Large-scale on-off switching of genetic activity mediated by the folding-unfolding transition in a giant DNA molecule: An hypothesis

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(Received 5 November 2007; published 6 March 2008)

We present a model to describe the on-off switching of transcriptional activity in a genetic assembly by considering the intrinsic characteristics of a giant genomic DNA molecule which can undergo a discrete structural transition between coiled and compact states. We propose a model in which the transition in the higher-order structure of DNA plays an essential role in regulating stable on-off switching and/or the oscillation of a large number of genes under the fluctuations in a living cell, where such a structural transition is caused by environmental factors. This model explains the rapid and broad transcriptional response in a genetic assembly as well as its robustness against fluctuations.

DOI: 10.1103/PhysRevE.77.031905 PACS number(s): 82.39.−k, 87.16.Yc

I. INTRODUCTION

The most widely accepted mechanism for the self-regulation of gene expression is currently a genetic network composed of a large number of specific interactions; i.e., key-lock interactions. In such a genetic network, a specific regulatory factor first binds to (dissociates from) a specific region on a DNA molecule, and then transcription starts. Proteins are produced following the production of mRNA molecules, and finally these proteins activate or inhibit their own or others' genes as specific regulatory factors. The occurrence of multistable states in such a network was suggested by Jacob and Monod in 1961 based on a couple of ordinary differential equations [1]. They hypothesized that the presence of multistability in the network is essential for the regulation of genetic activity, where genetic activity is regulated in biochemical networks by the interaction between regulatory factors and specific regions of base sequences on a DNA molecule. With this hypothesis, they claimed that genetic information is read out in the appropriate manner for the situation and the environment of the individual cells. This network concept and coupled nonlinear differential equations have been frequently used to explain the mechanism of rhythmic phenomena in biological systems. Recently, however, several reports have pointed out that there are serious problems with the network hypothesis [2,3]. They indicated that the abundance of gene expression based only on key-lock interactions is theoretically unstable under stochastic fluctuations, because a specific gene in one cell is usually presented as a single copy or at most several copies of a certain base sequence on a DNA molecule, and the number of keys, or regulatory factors, might be on the order of 10. In fact, the essential feature of the specific key-lock interactions in a living cell can never be interpreted in a precise manner with ordinary differential equations because the variables are integers of finite number, not continuous values. Nevertheless, in the case of the circadian clock in cyanobacteria, robust oscillation is observed even in a single cell [4]. Some reports have discussed possible solutions to these problems from the viewpoint of networks composed of specific interactions [5–9]. They evaluated the strength-to-noise ratios in systems or suggested an explanation for the regulation of systems. Currently, the regulation systems in these studies are always based on the interaction between a specific regulatory factor and a specific gene.

Recently, it was reported that a rapid and broad transcriptional response is observed, and that several hundreds or thousands of genes are up-regulated at the same time within a few minutes [10,11]. Other reports have shown that hundreds of genes oscillate cooperatively with a circadian clock [12,13]. The global genetic activities mentioned above are difficult to explain solely with the previous models for two reasons: it takes a long time to up-regulate so many genes, and many regulatory factors must work in cooperation at the same time to regulate many genes in the small intracellular space. Thus, a model that could make global regulation possible should be developed to solve these problems with previous models [2,3]. In this paper, we address the nonspecific interaction between a DNA molecule and environmental factors.

From a physical point of view, a DNA molecule that is longer than several micrometers [over 100 000 base pairs (bp)] shows the characteristics of a semiflexible polymer, which exhibits a discrete transition between coiled and compact states; i.e., a first-order phase transition for a single giant DNA molecule under the criterion of Landau's symmetry argument [Fig. 1(a)] [14]. In this transition, a specific key-lock interaction has a small effect and the transition is induced by "nonspecific" interaction between a DNA molecule and environmental factors within the cell, such as the concentrations of polyamine, mRNA, ATP, etc. [15,16]. Here we use the terms "specific" and "nonspecific" as follows: the switching induced by a few molecules is referred to as switching induced by specific interaction and that induced by a concentration of certain chemical(s) is referred to as nonspecific. Currently, such a discrete transition has not been

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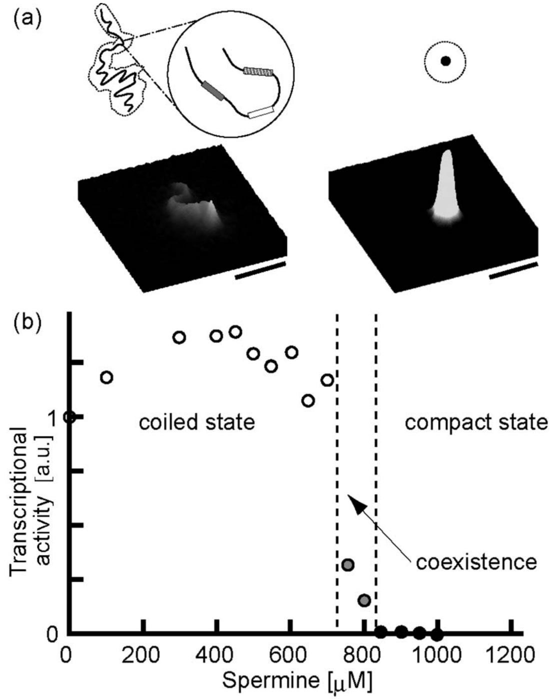

FIG. 1. (a) Structural transition of a giant DNA chain. Brightness distributions of fluorescence microscopy images and schematic representations of a DNA molecule (T4, 166 kbp) in a coiled (left) and a compact (right) state are shown. Due to the blurring effect, the bright molecules appear to be much larger than their actual sizes. Based on the measurement of the Brownian motion of individual DNA molecules, it has been confirmed that the volume ratio between the coiled and compact states is on the order of 10^4-10^5 [14]. Scale bars are 5 μm. (b) Change in transcriptional activity depending on the structural transition of DNA (BAC, 106 kbp), modified based on the report by Luckel *et al.* [17]. The transcriptional activities are on and off in the coiled and compact states, respectively.

observed in molecular biology, where giant DNA molecules are fractionated into several segments of a few kbp.

Recent studies have also revealed a relationship between the structural transition and the transcriptional activity of a DNA molecule. Based on observations of single DNA molecules as well as of a molecular ensemble, the transcriptional activity of a giant DNA molecule is switched on or off through the structural transition; transcriptional activities are activated or inhibited when a DNA molecule is in a coiled or compact state, as shown in Fig. 1(b) [17–19].

In the present paper, to support a model which is based on the inherent properties of a DNA molecule, we consider the higher-order structural transition as the essence of on-off switching of gene activity. Our model can solve the two above-mentioned problems inherent in previous models.

II. MODEL

We consider the global structural change in a giant naked DNA molecule for simplicity, as follows. When a DNA molecule is in a coiled state, all genes are activated at the same time. Over time, the number of environmental factors around the DNA molecule increases and the DNA molecule is driven to become compact. When a giant DNA molecule is in a compact state, products cannot be generated, the environment returns to its original condition, and finally the DNA molecule becomes coiled again.

To obtain a mathematical expression for the mechanism of the global structural change in a giant DNA molecule, we focused on the change in the free energy profile for a DNA molecule together with the surrounding environment. With the parameter α, which corresponds to R/R_0 (the molecular chain size is R, the Gaussian chain size is $R_0 \sim l_p N_k^{1/2}$, and l_p is the persistence length), the free energy per single DNA molecule is usually written as

$$F[\alpha] = F_{\text{DNA}} + F_{\text{env}}, \tag{1}$$

where

$$F_{\text{DNA}} = F_{\text{ela}} + F_{\text{int}} \sim \frac{3}{2}(\alpha^2 + \alpha^{-2}) + C^* \alpha^{-6} - N_k \ln\left(1 - \frac{\lambda}{N_k^{1/2}\alpha^3}\right) \tag{2}$$

for a molecule with N_k Kuhn segments [20]. F_{DNA} and F_{env} are the contributions directly from one DNA molecule and from the environmental effect per a single DNA molecule. F_{DNA} consists of F_{ela} and F_{int} terms, the elastic energy and the interaction energy between Kuhn segments, respectively. Equation (2) is derived by considering the local parallel ordering between segments in a semiflexible chain. C^* is the renormalized virial derived from the binary interaction between segments, and λ is the aspect ratio of the Kuhn segment; $\lambda = a/l_p$, where a is the width of a chain. Reflecting the semiflexible character, the profile of F_{DNA} has two local minimum values with a relatively high barrier, which correspond to the two different states: the coiled and the compact states. In the structural transition, the depths of the two minima change following a change in F_{env}.

In our model, we assume that the concentration of the products c is an essential part of the surrounding environment; i.e., $c = N/V$, where V is the volume of a nucleus, $N = \Sigma_k n_k$ is the total number of products, and n_k is the number of the kth product molecules. We assume that F_{env} becomes an increasing function of α when c is large, and vice versa. For simplicity, we set a new order parameter η as the normalized density, by considering that α^3 is proportional to the effective volume of a molecule:

$$\eta \sim \frac{(1/\alpha^3) - (1/\alpha_{\text{coil}}^3)}{(1/\alpha_{\text{compact}}^3) - (1/\alpha_{\text{coil}}^3)}, \tag{3}$$

where $\eta = 0$ and 1 correspond to the coiled and compact states, respectively. For the numerical simulation, by keeping the physical meaning of the transition kinetics in the double-minimum profile as shown in Fig. 2, we adopt Kramers' escape rates. Reflecting the change in F_{env}, it is assumed that the free-energy barrier from $\eta = 0$ to 1 is $\Delta F - \gamma(c - c_0)$ and that from $\eta = 1$ to 0 is $\Delta F + \gamma(c - c_0)$, where γ and c_0 are positive constants.

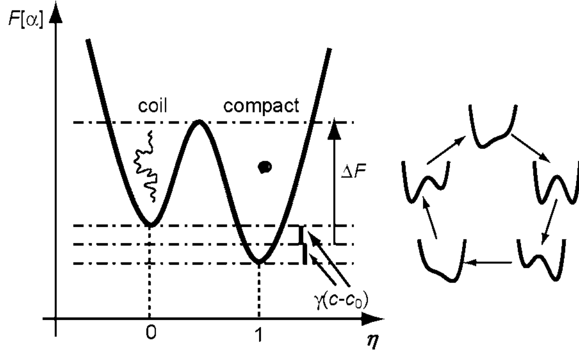

FIG. 2. Free-energy profile of a DNA molecule. The profile has two minima; $\eta=0$ and 1, which correspond to the coiled and compact states. The energy barriers of the transition from a coiled to a compact state and from a compact to a coiled state are $\Delta F-\gamma(c-c_0)$ and $\Delta F+\gamma(c-c_0)$, respectively. The right figure schematically shows the time change in potential profiles.

As shown in Fig. 2, the compact state becomes more stable with an increase in c. Thus, the transition probabilities of the conformational changes in a DNA molecule $P(\eta=0\rightarrow\eta=0)$, $P(\eta=0\rightarrow\eta=1)$, $P(\eta=1\rightarrow\eta=0)$, and $P(\eta=1\rightarrow\eta=1)$ are expressed as follows:

$$P(\eta=0\rightarrow\eta=1)=1-P(\eta=0\rightarrow\eta=0)=K\exp\left(-\frac{\Delta F-\gamma(c-c_0)}{k_BT}\right), \quad (4)$$

$$P(\eta=1\rightarrow\eta=0)=1-P(\eta=1\rightarrow\eta=1)=K\exp\left(-\frac{\Delta F+\gamma(c-c_0)}{k_BT}\right). \quad (5)$$

The transition probabilities of n_k are also represented with stochastic equations. When $\eta=0$ (coiled state), the transition probabilities $P(n_k\rightarrow n_k+1)$, $P(n_k\rightarrow n_k)$, and $P(n_k\rightarrow n_k-1)$ are introduced as follows:

$$P(n_k\rightarrow n_k+1)=a_k, \quad (6)$$

$$P(n_k\rightarrow n_k)=1-a_k-b_kn_k, \quad (7)$$

$$P(n_k\rightarrow n_k-1)=b_kn_k. \quad (8)$$

When $\eta=1$ (compact state), the transition probabilities $P(n_k\rightarrow n_k)$ and $P(n_k\rightarrow n_k-1)$ are introduced as follows:

$$P(n_k\rightarrow n_k)=1-b_kn_k, \quad (9)$$

$$P(n_k\rightarrow n_k-1)=b_kn_k. \quad (10)$$

The transition probability from n_k to n_k+1 equals zero in this case. Here we assume that the number of genes contained in the region is M and that the production and decay rates of the kth product from the kth gene are a_k and b_k $(1\leq k\leq M)$, respectively. The schematic representation of this system is shown in Fig. 3.

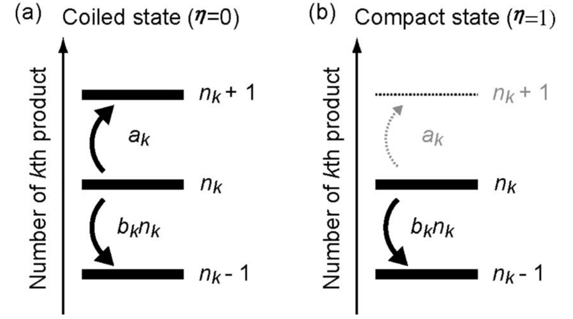

FIG. 3. Schematic representations of the transition probabilities of n_k. (a) Transition probabilities when DNA is in a coiled state. Each transition probability of the number of kth product molecules is given by Eqs. (6)–(8), respectively. (b) Transition probabilities when DNA is in a compact state. Each probability of the number of kth product molecules is given by Eqs. (9) and (10), respectively. It is noted that the probability of increase in the numbers of kth product (shown with dotted lines) is zero because the transcriptional activity of a compacted DNA molecule is completely suppressed.

III. RESULTS AND DISCUSSION

In this system, the changes in time in the number of kth products, n_k, and in the DNA molecular conformation, η, can be numerically obtained as shown in Fig. 4. In the numerical calculation, we set $M=100$ with different production and decay rates. The rates follow uniform distributions and range from A_1 to A_2 and B_1 to B_2, respectively.

Figure 4 demonstrates that the time change in the structure of a DNA molecule η and that in the surrounding environment c oscillate robustly even though the numbers of the kth product, n_k, fluctuate independently. Thus, although the number of molecules of transcriptional products from one gene is small enough to be affected by the fluctuation, the structural change in a DNA molecule can be robust. The model shows that robustness is achieved when the structural change is induced by the surrounding environment which is composed of many kinds of genetic products, such as mRNA and so on.

The entire DNA molecule is assumed to achieve a structural transition in the above numerical simulation. In an actual cell, however, such an entire structural transition could not occur. For example in a nucleus, a genome DNA (over several hundreds of micrometers or over several tens of Mbp) usually shows intrachain-segregated structures. The manner of segregation seems to depend on the degree of methylation, base specificities along the chain, and so on. In fact, various kinds of segregated structures produced by the change in environmental factors are observed with *in vitro* experiments [21]. Selective regulation would be possible due to the segregated structure in a living cell.

From a biological point of view, many experimental results suggest links between the structural transition and the transcriptional activity of DNA. One example is the segregated structure of chromatin: euchromatin and heterochromatin. Only the domains in euchromatin are actively transcribed, whereas the domains in heterochromatin are "gene-poor" or show transcriptional silencing [22,23]. It has also been reported that euchromatin is replicated earlier than het-

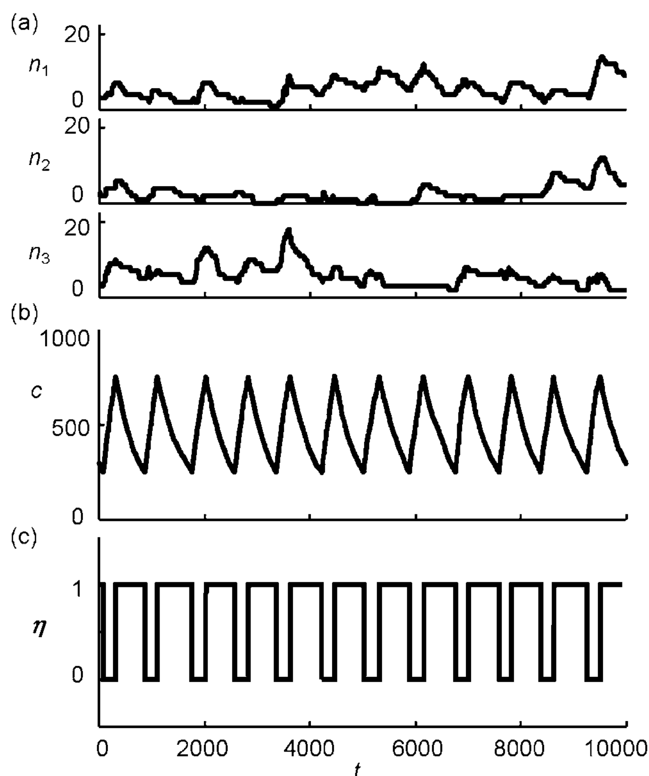

FIG. 4. Results of the numerical simulation based on Eqs. (4)–(10) with the initial conditions $\eta=1$ and $n_k=3$ for all k. The values of the parameters are as follows: $A_1=0.01$, $A_2=0.05$, $B_1=0.001$, $B_2=0.003$, $K=1$, $V=1$, $\Delta F/k_BT=25\,000$, $\gamma/k_BT=100$, and $c_0=500$. (a) Change in each n_k over time. We show representative profiles for $k=1$–3. (b) Change in the nonspecific parameter c over time. (c) Change in the order parameter η over time, which represents the time change in the structure of a DNA molecule; $\eta=0$ and 1 correspond to the coiled and compact states, respectively.

erochromatin in cell division [24], and this means that the change in environmental factors is closely related to the transcriptional activity of DNA. Another example is the stable oscillation of the circadian clock of a cyanobacteria [4]. Some experimental results have suggested that the rhythmic changes in the states of the chromosome might be related to genome-wide gene expression [25–28]. Indeed, Smith *et al.* observed the oscillation of DNA structure in a cyanobacterium following its circadian rhythm *in vivo* [28]. Shindo *et al.* also showed the links between the structural transition and the transcriptional activity of DNA by an *in vivo* experiment. They showed that, in the case of *Escherichia coli*, DNA molecules are packed loosely in the logarithmic growth phase, whereas DNA molecules exhibit a few compact regions together with elongated coiled parts in the stationary phase [29]. Therefore, we must consider the structural transition of a DNA molecule when we consider the regulation of genetic activity.

IV. CONCLUSION

In this paper, we have presented a model for the on-off switching of genetic activity by considering the characteristics of a genomic DNA molecule as a semiflexible polymer. Although this model gives only qualitative suggestions, it indicates a different possibility for regulating the genetic activity. By this model, the robustness against a noisy environment can be determined and the rapid and broad transcriptional response can be well explained. The robust and detailed regulation of a genetic network may be explained by a combination of our model, which includes the structural transition of a genomic DNA molecule, and the classical model, which includes a large number of specific interactions.

ACKNOWLEDGMENTS

This research was partly funded by the Japan Science Society through a Sasagawa Scientific Research Grant to Y.T., and by the Ministry of Education, Culture, Sports, Science and Technology of Japan through Grants-in-Aid for Scientific Research (Grant Nos. 17076007 and 18GS0421) to K.Y. and through a Grant-in-Aid for Young Scientists (B) (Grant No. 18740231) to H. K.

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