

Kinetic Proofreading in Chromatin Remodeling: The Case of ISWI/ACF

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ABSTRACT For activation or repression of genes in eukaryotic organisms, the chromatin structure has to be adapted. This action is performed at least in part by dedicated motor proteins, the chromatin remodeling complexes. Recently, investigators have shown some interest in explaining how specific nucleosomes are targeted for chromatin remodeling. For this purpose, two kinetic proofreading scenarios for gene activation and repression have been put forward. We reanalyze both scenarios and show their common points and differences. Further, we propose that in gene repression by ISWI/ACF remodelers, which involves the generation of regular nucleosomal arrays, an additional proofreading step may be active.

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Kinetic proofreading scenarios play a role in the biochemical regulation of several processes of molecular biology, including mRNA translation (1), DNA disentanglement (2), and signaling (3). Recently, such scenarios have also been postulated to be involved in chromatin remodeling (4,5). Chromatin remodeling is the modification of chromatin structure due to the repositioning or removal of nucleosomes. Such changes in chromatin structure can be caused by thermal fluctuations or ATP-generated mechanical forces exerted on the nucleosomes. The latter are brought about by chromatin remodeling complexes that encompass several families of enzymes that integrate multiple functions, including recognition of histone tails and DNA, and hydrolytic activity (6,7). All remodelers contain an ATPase subunit that is derived from the SWI2/SNF2 family. The distinction among the different remodelers arises mainly from the domains neighboring the ATPase domain, which determine the functionality of the enzymes. These differences currently allow us to distinguish four families: SWI/SNF, ISWI, CHD, and INO80 (7). Two particular cases are the SWI/SNF and ISWI families of remodelers. The former are implicated in activation of transcription, i.e., the repositioning of nucleosomes to allow the polymerase to gain access to promoter and gene sequences (8). The latter play a role in repression of transcription by forming arrays of well-positioned nucleosomes (9). Although numerous studies have examined this process using different experimental methods, a comprehensive picture of the functioning of a chromatin remodeler is not currently available.

Recently, we postulated that in the case of activation of transcription, a kinetic proofreading process involving the recognition of histone tail modifications coupled to ATP-dependent loosening of nucleosomal DNA might be employed to target specific nucleosomes for remodeling (4). Narlikar (5) put forward a kinetic proofreading scenario for the remodeler ACF, a member of the ISWI family. Here, we reanalyze these two kinetic proofreading scenarios

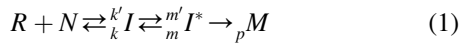
by going back to Hopfield's (10) original work, which underlies them both. We then discuss the limitations of the application of the Hopfield model to chromatin remodeling in the ISWI/ACF case, which need to be overcome before a more complete description can be achieved. We show that a secondary proofreading mechanism for regular positioning of the nucleosomes may be present.

Kinetic proofreading models for chromatin remodeling by ISWI/ACF must take into account three facts concerning the molecular recognition process:

1. Independence of the DNA sequence. Experimental results regarding the ACF remodeler show that, in contrast to nucleosomes, remodeler activity is not sensitive to the DNA sequence (11). A remodeler positions a nucleosome globally, and the nucleosome then relaxes locally into an equilibrium position in a sequence-dependent manner.
2. Dependence on the histone tail. Remodeler action depends on the presence of the histone tail and on its chemical state (i.e., whether or not it bears acetylated lysines) (12). In particular, a bromodomain has been identified in the C-terminal region of SWI/SNF, and SANT and SLIDE domains have been identified in ISWI. Bromodomains interact with acetylated lysines and are capable of recognizing correspondingly modified histone tails (8). By contrast, the SANT domain of the ISWI remodelers is known to interact with unmodified histone tails. The H4 tail has been shown to play a decisive role in ISWI remodeling, in that both the complete removal of the H4 tail (9,12) and its site-specific acetylation (13) suppress the remodeling action of ISWI.
3. Dependence on DNA length. Remodeling complexes may interact with extranucleosomal DNA. In particular,

the dimeric ACF remodeler (14) bears a DNA-binding domain that interacts with extranucleosomal DNA beyond a minimal distance from the nucleosome and below a maximal length. This structural element appears to play a key role in the positioning of nucleosomes in regular arrays, as the remodeler-induced motion of the nucleosome is biased toward the longer flanking DNA (15).

We now show that we can map the remodeler action in both the SWI/SNF and ISWI cases to the original Hopfield scenario of kinetic proofreading. This general biochemical mechanism allows us to favor a specific reaction among competing reactions based on the combination of equilibrium recognition steps and coupled ATP-dependent nonequilibrium steps. In our case, we denote the remodeler by R and the nucleosome by N . The complex formed between them is denoted by I for intermediate. After ATP activation, the complex is turned into an activated intermediate I^* . Finally, the activated intermediate is translocated and hence becomes a mobile nucleosome, which we call M . In terms of these variables, the Hopfield reaction becomes



together with the reaction branch from the activated intermediate state,



We can cast these reactions into three rate equations for the corresponding concentrations $[R]$, $[N]$, $[I]$, and $[I^*]$ (of course, we ignore the fact that the number of molecules intervening in each such reaction is actually small). The kinetic proofreading equations read as

$$\frac{d[I]}{dt} = k'[R][N] - (k + m')[I] + m[I^*] \quad (3)$$

$$\frac{d[I^*]}{dt} = \ell[R][N] + m'[I] - (m + \ell')[I^*] - p[I^*] \quad (4)$$

$$\frac{d}{dt}([R][N]) = -(k' + \ell)[R][N] + k[I] + \ell'[I^*] + p[I^*]. \quad (5)$$

We have already collected corresponding terms except for the term describing the generation of the product, the mobile nucleosome-remodeler complex M , for which we have $d[M]/dt = p[I^*]$. Assuming steady-state conditions, we have $d[I]/dt = d[I^*]/dt = d([R][N])/dt = 0$. By multiplying Eq. 4 by $(-k)$ and adding it to Eq. 5 multiplied by m' , we find the ratio of products to educts:

$$f = \frac{[I^*]}{[R][N]} = \frac{\ell(k + m') + m'k'}{(k + m')(\ell' + p) + km}. \quad (6)$$

We can now compare two reactions (reactions 1 and 2), which differ in their reaction rates (e.g., reaction 1 is energetically favored with respect to reaction 2). We obtain the

error fraction at equal remodeler concentrations, R , given by the ratio

$$F \equiv \frac{f_2}{f_1} = \frac{[I_2^*][N_1]}{[I_1^*][N_2]} = \frac{[I_2^*]}{[I_1^*]} \quad (7)$$

Proofreading conditions enforce the vanishing of the rate constants m_i and ℓ_i for $i = 1, 2$ so that F reduces to

$$F = \frac{m'_2 k'_2 (k_1 + m'_1)(\ell'_1 + p_1)}{m'_1 k'_1 (k_2 + m'_2)(\ell'_2 + p_2)}. \quad (8)$$

In the Blossey-Schiessel (BS) scenario (4), it is assumed that the specificity is in the off-constants k_i , just as in the original Hopfield scenario. Assuming $m'_i < k_i$, $m'_1 \approx m'_2$, $k'_1 \approx k'_2$, and $\ell'_i > p_i$, F simplifies to

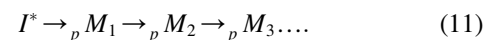
$$F_{BS} \approx \frac{k_1 \ell'_1}{k_2 \ell'_2} \approx \left(\frac{k_1}{k_2}\right)^2, \quad (9)$$

under the assumption that $k_i = \ell'_i$ (4), following Hopfield (10). Because the free energy enters into the rate via a Boltzmann factor, $F_{BS} \sim \exp(-2\Delta G/k_B T)$, the recognition of the correct substrate is significantly favored by the involvement of the remodeler. By contrast, in the Narlikar (N) scenario (5), we have, a priori, the full expression (Eq. 8) in which the notation $m' \equiv k_I$, $\ell' \equiv k_{off}$, and $p \equiv k_{tr}$ is used. Thus,

$$F_N = \frac{k_{I,2} k'_2 (k_1 + k_{I,1})(k_{off,1} + k_{tr,1})}{k_{I,1} k'_1 (k_2 + k_{I,2})(k_{off,2} + k_{tr,2})}. \quad (10)$$

Because Narlikar (5) does not specify the rate constants for the formation of the dimer, we assume in addition that $k'_1 = k'_2$ and we allow for $k_i = \ell'_i = k_{off,i}$. This now allows us to use the parameter values indicated in Narlikar's work. We have $k_{I,1} = 20/min$, $k_{off,1} = 8/min$, $k_{tr,1} = 80/min$ and $k_{I,2} = 1/min$, $k_{off,2} = 160/min$, $k_{tr,2} = 80/min$. When we put in these numbers, we find $F_N = 3.2 \times 10^{-3}$ and hence a selection factor of the correct substrate of $1/F_N \approx 313$.

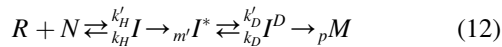
The Narlikar scenario is an effective description because it summarizes several of the recognition steps into two main steps described by the effective rates k_I and k_{tr} . To illustrate this, let us look first at the translation step. From the kinetic proofreading equations, it is already clear that the final product, the reaction with rate p , ultimately must be linked to a release of the remodeler-nucleosome complex. Because the remodeler is a processive motor, this does not happen immediately but occurs, say, after n steps. Then the translocation reaction $I^* \rightarrow_p M$ needs to be replaced by a scheme:



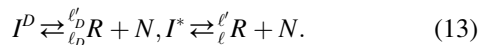
with the additional branches at each remodeling step $M_i \rightarrow_{\ell'} R + N$, where for simplicity we do not introduce new rates. Again assuming stationarity for such a process,

we find for dissociation after n steps $[M_n] \sim (p^n / (\ell' + p)^n) [M]$, which for $\ell' \ll p$ crosses over to Narlikar's result.

The second aspect concerns the formation of the complex I , which is not modeled explicitly by Narlikar. It involves at least two significant steps: interaction with the H4 tail and interaction with the DNA-binding domain SLIDE, which senses the DNA length and introduces a DNA length dependence into the process, which is needed to properly position the nucleosomes. We therefore expand the scenario to obtain



with the two branch reactions,



Recognition of the histone tail occurs with rates k'_H and k_H , and recognition by the DNA-binding domain occurs with rates k'_D and k_D (see Fig. 1). This distinction has experimental support because the complex remains stable after it has properly formed, but then it must sense the length of the neighboring DNA to decide in which direction to translocate (14,16,17). This decision is based on a strong dependence on DNA length in the on-rate k'_D , in contrast to the recognition step of the H4 tail, for which the off-rate is specific. ISWI remodelers display a sensitivity to extranucleosomal DNA length (basepairs) n if $20 < n < 70$. Because the probability of binding of the SLIDE-domain is $\propto n$, where n is the number of bases within the accessible range, directionality is decided on the basis of the higher binding probability to the longer flanking DNA. Further, the back-rate $\ell_D = 0$, because first the histone tail H4 must be bound. If $k'_D \gg k_D$, which one can generally assume, we therefore arrive at an additional proofreading step that biases nucleosome translocations toward a regular array.

Further experiments are clearly needed to validate the kinetic proofreading scenario for chromatin remodeling, which can be an important mechanism for transcriptional

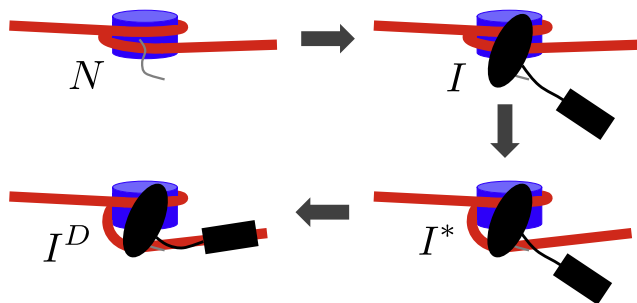


FIGURE 1 Kinetic proofreading steps: histone octamer (blue), DNA (red), and remodeler (black). Recognition of the histone tail H4 (gray) leads to the formation of complex I and to subsequent loosening of DNA on the nucleosome, the activated complex I^* . After binding to the H4 tail, the remodeler must sense DNA length to choose the direction for translocation, leading to the complex I^D . For simplicity, only one monomer of the remodeler is shown.

regulation in eukaryotes. We believe the most important point is the need to obtain further details on the sequence of binding events, particularly regarding the sensing of the DNA flanks. It would be highly interesting to devise experiments combining fluorescence resonance energy transfer and mutation studies to quantify this mechanism, and work on the ACF system in this direction is currently under way (G. J. Narlikar, University of California, San Francisco, personal communication, 2011). *in vivo* validation of the ISWI system also seems possible in the near future. Recent experimental studies by the Rippe group are at least qualitatively in accord with the scenario (18).

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