Reconciling the concurrent fast and slow cycling of proteins on gene promoters

Yaolai Wang, Feng Liu, Jun Li and Wei Wang

National Laboratory of Solid State Microstructures and Department of Physics, Nanjing University, Nanjing 210093, People's Republic of China

During gene transcription, proteins appear to cycle on and off some gene promoters with both long (tens of minutes) and short periods (no more than several minutes). The essence of these phenomena still remains unclear. Here, we propose a stochastic model for the state evolution of promoters in terms of DNA–protein interactions. The model associates the characteristics of microscopic molecular interactions with macroscopic measurable quantities. Through theoretical derivation, we reconcile the contradictory viewpoints on the concurrent fast and slow cycling; both the cycling phenomena are further reproduced by fitting simulation results to the experimental data on the \(pS2\) gene. Our results suggest that the fast cycling dictates how the proteins behave on the promoter and that stable binding hardly occurs. Different kinds of proteins rapidly bind/unbind the promoter at distinct transcriptional stages fulfilling specific functions; this feature is essentially manifested as the slow cycling of proteins when detected by chromatin immunoprecipitation assays. Thus, the slow cycling represents neither stable binding of proteins nor external modulation of the fast cycling. This work also reveals the relationship between the essence and measurement of transcriptional dynamics.

1. Introduction

Gene transcription is dynamically orchestrated by molecular interactions [1–6]. On a gene promoter, numerous proteins (including various chromatin-modifying enzymes and components of the transcription apparatus) intermittently associate to and dissociate from their binding sites, altering chromatin structure and inducing transcriptional initiation [1–4,7,8]. It seems that the individual binding time is no more than several minutes and the binding/unbinding of proteins occurs frequently. Such phenomena called ‘fast cycling’ were widely observed [8–16]. Interestingly, ‘slow cycling’ of proteins on promoters was also reported [15–21]: in a large cell population, the percentage of promoters bound by a specific kind of protein oscillates with a period of tens of minutes. The fast and slow cycling coexist on some promoters, typically on the \(pS2\) and \(CUP1\) genes [15–17]. The essence of these phenomena still remains elusive [15–17,22–24].

The fast cycling was inferred by using fluorescence recovery after photobleaching (FRAP) technology [8–15,25,26], which measured the exchange rate of promoter-bound proteins with the free ones. After the fluorescence-tagged proteins in the promoter region were photobleached to be non-fluorescent, the fluorescence intensity in that region restored within several minutes (figure 1a). This was interpreted as follows: the bleached proteins were substituted with the fluorescent ones, and the exchange was rapid and the binding time was rather short [8–16]. It was argued that such fast cycling mainly originated from frequent and purely unproductive transient associations, which were similar to molecular collisions and had no functional roles; by contrast, the functional binding that should rarely occur and be much more stable could not be differentiated by FRAP [16]. But this viewpoint was challenged by the claim that the fast cycling could be functional [15].

The slow cycling was revealed by a biochemical method called chromatin immunoprecipitation (ChIP) assays. A population of cells was first immersed in formaldehyde solution for some time (e.g. 5 min), so that the
protein–DNA complexes were fixed. With the use of antibodies, those promoters bound by a specific kind of protein were purified and counted (figure 1b). ChIP assays were performed per time interval (e.g. 5 min) [17]; simultaneously, the percentages of promoters bound by various kinds of proteins were recorded. The slow cycling revealed by ChIP assays was believed to reflect the productive associations that should rarely happen but persist for a long time once they occur [16]. The periodic repeat of a sequence of productive interactions was assumed to underlie the productive reaction to occur next is stochastic, and thus the next state is not unique. Therefore, the state evolution of promoters in a cell and variability in the routes of state evolution. It allows for analytic derivation of transcriptional dynamics and association of experimental observations with the properties of molecular interactions. Application of this model to the pS2 promoter shows that the promoter must be subject to very frequent molecular interactions and the slow cycling represents an integration effect. Thus, both the fast and slow cycling are interpreted within a unifying framework.

2. Model and a limiting condition

2.1. A stochastic model for the state evolution of a promoter

The state of a promoter is determined by how the promoter DNA is chemically modified and how the associated proteins are structurally assembled and chemically modified. During transcriptional processing, the promoter’s state evolves periodically (figure 2a), repeating stages from DNA demethylation, histone modification and nucleosome eviction, assembly of the transcription machinery, transcription initiation, destruction of the transcription machinery, to nucleosome recruitment, recovery of histone state and DNA methylation [17,28]. Every stage contains multiple processes, and each process involves many irreversible reactions [27,29–31]. For example, the demethylation of DNA traverses oxidation of cytosine, base excision and repair, with each process engaging definite irreversible reactions [29]. It is these irreversible reactions that drive transcriptional progression. After each irreversible reaction, the promoter irreversibly steps into the next state, i.e. the promoter remains in the same state between two successive irreversible reactions. Because the promoter–protein complex has many sites (such as the sites on histone tails to be chemically modified) for different proteins to interact with, which irreversible reaction to occur next is stochastic, and thus the next state is not unique. Therefore, the state evolution of promoters in a cell population may follow distinct routes, namely individual sequences of promoter states (figure 2b). The numbers of states on different routes are not necessarily the same.
Moreover, the number of possible routes is large. Let $p_l$ denote the probability that a promoter’s state proceeds along route $l$ (in this article, superscripts are bracketed to distinguish from exponents). Collectively, the states of promoters evolve along a ‘state annulus’ via different routes, and distinct routes share the same types of irreversible reactions.

As each route is essentially similar, we first describe the state evolution on route $l$ with the number of states being $n^l$ (i.e. the concrete states are presumed). At time $t$, the promoter is in state $S_l^0$ ($i = 1, 2, 3, \ldots, n^l$). The next reaction occurs at $t + \tau_l$ (see the electronic supplementary material, §II). It may be, with probability $\alpha_l$ ($0 \leq \alpha_l < 1$), an unproductive reaction leaving the promoter still in $S_l^0$ (figure 2c). If the three-dimensional conformation of the promoter–protein complex is currently unfavourable, for instance, a recruited protein may fail to function before its departure. That is, state $S_l^{0+1}$ cannot be reached until a specific irreversible reaction occurs. The average time required for the evolution from state $S_l^0$ to $S_l^{0+1}$ is

$$T_{l}^{0+1} = \frac{\tau_l}{1 - \alpha_l}, \quad (2.1)$$

where $\tau_l$ is the mean interval between two successive reactions on state $S_l^0$. (The detailed mathematical derivation of all equations is presented in the electronic supplementary material, §II.)

For a population of promoters that have initially been synchronized into the same state and evolve along the same route $l$, the mean time taken to finish one round of the state annulus is $\Psi_l = \sum_{i=1}^n \tau_{l,i}$. The average of $\Psi_l$ over various routes, i.e. the period of the slow cycling revealed by ChIP assays, reads

$$\Psi = \sum_l p_l \Psi_l = \sum_l p_l \sum_{i=1}^{n^l} T_{l,i}^{0+1}, \quad (2.2)$$

and the corresponding standard deviation satisfies

$$\sigma = \left[ \sum_l p^l \sum_{i=1}^{n^l} (T_{l,i}^{0+1})^2 \right]^{1/2}. \quad (2.3)$$

When these promoters have undergone $m$ rounds of state annulus along various routes, the standard deviation obeys $\sigma_m = \sqrt{m} \sigma$. $\sigma_m$ rises with increasing $m$, and the synchronization between promoters gradually drifts out. The tendency of such desynchronization can be characterized by comparing $\sigma_m$ with $\Psi$, i.e.

$$\chi_m = \frac{\sigma_m}{\Psi} = \sqrt{m} \sigma / \Psi. \quad (2.4)$$

Equations (2.1)–(2.4) characterize the state evolution of promoters. At the level of molecular interactions, the time intervals between two successive reactions including unproductive ones and between two consecutive promoter states, and the proportion of unproductive reactions are all depicted. At the level of promoter population, the heterogeneity in routes, the mean time taken to complete one round of the state annulus, the synchronization of state evolution and the number of slow cycles are all quantified. Together, the model associates macroscopically measurable features with microscopic molecular interactions and allows for a fully analytical investigation.

3. Results

3.1. Frequent molecular interactions on the promoter

For initially synchronized $pS2$ promoters, ChIP assays showed attenuating slow cycles with a period of 40 min persisting about 8 h; with the preclusive initial period excluded, the number of slow cycles is approximately 11 [23]. That is, the last slow cycle that can be differentiated is $m \approx 11$. Based on equation (2.5), the ranges of $\tau_l$, $\alpha_l$ and $\bar{T}$ for $\chi_{11} \leq 0.5$ can

![Figure 3. Percentage of promoters among $10^7$ cells in a specific state during the $m$th cycle under the limiting condition. The initial state of all promoters is $S_l$. The curves are well fitted by Gaussian distributions (solid lines). The grey line denotes the superposition of all the curves from $m = 1$ to $m = 15$. Here, $\alpha_l = 0.4$, $\tau_l = 30$ s and the focused state is $S_{10}$.

A limiting condition for synchronization

Decreasing $\alpha$ facilitates synchronization. As the same types of irreversible reactions are shared by all routes, the essential features of routes (such as $n^l$ and the distributions of $T_{l,i}^{0+1}$) do not substantially differ from each other. Equations (2.1) and (2.3) suggest that if all the routes satisfy $n^l = n$, $\Psi_l = \Psi$ and $T_{l,i}^{0+1} = \bar{T}$, $\sigma$ reaches its minimum $\sqrt{n} \bar{T}$. Under such a limiting condition, equations (2.1) and (2.2) are simplified into $\bar{T} = \tau_l / (1 - \alpha_l)$ and $\Psi = \bar{n} \tau_l / (1 - \alpha_l)$, respectively. Equation (2.4) is changed into

$$\chi_m = m \bar{T} = m \tau_l / (1 - \alpha_l). \quad (2.5)$$

Equation (2.5) correlates the microscopic properties described by $\bar{T}$ and $\alpha_l$ with the macroscopic quantities $\Psi$ and $m$. $\chi_m$ can be determined as follows. For a population of promoters that operate under the limiting condition and begin with the same initial state, the percentages of promoters in a specific state (e.g. $S_{10}$) during the $m$th cycle are displayed in figure 3. These curves are well fitted by Gaussian distributions, whose amplitude and width fall and rise with increasing $m$, respectively. The superposition of these curves, $\beta$, reflects the temporal evolution of the percentage of promoters in this specific state. A damped oscillatory behaviour is observed because the synchrony between promoters weakens progressively with increasing $m$. For the example shown in figure 3, two distributions corresponding to $m = 12$ and $m = 13$ overlap by half, and thus the sum of their overlapping is nearly a constant. This feature can be used as a criterion to determine the last oscillation in $\beta$ that is discernible, which gives $\sigma_m = (\Psi)/2$. Therefore, we have $\chi_m = 0.5$ based on equation (2.4). By contrast, the experimental discernibility is much weaker, i.e. $\chi_m < 0.5$ experimentally (see the electronic supplementary material, §III). In the following, we first compare the experimental data with the results obtained under the limiting condition.
be determined (figure 4a,b). Both \( \tau \) and \( T \) are no more than 1 min. On the other hand, the relationship between \( \tau \), \( \alpha \), and \( m \) with \( \chi_m = 0.5 \) is shown in figure 4c. \( m \) rises with decreasing \( \tau \) and \( \alpha \), and the changing rate of \( m \) also rises rapidly. These results indicate that even under the limiting condition, the promoters are subject to frequent molecular interactions including productive ones, although the fraction of unproductive ones can be widely distributed in \([0, 1)\).

To further test whether a long time interval may exist between two successive reactions, we assume a slight change in the limiting condition. While keeping \( n^{(i)} = n \) and \( \Psi^{(i)} = \Psi \), let \( T_\alpha = T + \delta \) and \( T_\delta = T - \delta/(n - 1) \) with \( \delta > 0 \) for \( e \in \{1, 2, 3, \ldots, n\} \) and \( \forall d \in \{1, 2, 3, \ldots, n\} \backslash \{e\} \). The resulting number of slow cycles, \( m' \), can be obtained based on equations (2.1)–(2.4), and the dispersion effect can be characterized by \( \gamma = m'/m = \Psi(\Psi - T)/\Psi(\Psi - T + \delta) \). \( \gamma \) drops dramatically with increasing \( \delta \) (figure 4d). For example, \( \gamma \approx 0.5 \) when \( \delta = 4 \) min and \( \bar{T} = 30 \) s, i.e. the number of slow cycles is almost halved. Thus, a slight perturbation to the limiting condition causes remarkable effects, excluding the possibility that there exist long intervals between two successive reactions. Furthermore, if the model included the extrinsic noise such as cell-to-cell variability and fluctuations in the concentration of various proteins, the synchronization between cells would be harder to keep with the same parameter setting. In fact, the time intervals between two successive reactions should be shorter than those shown in figure 4, so that 11 slow cycles can be observed. In sum, the promoter must be subject to rather frequent molecular interactions including productive ones, in contrast to the viewpoint that productive interactions rarely occur [16].

### 3.2. Concurrence of the fast and slow cycling

To evaluate the individual binding time of proteins, we analyse both the protocol and obtained data of ChIP assays. Experimentally, the protein–promoter complexes were first cross-linked by immersion in formaldehyde solution; to capture the current structure, the immersing time \( T_{im} \) was as short as 5 min [17]. For a promoter indexed by \( p \) \((p = 1, 2, \ldots, q)\) and evolving along route \( l \), the probability that it is fixed with specific kinds of proteins approximately equals the proteins’ temporal occupancy rate \( R_{TOR} \) at their binding sites, i.e. \( R_{TOR} = T_b^{(p,l)} / T_{im} \) with \( T_b^{(p,l)} \) being the total binding time of the proteins during immersing [3] (see the electronic supplementary material, §IV). The percentage of promoters bound by the same kind of proteins, \( P_{pro} \), equals the average of \( R_{TOR} \) over the immersed promoter population, i.e.

\[
P_{pro} = \sum_{p} \sum_{l} p^{(p,l)} \lim_{T_{im} \to 0} \frac{\sum_{k=1}^{q} T_b^{(p,l)} T_k^{(p,l)}}{T_{im} \sum_{k=1}^{q} T_b^{(p,l)} T_k^{(p,l)}} = \frac{1}{T_{im} \sum_{k=1}^{q} T_b^{(p,l)} T_k^{(p,l)}}
\]

(3.1)

where \( T_b^{(p,l)} \) and \( T_b^{(p,l)} \) separately denote the average of \( T_b^{(p,l)} \) over the promoters along route \( l \) and over all the promoters. Of note, this equation is applicable to ChIP data provided that a sufficiently large population of promoters is immersed for a short time and the target promoter–protein complexes are sufficiently extracted by antibodies. The ChIP data from different studies can be compared only when the immersing time is identical.

The values of \( P_{pro} \) for all proteins never approach 100% and are indeed much less than 100% at most moments [17]. According to equation (3.1), \( T_b^{(p,l)} \) thus tends to be far less than 5 min. This result may be interpreted from two different perspectives. The individual binding time of proteins is no more than several minutes, i.e. within the time scale revealed...
by FRAP analysis. Alternatively, the binding can persist for a much longer time; but for the same kind of proteins, the intervals between two successive binding are also much longer, and the time periods of binding on different routes rarely overlap (see the electronic supplementary material, figure S1). Given transcription involves a large number of protein species, the second interpretation means that the state evolution of promoters would hardly keep synchronized. This is in contrast to the fact that approximately 11 slow cycles were observed and any kind of protein was always detected at a specific stage in each cycle [17]. Therefore, proteins indeed cycle on and off the promoter rapidly, whereas stable binding occurs rarely. This supports the viewpoint that the fast cycling is functional [15] but contrasts the concept that functional binding is stable [16].

It is shown above that a promoter state lasts for a short time, far less than the immersing time, due to rather short intervals between two successive irreversible reactions. During immersing, a specific kind of protein may associate to and dissociate from the promoter for many times. An individual binding may traverse many promoter states. Therefore, for the promoter $p$ evolving along route $l$, the total time that it is bound by a specific kind of protein during immersing is $T^{(p,l)} = \sum_j T_{l,i}^{(p,j)}$, where $T_{l,i}^{(p,j)}$ denotes the contribution from the $i$th promoter state. Substituting this expression into equation (3.1), we have

$$P_{\text{pro}} = \sum_l \sum_{q=0}^{l-1} \frac{1}{q} \sum_i \sum_j T_{l,i}^{(p,j)} T_{l,i}^{(p,j)}$$ (3.2)

Let $p_{\text{sta}}^{(p)}$ denote the percentage of promoters in a specific promoter state $S^{(p)}$, i.e. $p_{\text{sta}}^{(p)} = \lim_{q \to \infty} \sum_l \sum_{p=1}^{168} T_{l,i}^{(p,j)} / T_{\text{imm}}$, and then $P_{\text{pro}} = \sum_l \sum_{q=0}^{l-1} \sum_{p=1}^{168} p_{\text{sta}}^{(p)}$. Because the states of promoters evolve periodically along the state annulus and the states are transient, $p_{\text{sta}}^{(p)}$ should oscillate with rather low amplitudes, almost the same periods, but different phases. The summation of appropriate $p_{\text{sta}}^{(p)}$ can thus represent any profile of the observed $P_{\text{pro}}$—this is similar to Fourier series analysis. In a word, the slow cycling is essentially an integration effect of the fast cycling; owing to the periodic evolution of promoter states, the transcriptional progression is manifested as slow cycling when detected by ChIP assays. The slow cycling itself thus represents neither stable binding nor external modulation as previously speculated [15,17].

### 3.3. Reproducing concurrent fast and slow cycling by simulations

To directly demonstrate the above conclusions, we performed numerical simulations so as to reproduce the ChIP data for four key proteins, whose temporal profiles are representative of others [17]. p/CIP is a member of p160 family, promoting histone acetylation and activation of transcription. RNA polymerase II (Pol II) acts to initiate mRNA synthesis. Brg1 is an ATPase of the SWI/SNF complex, remodelling the chromatin to inactivate transcription. Here, we make some simplifications. $P_{\text{pro}}$ is a linear summation of $\sum P_{\text{sta}}$ over various routes and the essential characteristics of different routes do not differ markedly; thus, it is sufficient for our purpose to demonstrate concurrent fast and slow cycling by considering a representative route $l$ that satisfies $\psi_l = 40$ min. Then, the temporal intervals between two successive productive interactions are to be determined, based on equation (2.2). We found that the ChIP data can be well reproduced provided that $T_l$’s are less than 15 s (see the electronic supplementary material §IV), strengthening our conclusion about the timescale of molecular interactions on promoters. In simulation, $T_l$ is assumed to obey the Gamma distribution $\Gamma(2.5)$ and $a_l = 0.3$ (see the electronic supplementary material, §IV).

Figure 5a shows time courses of $p_{\text{sta}}^{(p)}$ for 16 states, which are randomly chosen from 168 states. Clearly, the $p_{\text{sta}}^{(p)}$’s oscillate persistently, with the typical features as mentioned above. Figure 5b displays both the fitted simulation data and experimental data on the temporal evolution of $P_{\text{pro}}$ for the four proteins, showing good agreement. These curves oscillate with the same period of 40 min, representing the slow cycles (the phase diagrams are shown in the electronic supplementary material, figure S2). Accordingly, the binding patterns of these proteins on route $l$ are shown in figure 5c. Notably, the binding time is no more than several minutes. This again indicates that the proteins indeed bind/unbind the promoter rapidly. Moreover, it is evident that different kinds of proteins cycle on and off the promoter at distinct stages of each cycle.

### 4. Summary and discussions

In this work, a ‘state annulus’ model was proposed to depict the state evolution of promoters. Compared with previous studies [5,6,15,23,24], this model additionally took into account the unproductive molecular interactions, which is essential for addressing the debate about whether the fast
cycling mainly originates from unproductive interactions. Rather than assuming a single pathway for the state evolution of all promoters, this model represented the heterogeneity of individual promoters by assuming various routes. Mathematical equations were derived, connecting the characteristics of molecular interactions with macroscopic properties revealed by experimental data. Consequently, we revealed new features of transcriptional dynamics and reconciled the conflicting viewpoints on the fast and slow cycling.

This work clarified that the promoter–protein complexes are in rapid exchange of proteins with the nucleoplasm. The time intervals between two successive reactions including productive ones are within dozens of seconds. The fraction of unproductive reactions can be loosely dispersed in [0, 1) over different promoter states. It is thus not true that productive reactions rarely occur as believed [16]. This work further confirmed that proteins cycle on and off the promoter frequently, with the individual binding time no more than several minutes (i.e. within or comparable with the time scale revealed by FRAP); stable binding can hardly occur. That is, proteins accomplish their functions via fast cycling, supporting the viewpoint that the fast cycling is functional [15]. The short intervals between two successive reactions also suggest that a large number of reaction steps are involved in orbiting the state annulus for one round (168 steps are taken in our fitting to ChIP data).

What is the functional advantage of the fast cycling? The fast cycling of transcriptional activators such as NF-kB and liganded ERα allows their time-varying concentration to be timely detected by the transcription apparatus, such that the transcriptional activity of related genes is continuously re-tuned [3,12,14]. Our previous work showed that for the changes in concentration of activators, its components dynamics from quantitative ChIP data could be developed to decode the patterns of protein–DNA interactions.

We revealed that different kinds of proteins rapidly cycle on and off promoters at different transcriptional stages. Such a feature is essentially manifested as the slow cycling of proteins when detected by ChIP assays; the amplitudes and phases of slow cycling are determined by proteins’ binding patterns (equation (3.2) and figure 5). In other words, the slow cycling should not be interpreted as long-time stable binding of proteins or external modulation of the fast cycling as previously thought [15,17]. The slow cycling is also not analogous to stochastic fluctuations with well-defined timing [32,33]. How can the slow cycling be observed? Two conditions are necessary. (i) A large population of promoters—whose state evolution periodically repeats the stages from opening of condensed chromatin to transcriptional initiation and then to recovery of chromatin—are synchronized. As the synchronization fades out, the amplitude of the slow cycling gradually attenuates. (ii) The ChIP assays should be performed with high temporal resolution and acceptable measurement errors.

We can make two testable predictions. When ERα molecules are un-liganded, the period of slow cycling on the p52 promoter is halved to be 20 min [14,17]. Referring to equation (2.5), we predict that the number of slow cycles would be doubled, i.e. about 22 slow cycles would be discriminated under the same experimental condition. ChIP assays also revealed the slow cycling of the catalytic subunit of the proteasome [34]. We predict that this subunit should cycle on and off promoters rapidly during gene transcription. Moreover, it is worth noting that a novel framework is presented here for exploring transcriptional dynamics based on molecular interactions. The method for deriving molecular dynamics from quantitative ChIP data could be developed to decode the patterns of protein–DNA interactions.

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