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Self-regulation of Gene Activity with Structural Transition of a Giant DNA

By

Yoshiko Takenaka*

Abstract

In this study we examined the correlations between the microscopic properties and the macroscopic order in a DNA molecule. We particularly focus on the correlation between transcriptional activity and the higher-order structure, which is a typical characteristic of a large genome DNA. We present a new model to regulate the gene network which can solve two big problems inherent to previous models.

§ 1. Introduction

Many hierarchical classes are seen in a single DNA molecule: the solid-state property, one-dimensional base sequence, secondary structure, nucleosome structure, higher-order chromatin structure, the regulation of transcription and the gene network. While each class has been studied independently, it is thought that interactions between different classes should be studied to clarify "what is life?". Some previous studies show the interaction between different classes both experimentally and theoretically [1] - [5]. However, there have been few theoretical studies on the correlation between the structure and function of DNA from a physical perspective, although many biological experiments have indicated that they are indeed related [6] - [15]. Arney et al. and Kosak et al. reported that only the domains in euchromatin are actively transcribed genes whereas the domains in heterochromatin are "gene poor" or show transcriptional silencing. Mori et al., Xu et al. and Nakahira et al. suggested based on experimental findings that there is a relation between the cyanobacterial circadian clock and the DNA structure and Smith et al. observed their association in vivo. Akitaya et al. and Tsumoto et al. reported that

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*Department of Physics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan.

the transcriptional activity of DNA in vitro, when observed in the ensemble, switches through a structural transition due to a change in the environment. Moreover, Yamada et al. clarified the correspondence between transcriptional activity and the higher-order structure even at the level of a single DNA molecule. A change in the transcriptional activity of various kinds of DNA (short or long and linear or circular DNA) was also observed [15].

From a physical point of view, genome DNA is a polymer as well as a gene, and indeed can be classified as a semiflexible polymer ($D \ll \lambda \ll L$), with a chain thickness D of 2 nm, a persistence length λ of 50 nm and a contour length L of μm to mm. The persistence length of a common polymer (flexible polymer) approximately corresponds to the thickness of a chain. In the case of genome DNA, however, two poly-nucleotide chains organize into a double-helix structure with complementary hydrogen bonds between four kinds of bases: adenine (A), thymine (T), cytosine (C) and guanine (G). The persistence length of a genome DNA is much longer than its chain thickness because of these hydrogen bonds. Therefore, a semiflexible polymer shows interesting properties.

One of the interesting characteristics of a semiflexible polymer is the discrete transition between an elongated coil and a folded compact states ([16], [17]) with a change in the environment in solution (Fig. 1).

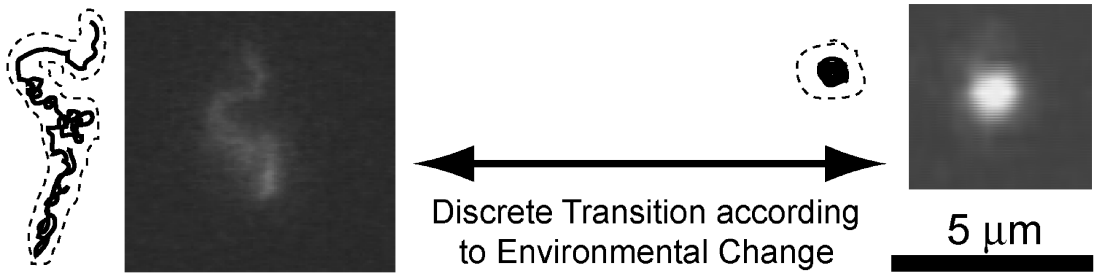

Figure 1. Images of a single DNA molecule observed by fluorescence microscopy (FM). The images are larger than the actual size because of the blurring of light. Schematic drawings of the effects of blurring are shown at the left of each image. The solid lines indicate polymer chains and the dotted lines correspond to the FM images.

Figure 1 shows the discrete structural transition of a semiflexible polymer: a double-helix DNA chain. As shown in Fig. 1, the radius of gyration of a semiflexible polymer changes drastically with a gradual change in the environment. Many kinds of condensing agents induce DNA compaction, including multivalent cations (polyamines), ATP and mRNA [18] - [20]. In addition to condensing agents, change in the pH or temperature can induce DNA compaction [21], [22]. In fact, the folding transition of a giant DNA is induced by a large number of nonspecific interactions, i.e., the transition is controlled

by the overall surroundings. Such a discrete transition is characteristic of a semiflexible polymer and occurs when the contour length of a DNA molecule is longer than at least 10 μm, which corresponds to several tens kbp and which should include more than several tens of genes. Therefore, this property of giant DNA could not be observed in molecular biology, where a giant DNA is fractionated into segments of a few kbp each.

DNA has a one-dimensional base sequence and some parts of this sequence correspond to functional proteins. How is the expression of different proteins in different organs controlled when all of the cells in an organism contain the same DNA? To explain this curious phenomenon, Jacob and Monod proposed the operon theory in 1961 [23]. They predicted the existence of a specific region (S-region) to control the transcription of a specific gene (S-gene). They proposed a model in which a specific control protein (S-protein) interacts with the S-region to switch on/off its transcriptional activity. With recent dramatic developments in molecular biology, the existence of the specific region and specific proteins has been confirmed and the most commonly accepted mechanism of transcriptional activity is now based on the operon theory (Fig. 2).

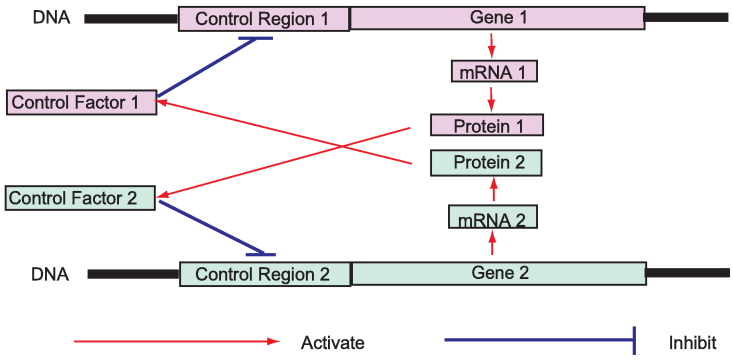

Figure 2. Schematic representation of the operon theory. The transcription of S1 gene starts when an S1 control factor binds to its specific control region. S1 mRNA and S1 proteins are produced and the S1 proteins inhibit the gene activity of S2 gene. The same applies to the S2 gene. Jacob and Monod indicated that two stable points exist when control factors bind to specific regions as dimers and that these stable points could correspond to the two stable states of a cell.

Recently, however, many papers have pointed out problems with operon theory: its weakness toward fluctuations in the number of molecules [24], [25] and the difficulty of explaining the rapid and broad transcriptional response [26] - [29]. While several reports have offered suggestions to overcome these problems [30] - [32] they do not address the nature of DNA as a semiflexible polymer.

In this paper, we focus on the correlation between transcriptional activity and the higher-order structure of DNA to present a new model for the self-regulation of

transcription. First, we present a novel model and then we examine whether the model can solve the two problems noted above. The results of this study may help us to clarify one aspect of the correlation between the "microscopic" properties and the spatio-temporal "macroscopic" order of a DNA molecule.

§ 2. Model

To express the regulation system mathematically, we consider the discrete structural transition of DNA from the viewpoint of its free energy. Due to the discrete nature of the transition and its finite system size, its free energy is given by a double-minimum profile [33].

To describe the bimodality in the structural transition of a giant DNA, it can be expressed as a quartic form with respect to an order parameter (Fig. 3), which corresponds to the DNA state [34]. In our model we set;

$$\eta = \frac{\rho - \rho_{\mathrm{coil}}}{\rho_{\mathrm{comp}} - \rho_{\mathrm{coil}}} \tag{2.1}$$

Thus, DNA is in the coiled state when $\eta = 0$ and in the compact state when $\eta = 1$. For a change in the DNA state due to an environmental factor ζ such as pH or the concentration of multivalent cations, ATP and mRNA, a cross-term should be added to the quartic form. The free energy of a giant DNA F can therefore be written as

$$F = \frac{k_1}{4}\eta^2(1-\eta)^2 - k_2(\zeta - \zeta_0)\eta, \tag{2.2}$$

where ζ_0 is the threshold of the environmental factor and k_1 and k_2 are positive constants that determine the shape of the free-energy profile. Since we consider a non-conservative system with respect to η, for simplicity the change in η is assumed to be proportional to the gradient of free energy with time constant ϵ:

$$\epsilon\frac{\mathrm{d}\eta}{\mathrm{dt}} = -\frac{\mathrm{dF}}{\mathrm{d}\eta} \tag{2.3}$$

In Fig. 4, we show a schematic image of the control system based on our model.

When DNA is in the coiled state, all of the genes are activated at the same time and many products are generated following the production of an individual mRNA. Hence environmental factors such as pH and the concentrations of cations or ATP around the DNA, change and enhance the compaction of DNA. With compaction, all of the products cannot be produced, the environment returns to the original state and finally DNA becomes the coiled state again. Here, let R and P be the sums of the numbers of individual mRNA and proteins, respectively (i shows the ith gene and n is the total number of genes ($1 \leq i \leq n$).). We introduce the environmental factor ζ as the sum of

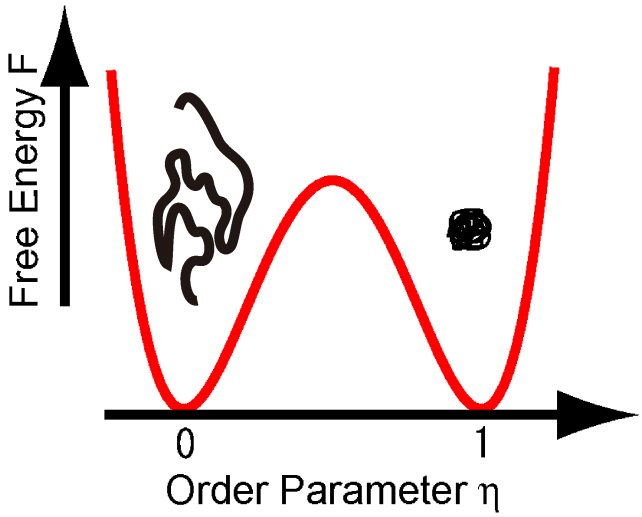

Figure 3. Free-energy profile of a DNA molecule with a size of over several tens of kbp. A simple bimodal profile is adopted to describe the intrinsic nature of the discrete structural transition in a giant DNA molecule. The order parameter η corresponds to the segment density of DNA: $\eta = 0$ and $\eta = 1$ indicate the coiled and compact states, respectively.

R and P for simplicity. The changes in each variable η, R and P obey the equations (2.4), (2.5), (2.6), (2.7):

$$\epsilon \frac{\mathrm{d}\eta}{\mathrm{dt}} = -\frac{\mathrm{dF}}{\mathrm{d}\eta} = -k_1\eta(\eta-1)(\eta-\frac{1}{2}) + k_2(\zeta-\zeta_0) \tag{2.4}$$

$$\frac{\mathrm{dR}}{\mathrm{dt}} = k_3(1-\eta) - k_4 R - k_R\zeta \tag{2.5}$$

$$\frac{\mathrm{dP}}{\mathrm{dt}} = k_5 R - k_6 P - k_P\zeta \tag{2.6}$$

$$\zeta = \sum_i^n r_i + \sum_i^n p_i = R + P, \tag{2.7}$$

where k_j $(1 \leq j \leq 6)$, k_R and k_P are positive constants. The constants k_R and k_P indicate the effects of the environmental factor on transcription and translation, respectively.

§ 3. Numerical Results

In our model, the temporal changes in the conformation of a giant DNA η and in the amount of environmental factors ζ can be numerically obtained as shown in Figs. 5 and 6.

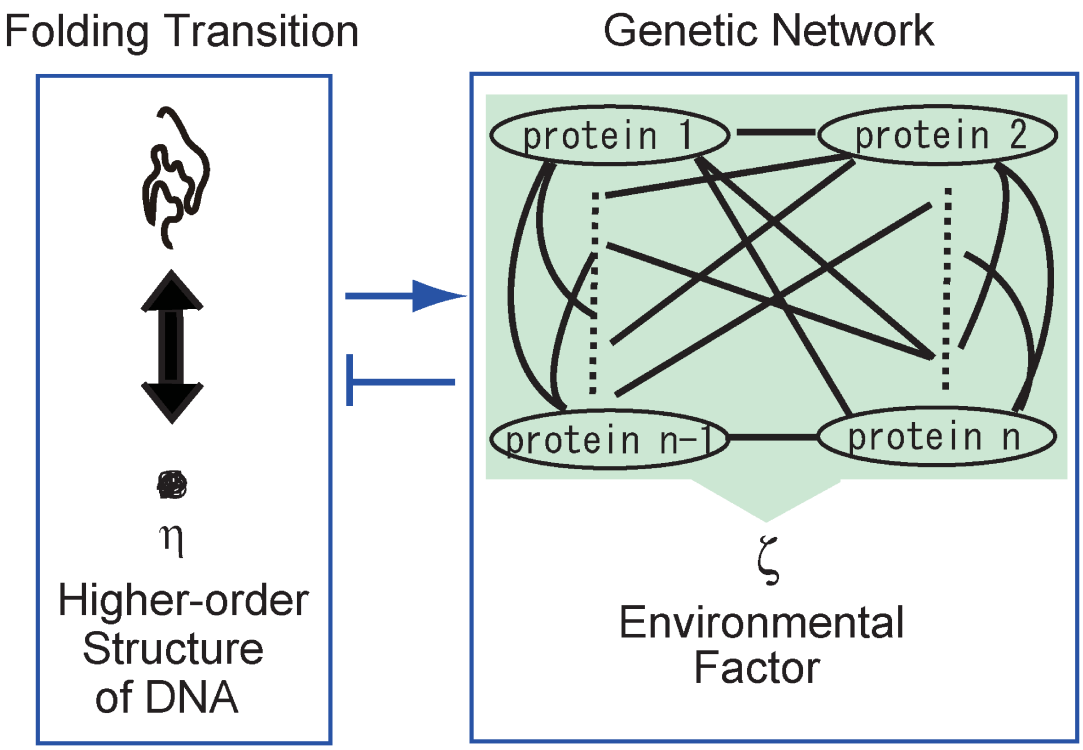

Figure 4. Interaction between DNA conformation and an environmental factor. When a DNA molecule is in the coiled state, its transcriptional activity is "on" and proteins transcribed from mRNA suppress the transcriptional activity of DNA by changing the free-energy profile of DNA; the DNA molecule is folded through the environmental factor (ζ). The transcriptional activity of DNA then turns off and neither mRNA nor proteins are produced, until the DNA molecule becomes unfolded again. This is the mechanism of self-regulation which includes the folding transition of DNA.

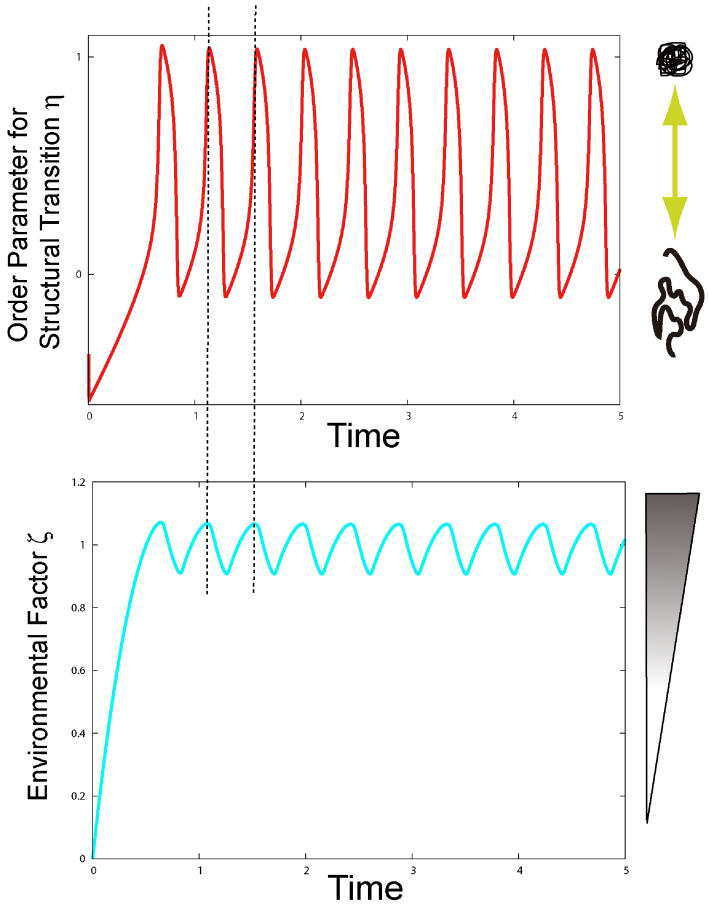

Figure 5. Results of the simulation with the system given in equations (2.4) - (2.7) with an initial condition of $(\eta, \zeta) = (0, 0)$. The values of the parameters are as follows: $k_1 = k_2 = k_3 = k_4 = k_5 = 2$, $k_6 = k_R = k_P = 0.5$, $\zeta_0 = 1.0$ and $\epsilon = 0.01$. Oscillations of the variables η (upper) and ζ (lower) are shown. The conformation of a DNA molecule η changes quickly between the coiled and compact states, reflecting a discrete transition. Environmental factor ζ oscillates following the DNA state η.

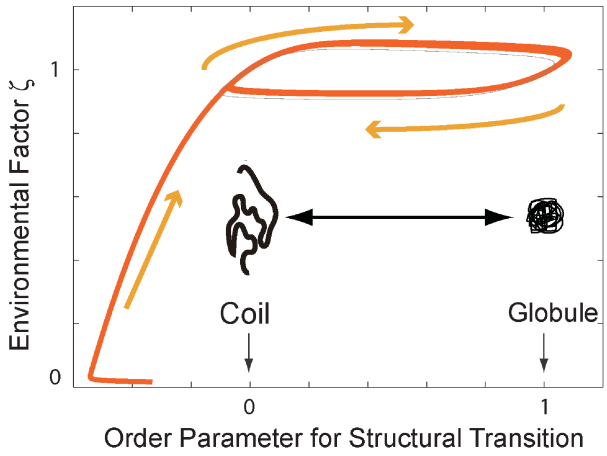

Figure 6. Dynamics in phase space (η - ζ plane) of the result shown in Fig. 5. The limit-cycle orbit is shown, with flow drawn with arrows.

The conformation of DNA shows a quick transition reflecting the small ϵ. The change in the environmental factor oscillates following that in the DNA conformation. Figure 7 shows the bifurcation structure of this system with a change in the threshold of environmental factor ζ_0, which would be controlled in an experiment. This system changes its state with an increase in ζ_0 from 0 to 1.

§ 4. Discussion

We now show that our model can solve the two problems inherent to the previous models: weakness toward the fluctuation of the number of molecules and the difficulty of explaining the rapid and broad transcriptional response. First, we demonstrate the robustness of this model against fluctuation in the number of molecules.

In our model, we can evaluate the robustness with a "law of averages" as follows. We assume that the numbers of individual mRNA r_i and product p_i fluctuate as follows;

$$r_i(t) = \langle r_i(t) \rangle + \xi_{ri}(t), \tag{4.1}$$

$$p_i(t) = \langle p_i(t) \rangle + \xi_{pi}(t), \tag{4.2}$$

$$\langle \xi_{ri}(t)\xi_{pi}(s) \rangle = 2D\delta_{ri,pi}\delta(t-s), \tag{4.3}$$

where D is the strength of the fluctuation. The standard deviation of the relative environmental factor is proportional to the inverse of the square root of the system size n;

$$\frac{\sqrt{\langle (\zeta - \langle \zeta \rangle)^2 \rangle}}{\langle \zeta \rangle} = \frac{1}{\sqrt{n}}, \tag{4.4}$$

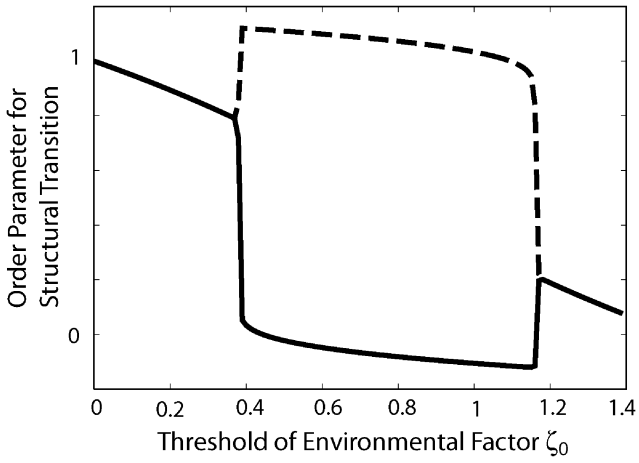

Figure 7. Bifurcation diagram of the system with equations (2.4) - (2.7) with a change in the threshold of environmental factor ζ_0. The vertical axis shows the maximum and minimum amplitudes of the oscillation generated by this system. For stable oscillation in this system, there is a proper region of the threshold for the environmental factor ζ_0.

where we assume an independent identical distribution for noise. Equation (4.4) means that as the system size increases, the effect of fluctuation decreases. In our model, we consider the global transition of a DNA, and n is on the order of 10^2; therefore, robustness against a noisy environment is guaranteed.

Next, we demonstrate that our model can explain the rapid and broad transcriptional response.

A DNA molecule undergoes a discrete structural transition when it is larger than 100 kbp, which usually includes more than 100 genes. Therefore, the global gene expression typified by the rapid and broad transcriptional response can be reproduced. In the cell, however, the proper genes should be activated at the proper moment and the selective activation of genes is necessary.

For the selective activation of genes, we suggest that the segregated structure (Fig. 9) could make it possible. In the section 3, although we considered a DNA molecule with one domain, a genome DNA molecule has a lot of domains. Here we assume for simplicity that there is no interaction between different domains; all domains can perform the structural transition independently. A segregated structure appears because of the variation in the local rigidity of a DNA molecule. This variation can result from the base composition, methylation and binding with proteins. For example, it has been reported that some domains can be folded more easily than others because of a transition in the secondary structure [35]. In fact, in vitro experiments have revealed various kinds of segregated structures [36], [37], [38].

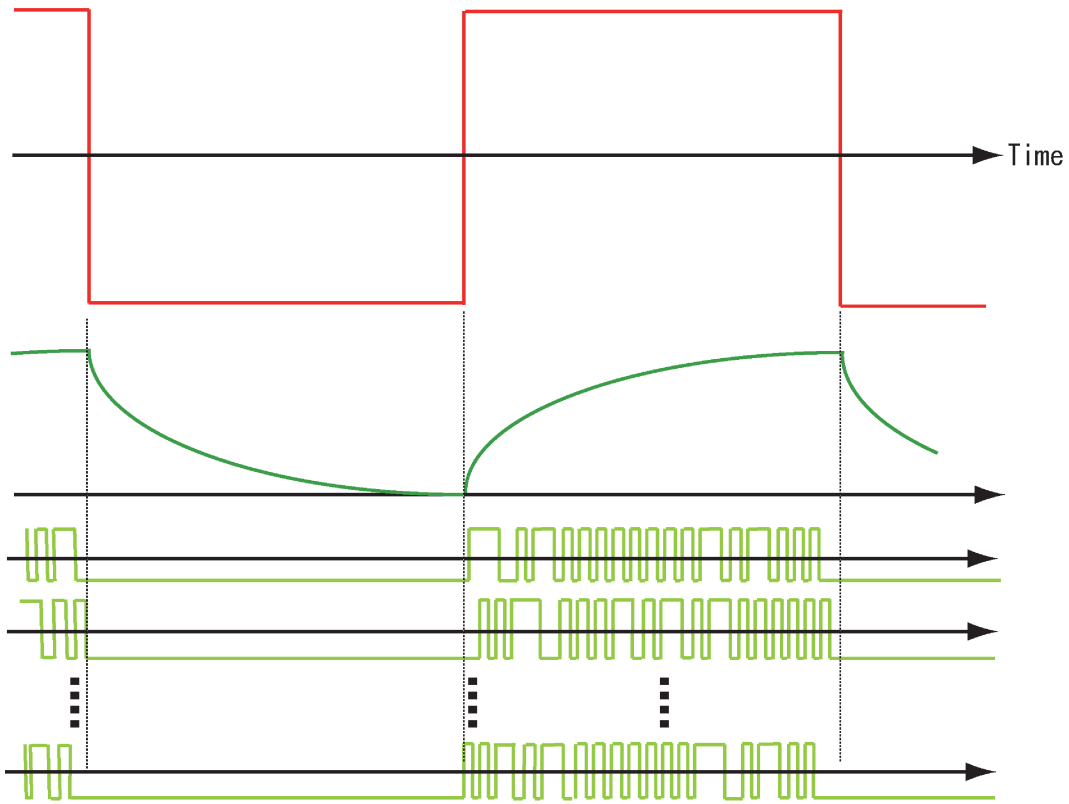

Figure 8. Schematic representation of the robustness against fluctuation in the number of molecules. In our model, the time-scale of the structural transition of a DNA molecule is around that of the cell cycle or the circadian cycle, i.e., it is sufficiently longer than that of the absorption/desorption of specific factors such as proteins. Therefore although the expression of each mRNA and protein is stochastic, the change in an environmental factor would be continuous. As a result, the structural transition which depends on the environmental change becomes robust and the on/off switching of gene activity which depends on the structural transition of DNA also becomes robust.

Figure 10 shows that different structures can arise from different polymers even under the same environmental conditions. We consider three different kinds of polymer: a G/C-rich polymer, an A/T-rich polymer and a di-block co-polymer with G/C-rich and A/T-rich domains. The G/C-rich polymer collapses more easily [35]. We calculated the total free energy of these polymers [33]. The results suggest that the selective activation of the proper genes is possible if the local rigidity of a DNA molecule changes with time.

Figure 9. Schematic image of a segregated structure of DNA. In a single molecule, there are two different domains: one of which is in the coiled state and the other is in the compact state.

§ 5. Conclusion

We have presented a novel model for the on/off switching of transcriptional activity by considering the characteristics of genomic DNA as a semiflexible polymer: discrete transition with a change in the environment. We have shown that this model is robust toward fluctuations in the number of molecules and can well explain the rapid and broad transcriptional response. However, the local and detailed control of gene expression is difficult to explain using only our model. Thus, with regard to the hierarchical hypothesis, we would like to point out that the robust and detailed regulation of a genetic network may be possible through a combination of our model which includes the conformational transition of genomic DNA and the classical model which include a large number of specific interactions.

Acknowledgment

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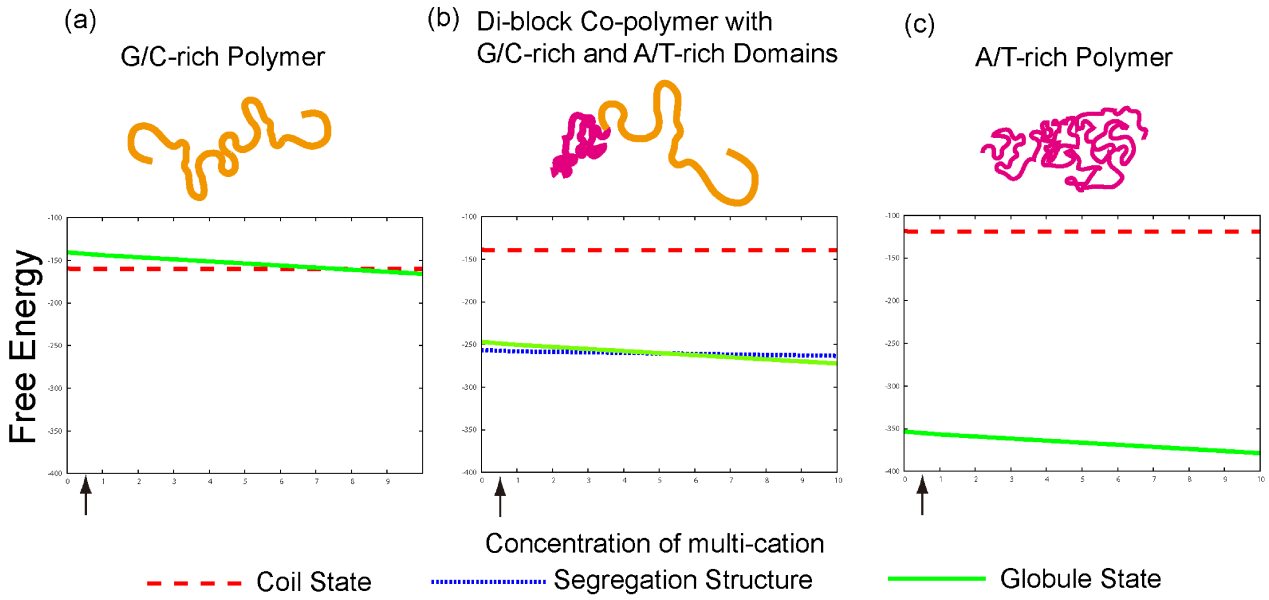

Figure 10. Numerical results regarding the most stable state under the same environmental factor in different polymers. In the graph, green solid and blue and red dashed lines show a compact, segregation and the coiled states, respectively. The images above the graph show examples of polymers; blue dotted parts represent G/C-rich (stiff) parts and red solid parts represent A/T-rich (flexible) parts. At the same concentration of multi-cation shown by the arrows, the three polymers show three different structures. (a) For the G/C-rich polymer, the coiled state appears at the indicated concentration. (b) For the Di-block co-polymer with G/C-rich and A/T-rich domains, a segregated structure, where the G/C-rich part is in the coiled state and A/T-rich part is in the compact state, appears. (c) For the A/T-rich polymer, the compact state appears.

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