve was obtained by using 5 MC runs executed up to $t = 2000$ min)

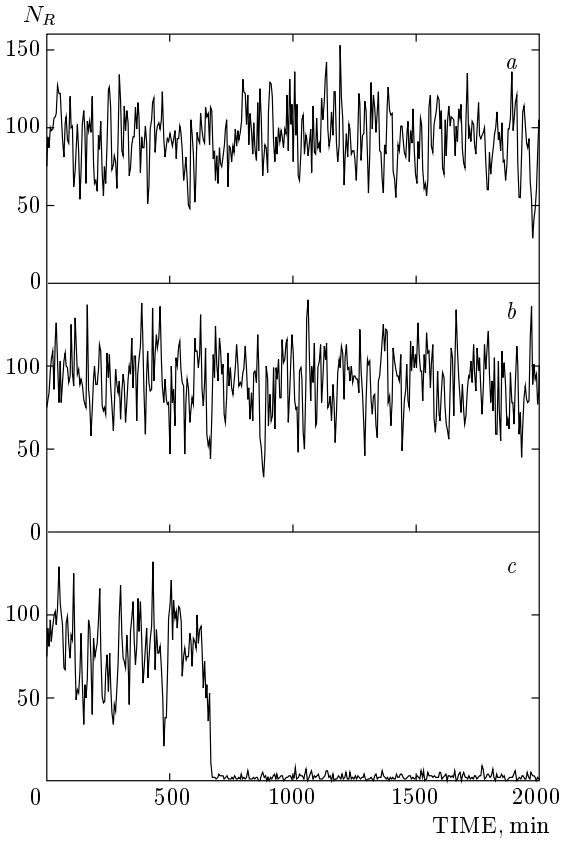

Fig. 6. The number of mRNA as a function of time for $n = 3$, $k_1 = 150$ min^{-1}, $\mathcal{K}_P = 30$, $k_R = 0.70$ (a), 0.71 (b), and 0.72 (c) min^{-1}. The initial mRNA number is 75

pecially for general readership) to explicitly show the transcriptional kinetics.

4. CONCLUSION

We have proposed a simple scheme showing that the stochastic behavior of a single stem cell may be related to (i) the existence of a short stage of decision whether it will proliferate or differentiate and (ii) control of this stage by stochastic bistability in gene expression. Our MC simulations of gene expression with positive feedback between the mRNA and protein production indicate that this scheme can be realized if the number of mRNA (or protein) generated during the high-reactive periods of gene expression is below or about 50. For the simplest models of gene expression, the stochastic-burst window in the space of kinetic parameters is found to be not too wide, however. For example, the mRNA degradation rate constant may be changed in the range narrower than or about $\pm 15\,\%$. Thus, one may question the plausibility of the suggested mechanism for cell fate determination, because it depends critically on the values of the rate

constants. For example, k_s depends on the number of ribosomes, and this parameter alone could easily vary by a factor of 2 or more. But this variation is primarily related to the growth of a cell. Specifically, the number of ribosomes increases simultaneously with the increase in the cellular volume. This results in the decrease of the mRNA concentration, which in turn compensates the increase in the number of ribosomes. Thus, the situation is not so dramatic as one could expect. On the other hand, the changes related to the cellular growth may of course influence the stochastic bistability of the gene expression. For the applicability of the proposed mechanism of differentiation, the stochastic bursts in gene expression should be generated during the decision stage at least. At the late stages, the bursts may disappear (if this is the case, the identification of the mechanism of differentiation may be complicated).

Concerning the robustness of the suggested scheme of the cell-fate determination, it is also appropriate to note that our analysis of stochastic bistability in gene expression is focused on the generic situation where the positive feedback between the mRNA and protein production occurs due to a few sites regulating the mRNA production. In more specific situations, e.g., with additional steps in protein processing and/or the interplay of two or more genes (for relevant mean-field models, see [16, 36] and the references therein), the stochastic-burst window may perhaps be wider and if this is the case, it may help to realize the scheme under consideration.

To relate our analysis to experiments, we repeat (cf. Sec. 2) that the special feature of the suggested scenario is that the average rate of expression of the gene(s) controlling proliferation and differentiation of a stem cell may be the same as that in differentiated cells. In addition, the stochastic bursts in gene expression may be generated only during a part of the cell cycle including the decision stage. This may hinder identification of the mechanism of differentiation. Concerning more constructive predictions, we note, e.g., that the degradation of proteins usually occurs in special compartments called lysosomes [32]. Thus, the corresponding rate constant (in Eq. (4)) is proportional to the ratio of the volume of lysosomes and the cell volume. Taking into account that the duration of the transcriptional bursts depends on this rate constant, one can try to study correlations between this ratio (or other kinetic parameters in Eqs. (3) and (4)) and the differentiation probabilities. Although the observation of such correlations cannot guarantee that the mechanism suggested is operative, it might be interpreted in favor of the mechanism.

Finally, we can articulate that stochastic effects in gene expression are common because most genes exist in a single or low copy numbers in a cell. Although the likely importance of such effects for proliferation and differentiation of stem cells has often been emphasized in general discussions of these two processes (see the Introduction), the corresponding mechanistic schemes illustrating in detail how the system may operate are lacking. We have tried to scrutinize this problem. Our analysis is speculative. The results obtained nevertheless make it possible to deeper understand the type of difficulties encountered here and to take further steps to clarify the interplay between stochastic gene expression and cell differentiation.

This work is a part of the FP6-project STREP NANOCUES (Nanoscale surface cues to steer cellular biosystems), funded by the European Commission, and is partially funded by the Chalmers Bioscience Program, the Swedish Science Council, and the Foundation for Strategic Research.

REFERENCES

1. J. E. Till and E. A. McClloch, Radiat. Res. **14**, 213 (1961).

2. L. Alonso and E. Fuchs, Proc. Nat. Acad. Sci. USA **100** (Suppl. 1), 11830 (2003).

3. J. I. Gordon, G. H. Schmidt, and K. A. Roth, FASEB J. **6**, 3039 (1992).

4. C. Lois and A. Alvarezbuylla, Proc. Nat. Acad. Sci. USA **90**, 2074 (1993); C. Klein and G. Fishell, Dev. Neurosci. **26**, 82 (2004).

5. Nature Insight: Stem Cells, Nature **414**, 87 (2001); A. Spradling, D. Drummond-Barbosa, and T. Kai, Nature **414**, 98 (2001).

6. N. L. Parenteau and J. H. Young, Ann. NY Acad. Sci. **961**, 27 (2002); M. Sykes and B. Nikolic, Nature **435**, 620 (2005); C. Zandonella, Nature **435**, 877 (2005).

7. F. Ulloy-Montoya, C. M. Verfaillie, and W.-S. Hu, J. Biosci. Bioing. **100**, 12 (2005).

8. L. Siminovitch, E. A. McCulloch, and J. E. Till, J. Cell. Compar. Physiol. **62**, 327 (1963); J. E. Till, L. Siminovitch, and E. A. McCulloch, Proc. Nat. Acad. Sci. USA **51**, 29 (1964).

9. S. Viswanathan and P. W. Zandstra, Cytotechn. **41**, 75 (2003); A. O'Neill and D. V. Schaffer, Biotech. Appl. Biochem. **40**, 5 (2004).

10. V. P. Zhdanov and B. Kasemo, Phys. Chem. Chem. Phys. **6**, 138 (2004); *ibid.* **6**, 4347 (2004); V. P. Zhdanov, D. Steel, B. Kasemo, and J. Gold, Phys. Chem. Chem. Phys. **7**, 3496 (2005).

11. K. Kaneko and T. Yomo, Bull. Math. Biol. **59**, 139 (1997); C. Furusawa and K. Kaneko, Bull. Math. Biol. **60**, 659 (1998); C. Furusawa and K. Kaneko, J. Theor. Biol. **224**, 413 (2003); H. Yoshida, C. Furusawa, and K. Kaneko, J. Theor. Biol. **233**, 501 (2005).

12. H. D. Preisler and S. Kauffman, Leuk. Res. **23**, 685 (1999).

13. S. Y. Shvartsman, AICHE J. **51**, 1312 (2005).

14. B. Schoeberl, C. Eichler-Jonsson, E. D. Gilles1, and G. Müller, Nature Biotechn. **20**, 370 (2002).

15. C. Athale, Y. Mansury, and T. S. Deisboeck, J. Theor. Biol. **233**, 469 (2005).

16. J. E. Ferrell, Trends Biochem. Sci. **23**, 461 (1998); J. E. Ferrell and W. Xiong, Chaos **11**, 227 (2001).

17. U. S. Bhalla and R. Iyengar, Chaos **11**, 211 (2001); U. S. Bhalla, Progr. Biophys. Mol. Biol. **81**, 45 (2003).

18. J. Hasty, J. Pradines, M. Dolnik, and J. J. Collins, Proc. Nat. Acad. Sci. USA **97**, 2075 (2000); M. Thattai and A. van Oudemaarden, Proc. Nat. Acad. Sci. USA **98**, 8614 (2001); T. Shibata, Phys. Rev. E **67**, 061906 (2003); Q. Liu and Y. Jan, Phys. Rev. E. **70**, 041907 (2004); Y. Morishita, T. Kobayashi, and K. Aihara, J. Theor. Biol. **235**, 241 (2005); V. P. Zhdanov, Chem. Phys. Lett. **424**, 394 (2006).

19. L. Mariani, M. Löhning, A. Radbruch, and T. Höfer, Progr. Bipohys. Mol. Biol. **86**, 45 (2004).

20. J. Paulsson, Nature **427**, 415 (2004); S. Bornholdt, Science **310**, 449 (2005).

21. M. Kaern, T. C. Elston, W. J. Blake, and J. J. Collins, Nature Rev. Genet. **6**, 451 (2005).

22. J. Yu, J. Xiao, X. Ren, K. Lao, and X. S. Xie, Science **311**, 1600 (2006).

23. *Computational Modelling of Genetic and Biochemical Networks*, ed. by J. M. Bower and H. Bolouri, MIT Press, London (2001).

24. A. Arkin, J. Ross, and H. H. McAdams, Genetics **149**, 1633 (1998); A. de Raniery, A. S. Virdi, S. Kuroda, S. Shott, Y. Day, and D. R. Sunner, Bone **36**, 931 (2005); O. Kobiler, A. Rokney, N. Friedman, D. L. Court, J. Stavans, and A. B. Oppenheim, Proc. Nat. Acad. Sci. USA **102**, 4470 (2005).

25. H. de Jong, J. Comput. Biol. **9**, 67 (2002); M. P. Styczynski and G. Stephanopolos, Comp. Chem. Eng. **29**, 519 (2005); R. Guthke, U. Moller, M. Hoffmann, E. Thies, and S. Topfer, Bioinfor. **21**, 1626 (2005); K. Noto and M. Craven, Reg. Genom. Lect. Not. Comp. Sci. **3318**, 52 (2005); M. Lappe and L. Holm, Biochem. Soc. Trans. **33**, 530 (2005); Y. Kaznessis, Chem. Eng. Sci. **61**, 940 (2006).

26. A. Paldi, Cell. Mol. Life Sci. **60**, 1775 (2003); A. Kurakin, Dev. Gen. Evol. **215**, 46 (2005).

27. K. Birnbaum, D. E. Shasha, J. Y. Wang, J. W. Jung, G. M. Lambert, D. W. Gabbraith, and P. N. Benfey, Science **302**, 1956 (2003); M. Schmidt, T. S. Davison, S. R. Hertz, U. J. Pape, M. Demar, M. Vingron, B. Schölkopf, D. Weigel, and J. U. Lohmann, Nature Genet. **37**, 501 (2005); B. J. DeYoung, K. L. Bickle, K. J. Schrage, P. Muskett, K. Patel, and S. E. Clark, Plant. J. **45**, 1 (2006).

28. L. S. Campos, J. Neurosci. Res. **78**, 761 (2004).

29. R. E. Gross, M. F. Mehler, P. C Mabie, Z. Y. Zang, L. Santschi, and J. A. Kessler, Neuron **17**, 595 (1996); A. Bonni, Y. Sun, M. Nada-Vicens, A. Bhatt, D. A. Frank, I. Rozovsky, N. Stahl, G. D. Yancopoulos, and M. E. Greenberg, Science **278**, 477 (1997); P. Rajan and R. D. G. McKay, J. Neuroci. **18**, 3620 (1998); K. Nakashima, T. Takitawa, W. Ochiai, M. Yanagisawa, T. Hisatsune, M. Nakafuku, K. Miyazono, T. Kishimoto, R. Kageyama, and T. Taga, Proc. Nat. Acad. Sci. USA **98**, 5868 (2001); M.-Y. Chang, H. Son, Y.-S. Lee, and S.-H. Lee, Mol. Cell. Neuroci. **23**, 414 (2003).

30. U. Gurok, C. Steinhoff, B. Lipkovitz, H.-H. Ropers, C. Scharff, and U. A. Nuber, J. Neurosci. **24**, 5982 (2004).

31. T. Burdon, A. Smith, and P. Savatier, Trends Cell Biol. **12**, 432 (2002).

32. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*, Garland, New York (2002).

33. D. P. Landau and K. Binder, *A Guide to Monte Carlo Simulations in Statistical Physics*, Cambridge University Press, Cambridge (2000).

34. V. P. Zhdanov, unpublished results.

35. M. Pineda, R. Imbihl, L. Schimansky-Geier, and Ch. Zülicke, J. Chem. Phys. **124**, 044701 (2006).

36. O. Cinquin and J. Demongeot, J. Theor. Biol. **233**, 391 (2005).