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X-ray and Cryo-EM structures reveal mutual conformational changes of Kinesin and GTP-state microtubules upon binding

Manatsu Morikawa, Hiroaki Yajima, Ryo Nitta, Shigeyuki Inoue, Toshihiko Ogura, Chikara Sato and Nobutaka Hirokawa

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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.)

1st Editorial Decision 05 December 2014

Thank you for the submission of your manuscript entitled "Mutual Conformational Changes of Kinesin and GTP-Microtubule Upon their Binding". We have now received the full set of reports from the referees, which I copy below.

As you can see from their comments, all three referees enthusiastically support the publication of your study in The EMBO Journal, but point out to a few concerns, mainly demanding clarifications of the text or the figures, that will require your attention before we can further proceed with the acceptance of your manuscript. We normally allow a single round of major revision only, which in any case will not be necessary here, and it should be submitted within the next three months. Do not hesitate to contact me if you have any questions, need further input on the referee comments or you anticipate any problems with the revision.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). However, we would appreciate if you contact me as soon as possible upon publication of any related work in order to discuss how to proceed.

When preparing your letter of response to the referees' comments, bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://emboj.msubmit.net/html/emboj_author_instructions.html - a2.12

As you have probably seen already, every paper now includes a 'Synopsis', displayed on the html and freely accessible to all readers. The synopsis includes a 'model' figure as well as 2-5 one-short-sentence bullet points that summarize the article. I would appreciate if you could provide this figure (a simplified version of figure 10 will do) and the bullet points. Take into account that the final size of the synopsis figure will be 550 pixels wide x 200-400 pixels tall. You do not need to provide the final sized figure, but keep in mind that small details might be lost in a busy image. Please check our website for examples.

REFEREE REPORTS

Referee #1:

There have already been several recent papers (1-3) reporting the structure of nucleotide-free kinesin bound to (GDP) tubulin, at higher resolution than this work. Also, Alushin et al already reported the structure of GMPPCP MTs with kinesin bound. What is unique about this work by Morikawa et al is (i) the use of an isotype of kinesin that is particularly sensitive to the nucleotide state of tubulin and also (ii) a comparison of undecorated and decorated GMPPCP MT structures, showing the somewhat surprising structural effects of kinesin binding. The authors first show that Kif5C binds more tightly to GMPPCP MTs, moves more quickly on them and hydrolyses ATP faster; these effects did not occur when loop L11 was replaced with that of Kif5A. To try to understand the molecular basis of Kif5C's preference for GMPPCP MTs, they have solved the crystal structure of Kif5C with sulfate bound and, more usefully, the cryo-EM structure of apo-Kif5C-decorated MTs. The insight regarding the key role of L11 came from the latter.

Abstract: KIF5 should be replaced by KIF5C throughout the abstract. The 2nd sentence should end at 'mechanism remains unresolved'.

Sindelar et al have shown that sulfate puts kinesin into an ATP-like state, so the new crystal structure reported here may not be relevant to the problem. Maybe it just deserves to be in supplementary data.

The discussion of nucleotide-dependent activity should take full account of the findings reported in the references listed below. Overall, this paper should focus on its new findings (i & ii). Other kinesins probably affect inter-protofilament contacts in the same way as shown in Fig. 2, to judge from the sructures of Alushin et al.

Figure 10 needs some explanation in the figure legend

The change in MT structure reported by Krebs et al showed up at much lower resolution, was most likely an artifact and is best forgotten!

Implications NOT imprecations (curses)

1. Atherton J, Farabella I, Yu IM, Rosenfeld SS, Houdusse A, Topf M, Moores CA, Conserved mechanisms of microtubule-stimulated ADP release, ATP binding, and force generation in transport kinesins.. elife 2014; 3:e03680

2. Shang Z, Zhou K, Xu C, Csencsits R, Cochran JC, Sindelar CV, High-resolution structures of kinesin on microtubules provide a basis for nucleotide-gated force generation. elife 2014 Nov 21; 3

3. Cao L, Wang W, Jiang Q, Wang C, Knossow M, Gigant B, The structure of apo-kinesin bound to tubulin links the nucleotide cycle to movement. Nat Commun 2014; 5:5364

Referee #2:

This manuscript provides useful new information about two areas of interest to kinesin-microtubule (MT) interaction. One is a more detailed analysis of the difference in structure and interaction with

kinesin of GDP/taxol versus GTP MTs. This focuses on the induction of mutual conformational changes in both the kinesin motor domain and the MT on interaction. The other focus is the X-ray structure of nucleotide-free kinesin and how it interacts with MTs. Although there are concerns about the interpretation of the X-ray structure, this does not distract from the cyroEM analysis of nucleotide-free kinesin on MTs.

1. The method of obtaining nucleotide-free kinesin crystals is unusual and not discussed in sufficient detail to allow evaluation for the limitations on its interpretation.
* It is stated that that crystals were first grown with ADP and then the ADP was caused to be released by addition of a very high concentration of the inhibitory tail peptide. Was this procedure based on solution measurement indicating that the tail peptide stimulated ADP release? Was this effect specific for this peptide? With dimeric constructs this tail peptide inhibits ADP release rather than favoring release. Is this a real difference of monomer heads versus dimer heads?
* A concern is that the initial crystallization locked the crystal into an ADP state and that subsequent release of ADP only produced a subset of the changes in the full rigor state (or even an artifactual conformation). Did the crystal lattice packing change on tail addition? Was the structure solved for the crystals before the tail was added to directly compare pre and post tail treatment?
* The expected hallmark of the nucleotide-free state is an undocked neck linker and the associated conformation changes in the rest of the protein that drive undocking. However, the neck linker in Fig. 3A appears to be fully docked. If this is true, it is a strong indication that the true nucleotide-free state has not been obtained due to crystal packing constraints. This is a serious limitation on the interpretation that needs to be addressed.

2. Both kcat and sliding velocity appear to be low. What are comparable literature values? For sliding velocity a processive dimeric construct would give a more meaningful comparison between MTs and unclear why that was not done.

Minor points:
* Should indicate that the K334 construct used for the X-ray had a partially truncated neck linker, whereas the K345 one used for motility and biochemical assays had a complete neck linker.
* K344 was purified by metal affinity chromatography, but it is never explicitly stated that it has a his tag and whether it is fused directly to amino acid 334.

Referee #3:

The manuscript by Morikawa et al. provides an extremely detailed structural and functional analysis of the Kif5 kinesin, both via a nucleotide-free crystal structure, as well as with high resolution EM structures of Kif5 bound to microtubules. There are a number of interesting and novel details presented in the manuscript, including:

- the nucleotide-free crystal structure of a plus-bed directed kinesin motor

- cryo-EM reconstructions of Kif5 with GTP-state microtubules, highlighting differences in both the motor domain, and perhaps more novel, changes in the microtubule conformation

- activity data is presented showing that Kif5 moves more rapidly along GTP-MT's, which complements the differences in the structures that are observed.

Taken together, the results present a compelling story for why Kif5 behaves in the manner it does, as well as clarifying the conformation of the so-called rigor state of kinesin binding. A detailed structural model is also presented that describes the various conformational changes occurring during the Kif5 mechanochemical cycle.

One general comment is that the text and figures are incredibly detailed and somewhat difficult to follow, especially for a non-kinesin specialist. It would be a great help if summary sentences could be included at the start or end of each section. It would also help to have simpler summary figures included, as even the model figure 10 is small, detailed, and hard to follow.

On page 8, the discussion of the MT-interface, referring to Figs 2A and 2B, and the differences

being with the "longitudinally long interface" for GDP-MTs and "around the intra-tubulin-dimer groove" for GMPCPP-MTs is confusing. I do not see the differences described when looking at Figs 2A and 2B. Please clarify. The other differences described with respect to Fig 2 are clearer.

The authors suggest that a sulfate ion is present near the P-loop and show of-fc density. Have they refined the structure with sulfate? How does that density look? Also, please provide a clearer contact for Fig 3B. The labeled ADP is difficult to see, and the overall position of the assumed phosphate density is not clear.

"devided" is misspelled on Page 11.

While I hesitate to ask for the structural figures to be remade, in general they are extremely busy, small, and difficult to follow (in particular Figs 5, 7, 8, and 10).

1st Revision - authors' response 17 January 2015

Point-by-point response
Referee #1:

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Abstract: KIF5 should be replaced by KIF5C throughout the abstract. The 2nd sentence should end at 'mechanism remains unresolved'.

We thank the referee for pointing these out. We have changed the parts accordingly.

Sindelar et al have shown that sulfate puts kinesin into an ATP-like state, so the new crystal structure reported here may not be relevant to the problem. Maybe it just deserves to be in supplementary data.

As suggested, Sindelar et al. have shown that sulfate puts the neck-linker conformation (and probably switch II conformation) into an ATP-like state (NSB 2002), that is also consistent with our structural result. However, the weakly ADP-bound form (before the exchange of ADP into sulfate), which has been newly added in the revised text as suggested by referee #2, also takes the ATP-like conformation. At least in our structure, therefore, ATP-like conformation of switch II and neck-linker was not induced by the addition of sulfate.
Because referee #2 was interested in our crystal structural results, we decided not to move the crystal structure into the supplement. In our nucleotide-free (or weekly ADP-bound) KIF5C, switch II takes ATP-like conformation, whereas switch I takes the open conformation. This conformation was also observed in the KIF1A structure in the weakly ADP-bound state that has the very different crystal packing from the KIF5C. Thus the crystal packing effect can be excluded and kinesins in the weakly-bound ADP state or the nucleotide-free state in the absence of microtubule (or tubulin) might take this type of conformation. We think that the metastable state in the nucleotide-free kinesin might be different between kinesin in the presence of microtubule and in the absence of microtubule. We have included this discussion briefly in the text to avoid misleading of readers (p.10-11).

The discussion of nucleotide-dependent activity should take full account of the findings reported in the references listed below. Overall, this paper should focus on its new findings (i & ii). Other

kinesins probably affect inter-protofilament contacts in the same way as shown in Fig. 2, to judge from the sructures of Alushin et al.

We have added the discussion about the difference between our structure and the recently solved three structures reported in the suggested references (p.24-25). In summary, the switch I conformation is similar among these four structures, albeit the switch II conformation is different between that on the GTP-MT and that on the GDP-taxol-MT (or GDP-tubulin). Since the switch I is the responsible region for the chemical cycle of kinesin motors, its conformation might be conserved even on the different type of the microtubule (nucleotide exchange factor). Switch II is one of the major interfaces for the microtubule so that switch II conformation should be altered depending on the difference of the microtubule conformation.

Figure 10 needs some explanation in the figure legend

We are sorry for the poor presentation. We have improved this figure to include the referee #3's point, and added an adequate legend.

The change in MT structure reported by Krebs et al showed up at much lower resolution, was most likely an artifact and is best forgotten!

We deleted the sentences and references about the previous works by Krebs.

Implications NOT imprecations (curses)

We thank the referee for pointing this out. We corrected.

1. Atherton J, Farabella I, Yu IM, Rosenfeld SS, Houdusse A, Topf M, Moores CA, Conserved mechanisms of microtubule-stimulated ADP release, ATP binding, and force generation in transport kinesins.. elife 2014; 3:e03680

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1. The method of obtaining nucleotide-free kinesin crystals is unusual and not discussed in sufficient detail to allow evaluation for the limitations on its interpretation.
** It is stated that that crystals were first grown with ADP and then the ADP was caused to be released by addition of a very high concentration of the inhibitory tail peptide. Was this procedure based on solution measurement indicating that the tail peptide stimulated ADP release? Was this effect specific for this peptide? With dimeric constructs this tail peptide inhibits ADP release rather than favoring release. Is this a real difference of monomer heads versus dimer heads?*
** A concern is that the initial crystallization locked the crystal into an ADP state and that subsequent release of ADP only produced a subset of the changes in the full rigor state (or even an artifactual conformation). Did the crystal lattice packing change on tail addition? Was the structure solved for the crystals before the tail was added to directly compare pre and post tail treatment?*

We thank this referee for this useful discussion. We would like to explain the time course to get the KIF5C structure in the nucleotide-free state. At first, we crystallized KIF5C without the tail peptide in the crystallization buffer. As a result, we only got KIF5C including the weakly-bound ADP (B-factor=100Å^2) without magnesium ion. Hence, we soaked conventional additives, EDTA and apyrase, to completely release ADP from the pocket. EDTA did not have any effect that is reasonable because KIF5C did not have magnesium ion in its pocket. The addition of apyrase resulted in the degradation of crystals. For the tail peptide, we only had information that it inhibits ADP release in the presence of magnesium ion. Because we have prepared the tail peptide for the other experiments, we soaked it as a trial to release ADP from the crystal. The mild effect of the tail peptide finally achieved the successful outcome to release ADP.
Currently, we did not have the biochemical results to directly prove that the tail peptide accelerates the ADP release after the magnesium ion has been released from the pocket. In the revised manuscript, however, we have presented both structures before and after the addition of the peptide, suggesting that the density corresponding to the weakly-bound ADP was in fact exchanged to the small round density possibly corresponding to the sulfate ion. The difference between them was only the treatment of the tail peptide. Therefore, we concluded that the tail peptide could mildly induce the release of ADP in the absence of magnesium ion.
Since the aim of the current project is getting the nucleotide-free structure of KIF5C, checking biochemically the effect of the tail peptide for the release of ADP is beyond the scope of this paper, albeit the biochemical confirmation will be necessary to prove the effect of ADP release by the addition of tail peptide.
In our nucleotide-free KIF5C, switch II takes ATP-like conformation with the two-third of the neck-linker being docked, whereas switch I takes the open conformation. This conformation was also observed in the KIF1A structure in the weakly-bound ADP state that has the very different crystal packing from the KIF5C. Thus the crystal packing effect can be excluded and kinesins in the weakly-bound ADP state or the nucleotide-free state in the absence of microtubule (or tubulin) might take this type of conformation. Especially, switch I adopts the favorable conformation to release Mg ion and ADP, which has not been observed in the presence of MT or tubulin.
We have included this discussion briefly in the text to avoid misleading of readers (p.10-11).

** The expected hallmark of the nucleotide-free state is an undocked neck linker and the associated conformation changes in the rest of the protein that drive undocking. However, the neck linker in Fig. 3A appears to be fully docked. If this is true, it is a strong indication that the true nucleotide-free state has not been obtained due to crystal packing constraints. This is a serious limitation on the interpretation that needs to be addressed.*

As described above, KIF5C structure solved here (weakly-ADP-bound or nucleotide free) shares the structural features of switch I and switch II with the previously solved KIF1A structure (weakly-ADP-bound), suggesting that this conformation was not due to the crystal packing constraints. Rather, the metastable state in the nucleotide-free kinesin might be different between kinesin in the presence of microtubule and the absence of the microtubule.

2. Both kcat and sliding velocity appear to be low. What are comparable literature values? For sliding velocity a processive dimeric construct would give a more meaningful comparison between MTs and unclear why that was not done.

We are deeply grateful for this comment. Our previous gliding velocities were low indeed. We performed the assay using dimeric construct K375 as well as the monomeric K345. The result of the dimeric KIF5C is now presented in Figure S1. The velocity and the ratio of the two MTs' movements were consistent with the previous reports (Coy et al, 1999; Vale et al, 1994). More importantly, we could get higher velocities for the monomeric construct than that we originally reported. The difference is the freshness of the motor. The previous motor was purified and stored at -80 degree, whereas this time we used it just after the purification. The results obtained by the lysate were the same. We also checked that the ATPase activity in Figure 1B is reproducible. We are sorry for the insufficient verification and thank this referee again for this insightful feedback.

Minor points:
** Should indicate that the K334 construct used for the X-ray had a partially truncated neck linker, whereas the K345 one used for motility and biochemical assays had a complete neck linker.*

We agree that the usage of the different constructs is misleading. We have attempted some constructs to make “nucleotide-free state” crystal. The reason that we finally selected K334 to get the high resolution structure is that this construct had best quality in expression, protein purification and crystallization. K334 has full ATPase activity tested biochemically (Okada et al, Nature, 2003) so that we consider that the partial truncation of the neck-linker does not fundamentally affect the motor domain structure. In the revised manuscript, we clearly note the difference of the construct in p. 10

** K344 was purified by metal affinity chromatography, but it is never explicitly stated that it has a his tag and whether it is fused directly to amino acid 334.*

We are sorry for the poor presentation. We modified that text of experimental procedures on p. 27 to:
The KIF5C motor domain K334 [mouse KIF5C residues 1–334 with a 7×His-tag in C-terminal] purified by immobilized metal affinity chromatography and cation exchange chromatography (AKTA Explorer 10S, RESOURCE S column; GE Healthcare) was dialyzed against…

Referee #3:

The manuscript by Morikawa et al. provides an extremely detailed structural and functional analysis of the Kif5 kinesin, both via a nucleotide-free crystal structure, as well as with high resolution EM structures of Kif5 bound to microtubules. There are a number of interesting and novel details presented in the manuscript, including:
- the nucleotide-free crystal structure of a plus-bed directed kinesin motor
- cryo-EM reconstructions of Kif5 with GTP-state microtubules, highlighting differences in both the motor domain, and perhaps more novel, changes in the microtubule conformation
- activity data is presented showing that Kif5 moves more rapidly along GTP-MT's, which complements the differences in the structures that are observed.

Taken together, the results present a compelling story for why Kif5 behaves in the manner it does, as well as clarifying the conformation of the so-called rigor state of kinesin binding. A detailed structural model is also presented that describes the various conformational changes occurring during the Kif5 mechanochemical cycle.

One general comment is that the text and figures are incredibly detailed and somewhat difficult to follow, especially for a non-kinesin specialist. It would be a great help if summary sentences could be included at the start or end of each section. It would also help to have simpler summary figures included, as even the model figure 10 is small, detailed, and hard to follow.

We are deeply sorry for the poor presentation. We condensed the discussion to make it suitable for the wide range of readers. We tried to present clearly how and why our new structure is different from the canonical structures. In the figure 5, the session of MT conformational change, we added schematic figures 5C, F and S5C, which placed more emphasis only on the moved helices. Informative but difficult difference maps were moved to the supplementary figure S5 for the specialist readers. As for the session of KIF5C conformational change, we improved the Figures 7B-D. In these new figures, we focused on showing the differences of switches I and II and neck-linker between our new structure and those of the ADP-like and ATP-like structures. For Figure 8 (MT-KIF5C interface), we added new panels to indicate the orientation of each panels (Figure 8A). Figure 10 were totally rewritten and added a figure to summarize MT conformational changes.

On page 8, the discussion of the MT-interface, referring to Figs 2A and 2B, and the differences being with the "longitudinally long interface" for GDP-MTs and "around the intra-tubulin-dimer groove" for GMPCPP-MTs is confusing. I do not see the differences described when looking at Figs 2A and 2B. Please clarify. The other differences described with respect to Fig 2 are clearer.

We are sorry for the unclear presentation “broken lines in Figures 2A and B”. There were multiple broken lines in the figures. We indicate each length of MT-KIF5C interface using bars to show the differences and modified the text.

The authors suggest that a sulfate ion is present near the P-loop and show of-fc density. Have they refined the structure with sulfate? How does that density look? Also, please provide a clearer contact for Fig 3B. The labeled ADP is difficult to see, and the overall position of the assumed phosphate density is not clear.

We are very sorry for our poor presentation. In the originally submitted manuscript, we overlaid following two electron density map to express that there is no electron density indicating ADP, and instead we can find small round density around P-loop.
Fo-Fc map "before" putting sulfate ion as a ligand
2Fo-Fc map after putting sulfate ion as a ligand (final structure)
As this referee pointed out, this may give a false impression. We also received some suggestions and opinions about the structure around P-loop from referee #2, thus we make new figures (Figures 3B and S3A) and explanations about the structure around P-loop (p.10-11).

"devided" is misspelled on Page 11.
We corrected the misspelled words.

While I hesitate to ask for the structural figures to be remade, in general they are extremely busy, small, and difficult to follow (in particular Figs 5, 7, 8, and 10).

We thank this referee for pointing these out. We improved the figures 5, 7, 8, and 10 as described above.

Accepted 13 February 2015

I am pleased to inform you that your manuscript has been re-evaluated by former referee #2 and given that only very minor concerns remain, it has been accepted for publication in the EMBO Journal.

Naturally, a final version of the manuscript text minimally discussing the issues mentioned by the referee should be provided (reply to this e-mail), but otherwise your manuscript is ready to be transferred to the production team.

You will find below the final comments from the referee as well as further important information regarding publication procedures and license requirements. If you have any questions, please do not hesitate to contact me by e-mail or on the phone. Thank you for your contribution to The EMBO Journal and congratulations on a successful publication.

REFEREE REPORT

Referee #2:

The manuscript has addressed most of the point previously raised. One issue is that a major part of the paper is the new X-ray structure without nucleotide, but that is not reflected in the title of the paper.
Two remaining concerns are:
1. The presence of the sulfate at the beta Pi binding site may prevent obtaining the full nucleotide-free conformation and this should be more explicitly included.
2. Although Fig. 10 may incorporate reasonable changes in the ATP binding site, it is still not clear that the movements of the neck linker are in agreement with the Rice et al model and this needs to be more explicitly included.

2nd Revision - authors' response 23 February 2015

Point by point response

Referee #2:
The manuscript has addressed most of the point previously raised. One issue is that a major part of the paper is the new X-ray structure without nucleotide, but that is not reflected in the title of the paper.

We appreciate the suggestion. We revised the title as to "Mutual Conformational Changes of Kinesin and GTP-Microtubule by X-ray and Cryo-EM Structural Studies" to emphasize that this work consists of X-ray crystallography and cryo-EM.
In addition we highlighted the new X-ray structure without nucleotides in short summary.

Two remaining concerns are:
1. The presence of the sulfate at the beta Pi binding site may prevent obtaining the full nucleotide-free conformation and this should be more explicitly included.

We agree with the referee that the sulfate ion might affect the conformation of the nucleotide-binding pocket. We thus added the following sentence in p.11: "This structure therefore represents nucleotide-free KIF5C, at the resolution of 2.9 Å (Table 1), although there remains the possibility that the presence of the sulfate ion might prevent from taking the "full" nucleotide-free conformation."

2. Although Fig. 10 may incorporate reasonable changes in the ATP binding site, it is still not clear that the movements of the neck linker are in agreement with the Rice et al model and this needs to be more explicitly included.

We thank the referee for the insightful comments. At the current stage, this discrepancy could not be firmly explained and further structural studies are needed in the immediate future. As you may know, the neck-linker conformation is completely coupled with the switch II conformation. Up to now, two different conformations of switch II-neck-linker have been reported. Nucleotide-free KIF5 on the GDP-taxol microtubule takes the ADP-like switch II with undocked neck-linker, as also suggested by the Rice's result. On the other hand, our nucleotide-free KIF5 on the GMPCPP-microtubule as well as nucleotide-free kar3 on the GDP-taxol microtubule take the Rigor switch II with NIS-docked neck-linker. NIS-docked conformation in the Mg-releasing intermediate of KIF1A was also observed in the absence of microtubule. Hence, there remain two possibilities to explain the discrepancy. One possibility is that nucleotide-dependent conformational difference of microtubule (GTP vs GDP) may induce the different mechanical cycles of kinesin. The other is that kinesins on the different types of microtubules will be stabilized at the different steps during the same mechanical cycle. Several results (KIF1A without microtubule can take the NIS-docked conformation or initial portion of neck-linker is necessary for the effective ADP release…etc.) indirectly suggest that the latter possibility might be likely, albeit more structural and biochemical studies are necessary to confirm this. We have added this discussion in pp.24-25 within the word limit.

