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Nucleosome sliding by Chd1 does not require rigid coupling between DNA-binding and ATPase domains

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Editor: Esther Schnapp

1st Editorial Decision 28 August 2013

Thank you for the submission of your manuscript to our journal. I am very sorry for the unusual delay in getting back to you, which is due to the recent holiday season. It is more difficult to find referees over the summer period. We have finally received the full set of referee reports that is pasted below.

As you will see, while referee 1 is more critical about the conceptual novelty of the findings, both referees 2 and 3 support publication of the study in EMBO reports and only ask for a minor revision. However, referee 1 points out in her/his report and again in the cross-comments that several previous studies have shown that the DBD is not required for sliding nucleosomes, which already indicates that a power-stroke is not required. S/he also feels that the Clapier and Cairns work published in Nature 2012 is relevant to your findings, and these two issues should therefore be discussed in your manuscript, as you feel appropriate.

Given these comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as mentioned above and in their reports) must be fully addressed and their suggestions taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

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Referee #1:

The Chd1 and ISWI chromatin remodeling enzymes share a similar overall organization, with a central ATPase domain flanked by a C-terminal DNA binding domain (DBD). Previous studies have shown that the DBD is required for optimal sliding of nucleosomes in vitro. These early results led to a model in which the ATPase domain engages the nucleosome, pulling the DNA-bound DBD into the nucleosome and thereby leading to nucleosome sliding. Thus, in this model, the DBD plays an integral role in the remodeling reaction pathway. In this manuscript, Bowman and colleagues extend a previous analysis (Patel et al., JBC, 2011) where small deletions have been created in the region between the DBD and the ATPase domain. In addition, the authors have also introduced either a glycine-rich, flexible peptide or a long (121) residue disordered peptide to test whether flexibilty of length of the linker region alters remodeling activity. In all cases, the authors find that the insertions and most deletions have very little impact on sliding activity (similar to their previous study). Finally, the authors replace the DBD with a bacterial AraC DBD and test activity on a variety of positioned nucleosomes that harbor AraC binding sites. These data indicate that a longer linker between the DBD and the ATPase core allows for remodeling when the DBD binds to DNA more distal to the nucleosome. This supports the authors conclusion that the DBD simply localizes the ATPase to the nearby nucleosome, and the linker plays a passive role.

Technically, this manuscript is outstanding. However, there does not seem to be a whole of lot of new information here. Essentially this work just adds a few more deletions that were not included in the early work from the Bowman lab, as well as adding two new constructs with flexible linker sequences. Second, I was surprised to find no discussion of the recent work from the Cairns and Becker labs on ISWI that are directly relevant to the central hypothesis tested here (Clapier and Cairns, Nature 2012; Mueller-Planitz et al., NSMB 2013). Those papers showed that the DBD is not required for nucleosome sliding, and furthermore, their work indicated that the DBD functions to antagonize a sequence element (NegC in ISWI, called the Bridge domain in Chd1) that blocks ATPase activity. This model is not even mentioned in the current manuscript under review. At the very least this model and the implications need to be discussed here in depth.

As a more minor point, this manuscript does show that linker sequences are important for coupling nucleosome-stimulated ATPase activity to sliding activity. This was also shown previously in the Patel paper for short deletions and substitutions closer to the ATPase core. If the DBD is simply providing a nucleosome binding surface, what is the role of these key linker sequences? There is clearly more going on that just an ATPase core and a DBD. This may also speak to the NegC model proposed by Cairns.

Referee #2:

The authors test a current model that has been invoked to explain how ATP-dependent nucleosome remodelers that possess both an ATPase domain and a C-terminally located DNA binding domain can mobilise nucleosomes. The ATPase domain interacts with DNA within the nucleosome whereas the DNA binding domain binds to free DNA outside of the nucleosome. The model proposes that a conformational change alters the spatial relation between the two domains resulting in the DNA binding domain pushing DNA into the nucleosome (via a socalled "power stroke"). A prediction of this model is that in order to exert such a power stroke the linker connecting ATPase and DNA binding domains must be rigid in structure in order to allow the exertion of the necessary force.

Nodelman and Bowman test this prediction by changing the linker region connecting the ATPase domain and the DNA binding domain in the yeast remodeler Chd1. They introduce deletions of 29 and 45 residues, replace 30 residues of the linker with a flexible peptide (9x GlyGlySer) or insert an additional disordered 120 residue region from the Notch receptor. These constructs are expressed as recombinant proteins and tested for ATPase activity, nucleosome binding and nucleosome sliding in vitro. With the exception of the 45 residue deletion these proteins where all capable of nucleosome sliding. The 45 residue deletion construct was still able to bind nucleosomes and to hydrolyse ATP. The authors conclude that although a minimum distance between the two domains is required to properly coordinate their functions during the remodeling process, length and sequence of the linker is very flexible.

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The authors then conduct an analogous series of experiments using analogous constructs where the Chd1 DNA binding domain is replaced with a sequence-specific DNA binding domain (AraC). Accordingly, nucleosomes with AraC binding sites in the linker DNA were used for remodeling assays. In agreement with previous results, moving the AraC binding sites away from the nucleosome lowered nucleosome sliding activity. Constructs with increased linker length were better able to slide nucleosomes with distant AraC binding sites, constructs with decreased linker length showed the opposite behaviour.

The authors conclude that their results indicate that the DNA binding domain is not physically pulled by the ATPase domain to transfer DNA into the nucleosome, in contrast to what is suggested by the power stroke model.
The authors suggest that an important function of the DNA binding domain is to tether the remodeler to the nucleosome and that it may increased ATPase processivity.

This is a straightforward study that tests an important, current model about nucleosome sliding. The experimental strategy is well thought out and the experimental data is of high quality. I find the results presented to be very convincing. This study is a major contribution to the remodeling field.

I have one minor request. I would suggest to include a figure demonstrating that the various recombinant proteins used are of the same "quality" with respect to purity, monodispersity etc. Given that differences in activity in these kinds of assays can vary from prep to prep this is quite important.

Referee #3:

ATP-dependent Chromatin remodeling enzymes carry out many different transformations of chromatin. Amongst these transformations the sliding of nucleosomes is important for setting up specific types of chromatin architectures and for exposing sites for factor binding. The two best-studied model systems for sliding are the ISWI and Chd1 remodeling enzymes. Both, the Chd1 class and ISWI class of enzymes contain an ATPase domain with homology to SF2 family helicases and a DNA binding domain (DBD) that contains SANT and SLIDE domains. Yet, how this ATPase activity is used to carry out the complex process of nucleosome sliding remains poorly understood. Two models have been proposed for how the DNA binding domains of ISWI and Chd1 remodelers help the ATPase domain achieve the sliding of nucleosomes. In one model (Model 1) the DBD acts as a mechanical element to help the ATPase domain exert force on the extra-nucleosomal DNA and pull the DNA into the nucleosome. In the second model (Model 2) the DBD acts as a passive tether to help correctly orient the ATPase and to locally guide and DNA into the nucleosome.

Here the authors design and perform an elegant series of experiments to directly test the first model in the context of Chd1. The premise is simple. If the ATPase domain exerts force on the DBD then increasing the flexibility of the connection should inhibit the mechanical coupling. The authors insert various linkers between the ATPase domain and the DBD that increase the length and flexibility of the connection between the two domains. Surprisingly they find that none of the insertions have large effects on the ability of the Chd1 to slide nucleosomes. The authors then build on their previous results in which they replace the endogenous DNA binding domain with an AraC binding domain. By increasing the length of the linker between the ATPase and the AraC domain they are able to increase the "reach" of the AraC domain on the extranucleosomal DNA.

The simplest interpretation of these results rules out the model in which the DBD acts as a mechanical element capable of exerting force. The second model then survives. This second model, which invokes a more passive and tethering role for the DBD also helps explain why exogenous DBDs like AraC can functionally substitute for the SANT-SLIDE domain of Chd1.

This work, together with the work by Ludwigsen at al. provides the first direct test of Model 1. The results lead to a clear conclusion that will be of much interest to the chromatin community and will help shape discussions of how complex chromatin remodeling functions can evolve from primitive helicase like functionalities.

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1st Revision - authors' response 11 September 2013

We appreciate the comments of all three Referees, and were pleased to see the strong positive responses from Referees #2 and #3. Below is our point-by-point response to Referee #1.

Referee #1:

The Chd1 and ISWI chromatin remodeling enzymes share a similar overall organization, with a central ATPase domain flanked by a C-terminal DNA binding domain (DBD). Previous studies have shown that the DBD is required for optimal sliding of nucleosomes in vitro. These early results led to a model in which the ATPase domain engages the nucleosome, pulling the DNA-bound DBD into the nucleosome and thereby leading to nucleosome sliding. Thus, in this model, the DBD plays an integral role in the remodeling reaction pathway. In this manuscript, Bowman and colleagues extend a previous analysis (Patel et al., JBC, 2011) where small deletions have been created in the region between the DBD and the ATPase domain. In addition, the authors have also introduced either a glycine-rich, flexible peptide or a long (121) residue disordered peptide to test whether flexibility of length of the linker region alters remodeling activity. In all cases, the authors find that the insertions and most deletions have very little impact on sliding activity (similar to their previous study). Finally, the authors replace the DBD with a bacterial AraC DBD and test activity on a variety of positioned nucleosomes that harbor AraC binding sites. These data indicate that a longer linker between the DBD and the ATPase core allows for remodeling when the DBD binds to DNA more distal to the nucleosome. This supports the authors conclusion that the DBD simply localizes the ATPase to the nearby nucleosome, and the linker plays a passive role.

Technically, this manuscript is outstanding. However, there does not seem to be a whole of lot of new information here. Essentially this work just adds a few more deletions that were not included in the early work from the Bowman lab, as well as adding two new constructs with flexible linker sequences.

> We respectfully disagree, and believe that our new constructs are informative and significantly extend our understanding of the linkage between the DBD and the rest of the remodeler. Our previous work (Patel et al., 2011) utilized shorter deletions that were designed to detect functionally critical residues, which were the focus of that study. Here we find that a larger deletion of 45 residues abolishes sliding activity, yet still allows for nucleosome-stimulated ATPase activity. This ATPase-active/sliding-inactive mutant is surprising because smaller, individual deletions spanning the same region (reported in Patel et al., 2011) had little impact on their own, and demonstrates that connecting the DBD too closely to the ATPase coupling region prevents productive nucleosome sliding.
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> The construct with the glycine/serine-rich segment that replaced 30 residues of the linker was specifically designed to test the impact of a highly flexible segment, while at the same time providing an alternative means for determining whether sliding required a particular linker DBD sequence. In contrast, the large 121-residue insertion was designed to test the limit of the tethering model, and we were surprised to see such robust nucleosome sliding activity. This construct is not an incremental change, but dramatically shows that increased flexibility and in fact increased distance of the DBD from the ATPase motor does not substantially interfere with remodeling activity.

Second, I was surprised to find no discussion of the recent work from the Cairns and Becker labs on ISWI that are directly relevant to the central hypothesis tested here (Clapier and Cairns, Nature 2012; Mueller-Planitz et al., NSMB 2013). Those papers showed that the DBD is not required for nucleosome sliding, and furthermore, their work indicated that the DBD functions to antagonize a sequence element (NegC in ISWI, called the Bridge domain in Chd1) that blocks ATPase activity. This model is not even mentioned in the current manuscript under review. At the very least this model and the implications need to be discussed here in depth.

> We have included the Clapier and Müller-Planitz references in the introduction where the

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point is made that Chd1 and Iswi remodelers can slide nucleosomes in the absence of their DNA-binding domains.

How the DBD antagonizes the inhibition of the C-terminal bridge/NegC element is not clear. However, we believe a simple interpretation is that inhibition by the C-terminal bridge is relieved by some epitope(s) on the nucleosome, and that tethering the remodeler to nucleosomes allows the DBD to antagonize the C-terminal bridge indirectly. This idea is supported by the ability of Chd1-AraC remodelers to slide nucleosomes, which lack the native DBD yet slide nucleosomes (flanked by the AraC binding site) as well as wild type Chd1. The question of how the ATPase motor is regulated by the C-terminal bridge is a fascinating one, but we refrain from delving into a discussion of this regulatory element as our experiments do not specifically focus on that region. We have expanded the first paragraph of the Results/Discussion and labeled the top schematic of Figure 1 to more clearly delineate the DBD linker we targeted, and where it is located relative to the NegC/bridge region.

As a more minor point, this manuscript does show that linker sequences are important for coupling nucleosome-stimulated ATPase activity to sliding activity. This was also shown previously in the Patel paper for short deletions and substitutions closer to the ATPase core. If the DBD is simply providing a nucleosome binding surface, what is the role of these key linker sequences? There is clearly more going on that just an ATPase core and a DBD. This may also speak to the NegC model proposed by Cairns.

The ATPase-coupling defect was unexpected. As mentioned above and now articulated in the first paragraph of the Results/Discussion, we focused on this linker region (residues 961-1005) precisely because we previously found it to lack functionally important residues. In contrast, the region defined as ATPase coupling in Patel et al. (2011) was sequence-specific, with complete abolishment of sliding activity with a single Trp-to-Ala (W932A) mutation. Therefore, although the ATPase-coupling defect due to the 45-residue deletion is similar to the W932A mutation, the deletion is interesting because it was achieved not by altering any key linker sequences (as the referee states above), but by shortening the distance between the DBD and the rest of the remodeler. This defect is therefore important because it suggests that the shortened linker may interfere with conformational changes or block necessary contacts that must occur to slide nucleosomes.

Although it is not clear how a linker deletion causes the ATPase coupling defect, one straightforward interpretation is that the shortened linker disturbs the ATPase-coupling residues (such as W932) during the remodeling cycle, thereby giving an ATPase-coupling defect. We agree with the Referee that remodelers are more complicated than simply an ATPase core and a DBD. We are not trying to oversimplify the remodeling process; it is clearly complex and poorly understood at present. By focusing on the role that the DBD plays in stimulating nucleosome sliding, our goal has been to help narrow down models for how sliding occurs. Though there is likely a mechanistic connection between the ATPase coupling region and the C-terminal bridge/NegC region, our results do not indicate how these regions may influence each other, and we therefore felt it best to omit speculative discussion on that point.

2nd Editorial Decision 17 Septermber 2013

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

Referee #2:

I only had one minor request - that the quality of the protein preps is documented to ensure that differences in activities measured are not a consequence of differences in prep quality. Figure 1

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C&D (SDS PAGE) as well as suppl. Figure 1 (Gel Filtration) document the high quality and comparability of the preps used nicely.

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