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A proposal for kinetic proof reading by ISWI family chromatin remodeling motors

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Summary

ATP-dependent chromatin remodeling motors play fundamental roles in nuclear processes by regulating access to DNA. Yet compared to other cellular motors less is known about how these motors couple the energy of ATP to alter their substrates. Here we use recent studies on a key chromatin remodeling motor from the ISWI class, human ACF and its yeast counterpart, ISW2, to propose a model for how these motors use ATP to read structural cues presented by nucleosomal substrates. Substantial earlier work has shown that ACF activity is strongly regulated by the length of the DNA flanking a nucleosome as well as by the histone H4 tail. Recent bulk and single-molecule studies of human ACF suggest that this complex functions as a dimeric motor. These studies, together with studies of yeast ISW2 imply that at least two types of ATP hydrolysis events accompany each cycle of nucleosome movement. We propose that ISWI motors may employ a kinetic proof reading type of mechanism to favor action on nucleosomes that are poised to be in condensed chromatin while inhibiting action on nucleosomes that are in fully active or fully condensed chromatin.

Introduction

Eukaryotic DNA is packaged in the form of chromatin, the smallest unit of which is a nucleosome. A nucleosome contains ~ 150 bp of DNA wrapped in ~ 1.5 turns around an octamer of four different types of histone proteins [1]. Even this smallest unit of chromatin greatly reduces the access of DNA binding factors, as interactions with the histones occlude a large surface of the DNA (Figure 1A). The DNA can get further occluded when strings of nucleosomes cooperatively fold up into more compact helical structures [2,3]. Such compaction occurs through inter-nucleosomal interactions that are mediated by the unstructured N-terminal histone tails [2,3]. Beyond these two levels of DNA packaging, there exist several additional levels of compaction, the mechanisms of which are not well understood but likely entail binding by other protein factors [2,3]. These different levels of DNA packaging enable functional compartmentalization of the genome into open, transcriptionally active states, and closed, transcriptionally silent states. However such compartmentalization is not static and switching between open and closed chromatin states is crucial for enabling the gene expression changes that accompany growth, development and differentiation. Such changes in chromatin states are catalyzed by chromatin remodeling motors, which use the energy of ATP to rearrange histone-DNA interactions.

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While there are many different classes of chromatin remodeling motors, two of the best-studied classes are those that have opposing functional effects [4,5]. SWI/SNF complexes generate a distribution of nucleosomal states, which contain nucleosomes with altered positions, altered composition and altered DNA paths. These products enable efficient exposure of short regions of DNA for localized binding of transcriptional activators or repressors. ACF complexes only generate nucleosomes with altered positions but do so to produce evenly spaced nucleosomes (Figure 1B). Regular spacing of nucleosomes has been suggested to promote higher-order chromatin folding, which is generally associated with long-term transcriptional repression [6]. Correspondingly, ACF has been directly implicated in gene silencing *in vivo* [7].

A key unanswered question is how the action of these two classes of motors is regulated and how cross-talk between them is prevented. This is clearly a complex problem and there are likely to be several inter-locking mechanisms that prevent cross-talk, including specific targeting of these complexes by sequence specific DNA binding factors. Here we discuss a mechanism for substrate recognition that is conceptually similar to kinetic proof-reading mechanisms proposed for explaining the high specificity achieved in translation [8]. We use recent mechanistic insights derived for human ACF and its yeast homolog, ISW2, to make the case for such a mechanism [9–11].

Substrate cues used by ISWI motors

The human ACF complex contains two subunits, an ATPase subunit, SNF2h, and a non-ATPase subunit, Acf1. A major yeast homolog of ACF, ISW2 contains a catalytic subunit, Isw2 and three other subunits. Both, SNF2h and Isw2 are homologous to the drosophila ISWI protein and are hence classified as part of the ISWI family of ATP-dependent chromatin remodeling complexes [5]. Earlier mechanistic characterization of ISWI family complexes has identified two specific features of the nucleosomal substrate that are important in regulating their remodeling activity. The first feature is the length of the DNA flanking the nucleosome and the second feature is the N-terminal tail of histone H4 (Figure 1).

Regulation of activity by flanking DNA length

It has been shown that shortening the flanking DNA causes a much larger reduction in the catalytic activities of ISWI complexes than in their binding affinities for the nucleosome [12–16]. As a result, once bound, these remodeling motors preferentially move the nucleosome towards the longer flanking DNA than towards the shorter flanking DNA (Figure 1B). Continuous sampling of either side of the nucleosome by the motor then results in a dynamic equilibrium in which nucleosomes with equal flanking DNA on either side accumulate [16]. The observation that shortening the flanking DNA has a larger effect on catalysis than on binding indicates that the motor makes more extensive contacts with the flanking DNA in an activated ATP state compared to the ground state. However, it is not known which step of the ATPase cycle the flanking DNA participates in.

While the ability of these motors to constantly move nucleosomes back and forth helps explain their ability to evenly space nucleosomes, it raises another question: how is remodeling activity regulated when the appropriate evenly spaced nucleosomal architecture is generated? The effects of the H4 tail described below help provide one type of explanation.

Regulation of activity by the H4 tail

A basic patch (K₁₆R₁₇H₁₈R₁₉) on the N-terminal tail of histone H4 is important for nucleosome remodeling by ISWI motors. Removal of the H4 tail or mutation of the basic patch does not appear to have significant effects on the binding affinity of these motors, but has large (> 10-fold) effects on their catalytic activities [11,12,17–19]. Acetylation of the H4 tail at lysine

16 also reduces the rate of remodeling by ACF [20]. Analogous to the flanking DNA, it is not known which step of the ATPase cycle the H4 tail participates in.

Regulation of activity by the H4 tail is biologically significant as the H4 tail plays a key regulatory role in mediating the transition between open and condensed chromatin [3]. The H4 tail helps mediate inter-nucleosomal interactions via its N-terminal basic patch (K₁₆R₁₇H₁₈R₁₉). Consistent with this role, acetylation of the H4 tail on K16 reduces higher-order chromatin folding *in vitro* and is associated with open and transcriptionally active chromatin *in vivo* [20]. This acetylation mark also helps recruit other transcriptional activators [21]. Because the biological role of many ISWI complexes appears to be the generation of repressive chromatin structures, it has been hypothesized that ISWI motors may use the unmodified H4 tail as part of their remodeling mechanism to allow discrimination against transcriptionally active loci [17–20]. The use of the H4 tail may also be a mechanism to ensure that remodeling activity is greatly reduced once evenly spaced nucleosomes are generated. This is because cooperative folding of the evenly spaced nucleosomes would occlude access to the H4 tail, thereby providing a negative feedback to remodeling activity.

Recent biochemical, structural and single-molecule studies have shed new light on the different steps in the remodeling reaction catalyzed by human ACF and yeast ISW2 [9–11,20]. Below we use these new results together with previous work to suggest a minimal set of reaction steps catalyzed by these motors. These reaction steps then provide a framework to discuss how the flanking DNA and the H4 tail participate in the overall reaction.

A minimal reaction scheme for nucleosome remodeling by ACF

Recent work suggests that ACF is able to move back and forth between the two sides of a nucleosome because it acts as a dimeric motor [9,10]. The nucleotide state appears to determine whether the dimer closely engages one vs. both sides of the nucleosome. Electron microscopy of the ATPase-nucleosome complex in an activated ATP state implies a dimer architecture in which the two SNF2h ATPases face each other [9]. At the same time, single-molecule FRET (smFRET) studies using nucleosomes labeled with FRET probes show that ACF can move a nucleosome back and forth several times without dissociating from the substrate [10]. Based on these results it has been proposed that the two ATPases work in a coordinated manner, taking turns to engage either side of a nucleosome, thereby allowing processive bidirectional movement [9].

The smFRET studies revealed new and interesting features of the ACF reaction [10]. Two types of ATP hydrolysis dependent conformational changes were observed during one remodeling cycle: an initiation type of event that does not result in a change in FRET, and a second type of event that results in a continuous change in FRET, consistent with movement of the DNA across the histone octamer. The first type of ATP hydrolysis dependent event observed in the case of ACF may be similar in character to a conformational change recently observed with the yeast ISW2 complex [11]. In this case, it was shown using elegant bulk studies that ATP hydrolysis catalyzes conformational changes in ISW2 that are necessary for template commitment, prior to actual movement of the nucleosome. Generation of this processive, ISW2-nucleosome complex, was shown to be dependent on the presence of the histone H4 tail.

These new observations provide an opportunity to develop a minimal reaction framework for ACF by building on previous observations and models (Figure 2). The framework is described in terms of steps that occur after the motor binds to a nucleosome. The first step entails hydrolysis of one of the two ATPases and results in the generation of loosened histone DNA contacts as well as a template committed complex. The second step entails ATP hydrolysis by

the second ATPase subunit and results in translocation of a defined amount of loosened DNA across the histone octamer.

The recent work on ISW2 indicates that upon formation of the template-committed complex, the motor makes more extensive contacts with the nucleosomal DNA [11]. As hypothesized by the authors, such an interaction could lead to loosened histone-DNA contacts if a region of the remodeling motor competes off the DNA contacts made with the histones. This would make the loosened DNA available for subsequent translocation in the second step. Translocation of the DNA across the histone octamer could be directly coupled to translocation of the ATPase motor on DNA as proposed previously [22]. In this type of model, the ATPase motor remains attached to a fixed location on the histone octamer as it translocates on DNA. This would result in movement of the DNA relative to the histone octamer. In this context it is interesting to note that the translocation velocity of SWI/SNF family complexes on nucleosomal DNA as measured using single-molecule optical trap based approaches (13 bs/s) is very similar to the translocation velocity of nucleosomal DNA observed during remodeling by ACF (14 bp/s) in the recent smFRET based work [10,23].

The basic reaction framework proposed in Figure 2 next allows us to identify the character of rate-limiting step under saturating motor concentrations. The smFRET data implies that the first type of ATP-dependent step is ~ 4 fold slower than the second type of ATP-dependent step (Figure 2, k_I vs. k_{tr}) [10]. This suggests that the rate-limiting step in the ACF reaction is the generation of the nucleosomal intermediate with loosened histone-DNA contacts. Consistent with this conclusion, the maximal rate constant for remodeling by ACF measured in bulk studies using similar FRET-based methods is comparable to k_I .

The results summarized above, and minimal framework, raise the question of why these motors have two types of ATP hydrolysis steps. As described below, we hypothesize that the first ATP hydrolysis dependent step provides an opportunity for the motor to “check” structural cues provided by the nucleosome and ensure that it is the correct substrate.

A case for kinetic proof reading

If, as analyzed above, the rate-limiting step for nucleosome movement is the formation of the template-committed complex, then the previously observed catalytic defects of shortening the flanking DNA and mutating the H4 tail, can have two origins: (1) these changes could directly affect the rate of formation of the intermediate (Figure 2, step 1), or (2) these changes could affect the actual rate of DNA translocation (Figure 2, step 2) and reduce it to such an extent that this step now becomes rate-limiting. While both models are possible and mutually compatible we would like to argue that there is a mechanistic and biological advantage to the first model as discussed below.

Model 1 allows for a larger quantitative discrimination between the correct and incorrect substrate if both, the rate of formation of the intermediate and the stability of the intermediate are sensitive to the H4 tail and flanking DNA length. In other words, relative to the ground state, the motor would make stronger contacts with the H4 tail and the flanking DNA in the transition state, and product of the first ATPase step. Further if these contacts are similar in both the transition state and the intermediate, we can make the simple assumption that they make the same energetic contribution in both states. Earlier results with ISWI motors showed that the rate of remodeling is reduced upon removal of the H4 tail. The recent ISW2 study implies that the stability of the intermediate is also reduced upon removal of the H4 tail [11]. Together, these data are consistent with Model 1. A lower stability of the high-energy intermediate in the absence of the H4 tail would translate to a faster rate of dissociation of nucleosome from the motor. Because this dissociation occurs from a high energy intermediate, dissociation will, in effect, be irreversible and the intermediate would rapidly collapse to the

starting canonical nucleosome structure. In such a scenario, a 20-fold reduction in nucleosome-motor interaction due to a loss of contacts with the H4 tail or parts of the flanking DNA can result in a ~40-fold decrease in the overall rate of nucleosome movement (Figure 2). In addition to the overall reduced rates of remodeling, the increased rate of dissociation would provide the motor an opportunity to find the correct substrate before additional energy is invested in translocating the wrong substrate. The increased dissociation rates would also result in lower processivity, as observed with ISW2 in the absence of the H4 tail. For comparison, In Model 2, a 20-fold reduction in the rate of translocation will have a smaller overall effect (6-fold) on the rate of nucleosome movement as this step is not rate limiting in the context of the correct substrate. However, the processivity defect will be the same as in Model 1.

The first model gives the motor a bigger opportunity to determine whether the substrate is appropriate before the actual movement of DNA across the histone octamer. Such proof reading before translocation of the nucleosome can avoid unwanted downstream changes that are coupled to transient movement of the incorrect nucleosomal substrate. For example, small movements of a few nucleosomes within a folded chromatin structure that have less accessible H4 tails and less accessible flanking DNA could cause cooperative unfolding of the chromatin. Similarly, transient movement of a nucleosome acetylated on H4 at lysine 16 could occlude the binding site for a transcription activator and inhibit downstream transcription events.

The mechanism in model 1 is similar in concept to the proof reading mechanism proposed by Hopfield in 1974 [8]. The basic concept entailed an NTP hydrolysis driven step that generates a high energy intermediate whose irreversible dissociation allows iteration of the discrimination between substrates achieved in a prior non-energy dependent step. The relative rate constants of the ACF reaction do not allow for the maximum achievable discrimination proposed in the original proof-reading models. Maximum iteration of the energetic difference between two substrates can occur if $k_{tr} < k_{off}$ for correct and incorrect substrates. In the context of ACF, this condition is only satisfied for nucleosomes lacking the H4 tail.

Conclusions

ATP-dependent chromatin remodeling motors perform complex mechano-chemical reactions on nucleosomes to alter histone-DNA contacts and move the histone octamer across DNA. Not much is known about how these motors couple the energy of ATP to effect these changes. Based on recent results, we propose that the mechano-chemical reactions of dimeric chromatin remodeling motors like ACF contain two types of ATPase cycles: the first type of ATPase cycle generates an activated intermediate that provides ACF an opportunity to check for specific structural cues on the substrate, while the second type of ATPase cycle enables translocation of the DNA across the histone octamer. Such a mechanism allows rapid rejection of the wrong substrates before the histone octamer is moved along the DNA. Testing this hypothesis will require direct comparison of the individual rate constants shown in Figure 2 for the correct and incorrect nucleosomal substrates. It will be interesting to determine whether conceptually similar mechanisms could also occur in monomeric chromatin remodeling motors such as SWI/SNF.

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Figure 1.

(A) Two views of the x-ray crystallography based structure of a nucleosome [1,24]. The DNA is shown in black, histone H2A in red, histone H2B in yellow, histone H3 in green and histone H4 in blue. The unstructured N-terminal tails of the individual histone extend out from the core structure. (B) The ACF complex generates evenly spaced nucleosomes. A cartoon representation of nucleosomes is shown with the nucleosomes in orange and the inter-nucleosomal DNA (also called flanking DNA) in black. Only the histone H4 tail is shown in blue. The dotted lines signify inter-nucleosomal interactions mediated by the H4 tails. The inset in grey shows the action of ACF on a single nucleosome. ACF constantly moves the nucleosome towards the longer flanking DNA faster than towards the shorter flanking DNA.

Figure 2.

(A) The proposed structural transformations catalyzed by ACF are shown in the absence of bound enzyme for clarity. The histone octamer is shown in yellow, the DNA in dark grey and the histone H4 tail as a blue strand with a black circle. The untransformed nucleosome is designated as "S". The intermediate with loosened histone DNA contacts is designated as "I". The direction of movement of the flanking (or extranucleosomal) DNA relative to the histone octamer is shown by the red arrow. (B) Model for the action of a dimeric ACF motor (**E**) on a nucleosome. Each monomer is colored differently (purple and blue). The DNA binding domains of each monomer are shown as rectangles binding to the flanking DNA and the ATPase domains are shown as binding to a specific internal region of the nucleosome. This model of how each motor domain interacts with the nucleosome is based on published cross-linking, footprinting and electron microscopy studies [9,11,13,14,25]. In the first type of

ATPase reaction, the blue monomer hydrolyzes ATP to generate an intermediate with loosened histone-DNA contacts as shown. In the second type of ATPase reaction, the purple motor hydrolyzes ATP to promote movement of the loosened DNA across the histone octamer. This movement can arise from translocation of the purple motor on the DNA while remaining attached to the histone octamer. In Model 1, the blue motor engages the H4 tail proximal to it and the flanking DNA in the transition state of step 1 and in E.I. in Model 2, the purple motor engages the H4 tail proximal to it and the flanking DNA during the translocation step (step 2). Based on smFRET experiments, k_{tr} is estimated to be $\sim 80 \text{ min}^{-1}$ and k_l is estimated to be $\sim 20 \text{ min}^{-1}$ [10]. To calculate the effects mentioned in the text of reducing motor-nucleosome interactions by 20-fold, a value of 8 min^{-1} was used for k_{off} . This value of k_{off} has not been directly measured but is a rough upper limit that is estimated using the high processivity of ACF and ISW2 observed in smFRET and bulk experiments, respectively [10,11].