EMBO reports

Manuscript EMBO-2013-37751

No need for a power stroke in ISWI-mediated nucleosome sliding

Johanna Ludwigsen, Henrike Klinker and Felix Mueller-Planitz

Corresponding author: Felix Mueller-Planitz, Adolf-Butenandt-Institute, Ludwig-Maximilians-Universität, Munich

Review timeline:

Submission date:	23 July 2013
Editorial Decision:	28 August 2013
Revision received:	04 September 2013
Accepted:	16 September 2013

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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 very minor revisions. After cross-commenting on each others' reports, both referees 2 and 3 think that it is beyond the scope of this study to investigate the role of the NegC domain, and such experiments therefore do not need to be performed. However, referee 1 also 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. Referee 1 further suggests a change to the title, however, referee 2 does not agree, given that the term power stroke is well known in this context, and I also think that it does adequately reflect the findings reported in your manuscript.

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.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have any further questions or comments regarding the revision.

Referee #1:

The Chd1 and ISWI chromatin remodeling enzymes are characterized can function in the absence of accessory subunits, and these enzymes are characterized by an Snf2-like ATPase domain and a C-terminal DNA binding domain (DBD). Previous studies from several groups have shown that remodeling activity does not absolutely require the C-terminal DBD, though it does stimulate activity (and nucleosome binding) about 10-fold. Since the DBD binds to linker DNA, the Bartholomew lab first proposed that the DBD may play an essential role in the remodeling reaction by functioning in a power stroke reaction where the ATPase domain communicates with the DBD, leading to pulling of DNA into the nucleosome. This power stroke model has remained popular even though studies have shown potent remodeling in the absence of the DBD(including work from this group).

More recently, work from the Cairns group has shown that the DBD becomes dispensable for ISWI remodeling activity if a small peptide, called NegC, is deleted from ISWI. The NegC region is located directly adjacent to the ATPase domain, and the structure of the Chd1 ATPase implicates this region (also called the brace or bridge peptide) in contacting the ATPase lobes. The Cairns work suggested that communication between the ATPase and DBD leads to functional antagonizm of NegC, leading to activation of the remodeling reaction.

In this manuscript, the authors have asked whether insertion of a glycine rich, 10 or 20 amino acid peptide into various positions within the C-terminus of ISWI disrupts remodeling activity. These insertions were designed in the context of the power stroke model, assuming that the peptide linker between the DBD and ATPase domain must be rigid in order to "pull" the DBD towards the nucleosome bound ATPase domain.

Technically, the data are of very high quality. However, the authors obtain the somewhat unsatisfying result that the insertions have no impact on any activity of ISWI. Thus, all the data are essentially negative. The authors conclude that these negative results rule out the power stroke model. One might also conclude that the glycine-rich peptides simply didn't work as predicted. Perhaps they are not flexible in this context. Given that there are lots of data showing that the DBD is not essential for remodeling, perhaps it is not surprising that there does not need to be a rigid linker region.

The authors might have the tools in hand to not only test their version of the power stroke model, but to also test the Cairn's NegC model. They appear to have made insertions that separate NegC from the ATPase lobes. They state in the text that they were surprised that these insertions had not impact. However, if these insertions disrupted NegC function, the prediction would be that the DBD would become dispensable. Perhaps the authors could assays several of their linker insertions derivatives in the context of ISWI that lacks its DBD.

On a minor note, the title is a bit misleading. It is already known from previous data that a power stroke (as defined here) is not needed for remodeling. I guess the only question would be whether a power stroke enhances remodeling. The title should more accurately reflect what is actually done in the paper, such as: "ISWI remodeling does not require a rigid connection between ATPase and DNA binding domains".

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.

Ludwigsen et al. test this prediction by varying the linker length between the ATPase domain and the HAND-SANT-SLIDE DNA binding domain of the Drosophila ISWI remodeler. They generate recombinant proteins with insertions of flexible GS-rich sequences at four different positions. They then monitor ATPase activity and the generation of restriction enzyme accessibility / inaccessibility on polynucleosome substrates. The authors also investigate a construct lacking the DNA binding domain and confirm that the remodeling activities of this enzyme is orders of magnitude lower than those of the wild type enzyme. Surprisingly, all the constructs with insertions of flexible linker sequences display activities similar to wild type in all assays employed.

The authors conclude that their results strongly argue against the power stroke model. They propose that the main function of the DNA binding domain lies in tethering the ATPase to the nucleosome substrate. They suggest an alternative "ratcheting" mechanism for the cooperation between ATPase and DNA binding domain during the remodeling process.

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 remodelling field.

I have one minor comment. Figure 1B show Coomassie gels of the purified recombinant proteins studied. The authors also state that all proteins were checked for monodispersity by size exclusion chromatography. I would request that (examples of) these data are shown. This is important in general, since enzyme activities can vary from prep to prep. I realize that in this study most of the proteins tested retain full activity, so this is a minor point.

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 the 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 perform an elegant and rigorous series of experiments to directly test the first model in the context of Drosophila ISWI. The premise is simple. If the ATPase domain exerts force on the HAND-SANT-SLIDE domains then increasing the flexibility of the connection between the ATPase and these domains should inhibit the mechanical coupling. The authors insert 10-20 aa Ser-Gly linkers at different locations between the ATPase domain and the HAND, SANT and SLIDE domains. Surprisingly none of the insertions, even the ones flanking the brace-bridge region significantly affect nucleosome stimulated ATPase or nucleosome sliding. The simplest

interpretation of these results rules out the model in which the HAND, SANT or SLIDE domains act as mechanical elements 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 in the related Chd1 enzyme, exogenous DBDs like AraC can functionally substitute for the SANT-SLIDE domain.

I have one comment on the language. In the introduction, the authors imply that previous papers have used the term "power stroke" to describe the role of the DBD. However, to the best of my knowledge, none of the papers quoted by the authors (refs 10-13) seem to have used this specific term. These previous papers do however describe models that can be explained by using the term "power stroke". I would therefore ask the authors make this distinction clear. For example, the authors can describe the specific language used by the previous authors and then add something like " we classify these models in terms of a power stroke hypothesis". This will help prevent confusion in future literature.

Overall this work, together with the work by Nodelman and Bowman 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.

1st Revision - authors' response 04 September 2013

Referee #1:

The Chd1 and ISWI chromatin remodeling enzymes are characterized can function in the absence of accessory subunits, and these enzymes are characterized by an Snf2 like ATPase domain and a C terminal DNA binding domain (DBD). Previous studies from several groups have shown that remodeling activity does not absolutely require the C terminal DBD, though it does stimulate activity (and nucleosome binding) about 10 fold. Since the DBD binds to linker DNA, the Bartholomew lab first proposed that the DBD may play an essential role in the remodeling reaction by functioning in a power stroke reaction where the ATPase domain communicates with the DBD, leading to pulling of DNA into the nucleosome. This power stroke model has remained popular even though studies have shown potent remodeling in the absence of the DBD(including work from this group).

More recently, work from the Cairns group has shown that the DBD becomes dispensable for ISWI remodeling activity if a small peptide, called NegC, is deleted from ISWI. The NegC region is located directly adjacent to the ATPase domain, and the structure of the Chd1 ATPase implicates this region (also called the brace or bridge peptide) in contacting the ATPase lobes. The Cairns work suggested that communication between the ATPase and DBD leads to functional antagonizm of NegC, leading to activation of the remodeling reaction.

In this manuscript, the authors have asked whether insertion of a glycine rich, 10 or 20 amino acid peptide into various positions within the C terminus of ISWI disrupts remodeling activity. These insertions were designed in the context of the power stroke model, assuming that the peptide linker between the DBD and ATPase domain must be rigid in order to "pull" the DBD towards the nucleosome bound ATPase domain.

Technically, the data are of very high quality. However, the authors obtain the somewhat unsatisfying result that the insertions have no impact on any activity of ISWI. Thus, all the data are essentially negative. The authors conclude that these negative results rule out the power stroke model. One might also conclude that the glycine rich peptides simply didn't work as predicted. Perhaps they are not flexible in this context.

Reply: We thank the reviewer for her/his thoughts. We disagree in most aspects, however. First, we could interpret our data so rigorously only *because* the activities of all insertion mutants were (surprisingly) quantitatively the same. Had the activity of any insertion mutant suffered, numerous other explanations (global or local misfolding, tendency to aggregate, tendency to

stick to tube walls, idiosyncratic behavior of the inserted loop at that particular site, lack of proper substrate affinity, varying levels of unproductive binding to nucleosomes, changes in the ability of ISWI to dimerize on nucleosomes, changes in the ability to simultaneously bind two different nucleosomes, etc.) would have been just as likely as the explanation that we destroyed the power stroke by the insertion. In essence, damaging the activity of an enzyme is always easy but being able to derive biologically meaningful conclusions from such a result can be exceedingly difficult. For that reason, we felt extremely lucky that the results turned out as they did and therefore disagree that our results are "essentially negative".
Second, crystallographic evidence and biophysical experiments that directly measured the flexibility of glycine containing linkers solidly showed how these linkers support the highest possible degree of flexibility. These linkers essentially behaved as a random coil that can be well described by a worm like chain with a persistence length of roughly the size of an amino acid (4.5 Å; refs. 23 and 24). These results provide the rationale for the use of glycine rich linkers in mechanistic dissections of other motor proteins and for the near ubiquitous use of these linkers between proteins and affinity tags in innumerable protein expression vectors. The possibility that any given insertion loop unexpectedly forms rigid interactions is rather low given that we inserted the loops in several places and that similar results were obtained by the Bowman lab with Chd1.

Given that there are lots of data showing that the DBD is not essential for remodeling, perhaps it is not surprising that there does not need to be a rigid linker region.

Reply: We'd like to reiterate why we think our study stands out from previous ones and why it comes at a critical time. Further above, the reviewer states that the "power stroke model has remained popular even though studies have shown potent remodeling in the absence of the DBD". We can only speculate why it has remained popular (see for example Deindl et al. (Cell), 2013 and Hota et al. (NSMB), 2013) despite mounting evidence. Perhaps the field remained skeptical because previous studies that were critical of the power stroke hypothesis relied on heavily truncated mutants or unnatural, chimeric proteins. In any case, there is a controversy in the field that needs to be resolved. In our study, we directly tested the power stroke model.
The reviewer is right of course that there have been a n umber of reports that showed that remodeling can in principle occur without the DBD, and we appropriately cited them. To the best of our knowledge we've come closest to actually quantitate the functional importance of the DBD in our recent NSMB study (ref. 15). We found that the DBD optimizes catalysis by an order of magnitude. These data showed that a power stroke is not necessary for rudimentary remodeling, but left open the possibility that a power stroke is responsible for the order of magnitude effect. We have laid out the entire history in the introduction:
p. 3: "Other data appear to be in conflict but can be reconciled with the power stroke model. We and others have shown that ISWI can remodel nucleosomes even if the HSS module is missing [15, 16]. Similarly, the C terminal DBD of Chd1, composed of a related SANT SLIDE module [17], is also not required for remodeling [18, 19]. Nevertheless, the remodeling activity of ISWI decreases an order of magnitude upon deletion or mutation of the HSS module [10, 15]. This drop in activity could potentially be attributed to a missing power stroke in the deletion mutants."
In summary, there is a need to resolve a controversy in the field and to put the power stroke model to a rigorous test. In referee #3's words, we –together with Nodelman and Bowman– are the first to do so.

The authors might have the tools in hand to not only test their version of the power stroke model, but to also test the Cairn's NegC model. They appear to have made insertions that separate NegC from the ATPase lobes. They state in the text that they were surprised that these insertions had not impact. However, if these insertions disrupted NegC function, the prediction would be that the DBD would become dispensable. Perhaps the authors could assays several of their linker insertions derivatives in the context of ISWI that lacks its DBD.

Reply: We thank the reviewer for the suggestion. Experiments designed to better understand the function of the brace bridge (a.k.a. NegC) region would indeed be important for the field. Though interesting, these experiments would not directly test the power stroke hypothesis. Thus, we agree with reviewer 2 and 3, who reportedly mentioned in their cross comments that experiments aimed at a dissection of the function of the brace bridge region are beyond the

scope of the study. Nevertheless, we added a few sentences to the discussion that are intended to better interpret our data in the context of Cairns' NegC hypothesis:
p. 5: "We were particularly surprised that the 10 to 20 aa long insertions on either side of the brace bridge polypeptide did not hamper catalysis as this polypeptide makes intimate contacts with the ATPase domain and was proposed to regulate the enzyme [15, 16, 19, 25]. Depending on whether or not the structure of the brace and bridge is disrupted by the insertions, we can either conclude that this region may be of lesser importance for remodeling than previously hypothesized [16] or that build up of force is not necessary for proper function of the brace bridge polypeptide."

On a minor note, the title is a bit misleading. It is already known from previous data that a power stroke (as defined here) is not needed for remodeling. I guess the only question would be whether a power stroke enhances remodeling. The title should more accurately reflect what is actually done in the paper, such as: "ISWI remodeling does not require a rigid connection between ATPase and DNA binding domains".

Reply: We agree that the title carries a strong message but don't think that it is misleading as it reflects well the major conclusion from our work. As the reviewer notes, the power stroke model has been and still remains "popular" in the field and we tested this model as well as a more specific model in which the power stroke is "only" responsible for enhancing remodeling by an order of magnitude. Given that reviewer 2 in the cross comments reportedly disagreed that a change in the title is required and with the consent of the editor we opted to leave the title unchanged.

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.

Ludwigsen et al. test this prediction by varying the linker length between the ATPase domain and the HAND SANT SLIDE DNA binding domain of the Drosophila ISWI remodeler. They generate recombinant proteins with insertions of flexible GS rich sequences at four different positions. They then monitor ATPase activity and the generation of restriction enzyme accessibility / inaccessibility on polynucleosome substrates. The authors also investigate a construct lacking the DNA binding domain and confirm that the remodeling activities of this enzyme is orders of magnitude lower than those of the wild type enzyme. Surprisingly, all the constructs with insertions of flexible linker sequences display activities similar to wild type in all assays employed.

The authors conclude that their results strongly argue against the power stroke model. They propose that the main function of the DNA binding domain lies in tethering the ATPase to the nucleosome substrate. They suggest an alternative "ratcheting" mechanism for the cooperation between ATPase and DNA binding domain during the remodeling process.

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 remodelling field.

I have one minor comment. Figure 1B show Coomassie gels of the purified recombinant proteins studied. The authors also state that all proteins were checked for monodispersity by size exclusion chromatography. I would request that (examples of) these data are shown. This is important in general, since enzyme activities can vary from prep to prep. I realize that in this study most of the proteins tested retain full activity, so this is a minor point.

Reply: Following the request of the reviewer, we now show the SEC data for all proteins used in this study in the new Supplementary Figure 1. We thank the reviewer for the positive evaluation of our work!

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 the 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 perform an elegant and rigorous series of experiments to directly test the first model in the context of Drosophila ISWI. The premise is simple. If the ATPase domain exerts force on the HAND SANT SLIDE domains then increasing the flexibility of the connection between the ATPase and these domains should inhibit the mechanical coupling. The authors insert 10 20 aa Ser Gly linkers at different locations between the ATPase domain and the HAND, SANT and SLIDE domains. Surprisingly none of the insertions, even the ones flanking the brace bridge region significantly affect nucleosome stimulated ATPase or nucleosome sliding. The simplest interpretation of these results rules out the model in which the HAND, SANT or SLIDE domains act as mechanical elements 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 in the related Chd1 enzyme, exogenous DBDs like AraC can functionally substitute for the SANT SLIDE domain.

I have one comment on the language. In the introduction, the authors imply that previous papers have used the term "power stroke" to describe the role of the DBD. However, to the best of my knowledge, none of the papers quoted by the authors (refs 10 13) seem to have used this specific term. These previous papers do however describe models that can be explained by using the term "power stroke". I would therefore ask the authors make this distinction clear. For example, the authors can describe the specific language used by the previous authors and then add something like " we classify these models in terms of a power stroke hypothesis". This will help prevent confusion in future literature.

Reply: We think this is a very good idea and inserted a couple of sentences:
p. 2: “For remodeling by ISWI enzymes, it has been proposed that a conformational change mechanically pulls flanking DNA into the nucleosome [10 13]. The energy required for this conformational change would come from hydrolysis of ATP. A step that uses chemical energy to perform mechanical work is often called a power stroke, a terminology that we adopt herein.”

Overall this work, together with the work by Nodelman and Bowman 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.

We thank the reviewer for the positive evaluation of our work!

2nd Editorial Decision 16 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:

The authors have fully addressed my minor concern by providing SEC data for all constructs used in the new Suppl Fig 1.

