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Specific KIF1A-adaptor interactions control selective cargo recognition

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May 18, 2021

Re: JCB manuscript #202105011T

Dr. Casper C Hoogenraad
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Netherlands

Dear Dr. Hoogenraad,

Thank you for submitting your manuscript entitled "Specific KIF1A-adaptor interactions control selective cargo recognition" from Review Commons. We have discussed your study, the original reviews, and your revision plan. We agree that your study is of interest to the readership of JCB, therefore we invite you to submit a revision as you have outlined in your response.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

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We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Patrik Verstreken, PhD
Monitoring Editor

Andrea L. Marat, PhD
Senior Scientific Editor

Journal of Cell Biology

Reviewer #1

In this study Hummel and Hoogenraad explore how the kinesin-3 family member Kif1A engages certain types of vesicle cargo. Working in hippocampal neurons the authors use an inducible dimerization system comprising Kif5C motor domains and the cargo-binding tail of Kif1A to examine which vesicles Kif1A can associate with and transport. They conclude that Kif1A can bind to dense core vesicles (DCVs), lysosomes and synaptic vesicles. Using the same system and a series of truncations/deletions, the authors implicate the coiled coil 3 (CC3) and C-terminal PH domains as both required for vesicle recruitment. This is reinforced by a series of knockdown and rescue experiments. These are also used to show that the CC2 region of the protein can be replaced with a leucine zipper, suggesting that its main role is in dimerization of the motor. Next, the role of a S to Q mutation in the PH domain (linked to neurological disease) is examined. This mutant is shown to be transport defective whereas an S to D mutant is not, leading the authors to suggest that serine phosphorylation may be important for its function. Previously, the same group has shown that Kif1A has a calmodulin binding site, mutation of this site also prevents transport, as does chemical inhibition of the Ca2+/calmodulin-dependent protein kinase II (CaMKII). The authors suggest that CaMKII phosphorylates the PH domain to allow vesicle association. Finally, the authors consider the role of adaptor proteins, showing that several previously described adaptors function in hippocampal neurons as in other systems, bind to CC3 and implicate Arl8A (over Arl8B) in lysosome transport.

****Major Comments****

The case for the overall conclusion that cargo selection requires both CC3 mediate cargo adaptor interactions and membrane interactions via the PH domain is convincing, as is the role of CC2 in contributing to dimerization of the motor. However, two of the key mechanistic propositions are less well supported and I suggest that these are addressed experimentally or qualified.

1. The role of phosphorylation of S1665. On the basis that a Q substitution (non-phosphorylatable) causes loss of function and a D substitution (phosphomimetic) retains function, the authors conclude that 'Phosphorylation of the PH domain enables binding to any vesicle .... (Abstract)'. In my opinion, this conclusion is not yet well supported by experimental data. No evidence is presented that S1665 is actually phosphorylated in any circumstance (also a brief examination of phosphoproteomic databases by this reviewer couldn't find any either). The effect of CaMKII inhibition is consistent with a role for phosphorylation but is not linked to this site. Unless the authors can show that this residue is indeed phosphorylated and that manipulations affect is phosphorylation state (mass spectrometry analysis perhaps) the authors should qualify their statements in this area.

The reviewer is correct in that the S1665 site has so far not been identified as a phosphorylation site in the current databases. We do believe that our data strongly suggests that phosphorylation of this site is involved in the cargo interaction, however we agree with the reviewer that these results should be

further validated. In the revised manuscript we toned down our statements regarding phosphorylation of the PH domain and suggest a mechanism in which KIF1A phosphorylation, potentially in the PH domain, is required for cargo interaction.

2. The authors conclude that 'CC2 domain of KIF1A is responsible for motor dimerization'. Again, I think this overstates the data. The substitution with the LZ gives a good indication that CC2 dimerization is a key function of this sequence. However, the implication that the absence of higher bands in 293T extracts run on native cells of GFP-fusions of the other domains suggests that this is the most important driver of self-association for the full length molecule is not well founded. This is not a robust dimerization assay and the CC1-FHA module apparently exists as a stable dimer (PDB: 4EJQ; Hou et al. Structure 2012). As such, statements around this should be qualified appropriately or tested biochemically by comparing self-association of full length proteins carrying CC1 and CC2 deletions in, for example, immunoprecipitation assays.

We agree with the reviewer that the conclusions based on these results are too strong. Therefore, in the revised manuscript we have toned down these conclusions.

****Minor Comments****

It would be helpful if the basis for making the S1665 mutation in their Kif1A isoform were better described. Gabrych et al. is cited but this appears to be a review and here I believe it is specified as S1758Q in a supplementary table. How does this isoform relate to the one used by the authors? - I don't doubt that this is the correct residue, but it will be helpful for the reader to understand how various Kif1A isoforms/constructs may differ to be able to link this work in with theliterature. Also, the original paper reporting this mutation appears to be Riviere et al. (10.1016/j.ajhg.2011.06.013) and so should be cited if so.

The reviewer is correct that in literature the S1758Q mutation has been reported. However, this is in human KIF1A. The KIF1A construct we have used in our study is mouse KIF1A. The human S1758Q mutation corresponds with the S1665Q mutation in mouse KIF1A. In the revised manuscript we have clarified this in the corresponding results section. We also included the reference to the original paper of Riviere et al.

****Significance****

This is a carefully performed study that integrates and tests somewhat disparate existing knowledge and hypotheses in a systematic fashion and builds them on to draw some insightful conclusions, and proposes a general model for Kif1A-cargo selection in neurons. It will be of interest to those studying neuronal transport generally as well as those with a more specific interest in kinesin-3 family function and regulation. Part of the potential impact of the study comes from conclusions that are not fully

supported by the data, particularly the phosphorylation of the PH domain (see above). Qualification of of this would reduce the significance, further data to support this would enhance the significance.

Reviewer #2

The manuscript by Hummel and Hoogenraad is a cell biological structure-function study dissecting the role of coiled-coil domains in the kinesin KIF1A. The authors find specific roles of CC1, 2 and 3 with respect to autoinhibition, dimerization and cargo binding using dissociated neurons. They apply the rapalog-system, coupling the KIF1A tail with the KIF5 motor domain in combination with several deletion constructs. They further study full-length KIF1A on the background of KIF1A knockdown conditions. Finally, they identify Arl8A and MADD as cargo adapters for DCV/lysosomes or SVs, respectively.

A major concern is that the study is still premature, since many experiments are based on single or maximally two independent experiments. Figure legends mention N-values of 'one' for 1E, 1H, 1I, 2D, 2H, 2L, 5B/C. Likewise, subfigures 2C, 2G, 2K, 4I, 4J, 5G and 5I are based on 'two' independent experiments, only. To meet scientific standards and to control the use of identical constructs, a minimum of 'three' independent transfection experiments (N) is required per condition.

We agree with the reviewer that for biological experiments a minimum of three independent transfection experiments are required. Therefore, in the revised manuscript we have added data to finalize N=3 for the experiments in Figures 1G, 1H, S2A, S2B, 2C, 2G, 2K, 4I, 4J, 5G and 5I. The data shown in Figures 1C, 2D, 2H, 2L, 5B, 5C were obtained by an initial screen to identify potential interactions. Increasing the N number for the specific subset of data is unfortunately not possible in the current lab situation. All the results obtained from the initial screen are further validated using other biological experiments for which we included three independent replicas.

****Specific Points:****

1. Figure 1C: it is not clearly mentioned how KIF1Atd is detected (fusion protein or staining)? In the KIF1Atd condition +Rap, there is less signal in the soma but hardly any signal in neurites. Images should be displayed with larger size and contrast.

All the FRB constructs used throughout the paper are fused to a 3myc tag and visualized by immunofluorescence staining with a myc antibody. In the revised manuscript we have explained this in the first results section. In the revised manuscript we have moved Figure 1C to Figure S1A, where we included larger images to more clearly show the rapalog induced binding of the tail domain to the motor domain shown.

2. Figure 1D: in the KIF5Cmd image -Rap one can hardly identify the KIF5 signal. How will this small amount of KIF5 mediate the transport effect? The KIF1Atd channel should be included to 1D.

We agree with the reviewer that KIF5C signal seems to be low, which is mainly due to the motor accumulation at very specific points, namely in distal axonal tips. However, this is no indication for the amount of motor present in the cell as there might be a lot of motor present in one tip. We expect that there is sufficient amount of motor present as we do see that axonal tips are larger than normal, suggesting high protein content. Also, as we do observe clear transport of cargo upon rapalog induced binding of the motor and tail domain and we believe that there is sufficient KIF5C present to facilitate this transport. In the revised manuscript we have added the KIF1Atd channel to Figure 1D.

3. A KIF1A motor domain should be used as control in the rapalog assay.
The KIF1A motor domain is actively translocating into both axons and dendrites (Yang *et al.*, 2016). Using this motor domain in our assay will therefore result in cargo translocation into both axonal and dendritic tips. As the motor-cargo complex will be distributed over a larger cell area, re-localization of cargo is less clear. The KIF5C motor domain provides a robust and clear readout and we therefore focused on this motor domain in our assay.

4. Figures 1-5: there is many boxed areas displaying putative axons, however some axons are clearly dendrites. Either select clear examples or use an AIS marker for instance in Figure 2F.
For all images where an axon is selected, the axon was selected based on a TRIM46 staining. In the revised manuscript we have added images of the TRIM46 staining to all relevant figures.

5. Figure 2B: why is the isolated PH domain diffusely distributed if it binds to vesicular cargoes? Does overexpression of the PH construct compete with cargo binding?
We identified that the PH domain is involved in the cargo interaction. However, our data show that the PH domain alone is not sufficient for cargo binding. This conclusion is made based on our observations that the PH domain is diffusely distributed in cells (Figure 2B) and that the PH domain alone is not sufficient to translocate cargo in our engineered motor assay (Figure 2D).

6. Figure 4A-D: some labeling is mixed up here. For instance, in Figure 4B NPY labels a punctate pattern in the grey channel and labels the diffuse (green) staining in the merged channel.
We thank the reviewer for carefully addressing our Figures. Indeed, there is mistake in the labelling of Figure 4A-D. We have corrected the labelling in the revised manuscript.

7. In general, cotransport is hardly visible in Figures 4A-D. Data should be quantified.
We agree with the reviewer that the data in Figures 4A-D should be quantified. In the revised manuscript we have added a quantification of NPY co-localization with KIF1A during live-cell imaging in Figure S4B.

8. Figure S3E: under SDS conditions, GFP is about 30 kDa. In the blot shown here it runs at 50 kDa (control lane, left). Please clarify whether loading was correct.

We agree with the reviewer that the size of GFP is larger than one would expect. The GFP-construct we used contains an additional sequence of 150 basepairs containing a multiple cloning site. This explains why it runs at a larger size on both our normal and native gel. We have added this information on the construct in the method section of the revised manuscript.

9. Figures 3 and 5 (knockdown KIF1A, Arl8A/B, MADD): how efficient are the knockdown constructs used here? Although the kymographs reduce transport and can be considered as functional controls, it is important to measure the reduction of the protein of interest by immunostaining.

The shRNA constructs used for KD of Arl8A/B were designed in this study and validated by immunofluorescence staining. This quantification is shown in Figures S4A and S4C. In the revised manuscript we have also added example images showing the immunostaining of the KD of Arl8A and Arl8B in Figures S4B and S4D. The shRNAs used for KIF1A and MADD have been described and validated before (Niwa, Tanaka and Hirokawa, 2008; Kevenaar *et al.*, 2016).

****Significance****

The project is a nice piece of work that is of interest to the neuroscience and cell biological communities. It provides novel insights into the structure and function of the kinesin KIF1A. Although motor dimerization via CC2 has been previously published, it extends published knowledge about motor regulation significantly.

Following a major revision, I expect that it will become suitable to Review Commons journals.

Reviewer #3

This manuscript takes on an important question in understanding mechanisms responsible for regulation of axonal transport. The focus is an important form of kinesin - Kif1a - which has been implicated in transport of synaptic vesicles, dense core vesicles, and lysosomes. Kif1a is a dimer of very large polypeptides. Even the most basic questions regarding Kif1a structure vs. function - e. g., the requirement for dimerization for motor activity - have proven challenging. Multiple forms of subcellular cargo have been identified, but evidence for distinct mechanisms for their recruitment to the motor protein have been incomplete and confusing.

The authors (only two) have devised and undertaken a heroic program of investigation to sort out these basic issues. Using a variety of fixed and live in situ assays for the behavior of cargo and motor behavior, they propose a comprehensive model for Kif1a transport. Cargo binding is mediated by two domains within the Kif1a "stalk", CC3 and the C-terminal PH domain. The latter are proposed to function cooperatively in cargo selection.

An entirely new finding is a calmodulin/Cam Kinase-dependent mechanism for phosphorylation of the PH domain at a discrete residue, activating this region to participate in cargo recruitment along with the Kif1a CC3 region .

This paper should serve as a potent stimulus to further research on this surprisingly complex motor protein. It should also help provide a timely framework for correlating human disease mutations with their individual phenotypic effects.

****Specific issues:****

1) It would be helpful to include more about known or potential mechanisms for calmodulin regulation of microtubule-based motor protein transport. How might Ca++ levels affect the redistribution of known cargo forms, for example.

It is indeed interesting to include some more information on the Ca^{2+}-mediated regulation of MT based transport by kinesin motors. Interestingly, within the field of bionanotechnology Ca^{2+} regulated kinesin motors have been engineered. Monomeric kinesin motors are fused to a CaM binding domain. Increased Ca^{2+} levels induce dimerization of CaM, which binds and dimerized the monomeric kinesin motors. These Ca^{2+}-CaM activated motors were found to move along MTs, suggesting a role for Ca^{2+} regulation in kinesin motor activity (Shishido and Maruta, 2012) . Furthermore, it was found that Ca^{2+} levels regulate kinesin-1 mediated transport of mitochondria via its interactor Miro and increased Ca^{2+} levels arrest mitochondrial transport (Wang and Schwarz, 2009). We have included a more in-depth discussion on this in the revised manuscript.

2) Is there precedent for phosphoregulation of PH domains in other proteins? How would the site of altered charge affect phosphoinositide binding? Do the known forms of Kif1a cargo binding contain any common phosphoinositides?

The reviewer makes very interesting comments about the PH domain and we would like to speculate that the phosphorylated residue increases interaction with phosphoinositides on the membrane of cargo vesicles. In the revised manuscript, we have added some discussion on this.

3) For completeness, the authors might comment on the function of the FHA domain, and how its behavior might would fit with cargo selection, motor activation, etc.

We agree with the reviewer that for completeness the FHA domain should also be included. Previously, it has been shown that the FHA domain is involved in motor activation (Lee *et al.*, 2004; Huo *et al.*, 2012). In the revised manuscript we have added a few sentences in the discussion on the function of the FHA domain.

4) Is it possible that CC3 might affect cargo binding indirectly via changes to autoinhibition or motor activation?

In our engineered motor assay, we find that removal of the CC3 domain from the KIF1A tail domain diminishes the interaction with cargo vesicles. As we use the constitutively active KIF5C motor domain in this assay, we look at vesicle interaction and not directly at KIF5C motor activity regulation. These results therefore suggest that cargo binding (and not KIF5C motor activity) is directly regulated by CC3. In addition – in a KIF1A motor context - we show that removing the CC3 domain from full-length KIF1A results in a non-processive motor in COS7 cells (Figure S3B) and in neurons (Figure 4D), suggesting that CC3 is involved in regulation of KIF1A motor activity. This is clarified in the discussion.

Over-all the work is of high quality, using state-of-the-art techniques. Although much is already known of Kif1a mechanism, part of the value of the current study is in providing an advanced framework for making sense of Kif1a structure and function. The work should also e be of timely value in understanding the genetic basis of KIF1A-associated neurological disease (KAND).

****Significance****

Kif1a is a very important, but very complex member of the kinesin family of motor proteins. Mutations throughout the Kif1a polypeptide cause human neurological disease. Progress in understanding Kif1a mechanism has been delayed by its large size and multiplicity of functional and structural domains. This study completes our basic understanding of how Kif1a works, and provides new insight into coordinate roles of the two known Kif1a cargo binding domains.

This study does revisit some known material regarding Kif1a function, but there is clear novelty in the CC3-PH domain interactions, and in the advanced framework provided for understanding Kif1a behavior.

References

Huo, L. *et al.* (2012) 'The CC1-FHA tandem as a central hub for controlling the dimerization and activation of kinesin-3 KIF1A', *Structure*. Elsevier Ltd, 20(9), pp. 1550–1561.

Kevenaar, J. T. *et al.* (2016) 'Kinesin-binding protein controls microtubule dynamics and cargo trafficking by regulating kinesin motor activity', *Current Biology*, 26(7), pp. 849–861.

Lee, J. R. *et al.* (2004) 'An intramolecular interaction between the FHA domain and a coiled coil negatively regulates the kinesin motor KIF1A', *EMBO Journal*, 23(7), pp. 1506–1515.

Niwa, S., Tanaka, Y. and Hirokawa, N. (2008) 'KIF1Bβ- and KIF1A-mediated axonal transport of presynaptic regulator Rab3 occurs in a GTP-dependent manner through DENN/MADD', *Nature Cell Biology*, 10(11), pp. 1269–1279.

Shishido, H. and Maruta, S. (2012) 'Engineering of a novel Ca 2+-regulated kinesin molecular motor using a calmodulin dimer linker', *Biochemical and Biophysical Research Communications*. Elsevier Inc., 423(2), pp. 386–391.

Wang, X. and Schwarz, T. L. (2009) 'The mechanism of Ca2+-dependent regulation of kinesin-mediated mitochondrial motility', *Cell*. Elsevier Inc., 136(1), pp. 163–174.

Yang, R. *et al.* (2016) 'Analyzing kinesin motor domain translocation in cultured hippocampal neurons', *Methods in Cell Biology*, 131, pp. 217–232.

June 21, 2021

RE: JCB Manuscript #202105011R

Dr. Casper C Hoogenraad
Utrecht University
Faculty of Science Padualaan 8
Utrecht 3584CH
Netherlands

Dear Dr. Hoogenraad:

Thank you for submitting your revised manuscript entitled "Specific KIF1A-adaptor interactions control selective cargo recognition". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

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10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles/Tools may have up to 5 supplemental display items (figures and tables). Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

11) eTOC summary: A ~40-50-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.

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14) A separate author contribution section following the Acknowledgments. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

B. FINAL FILES:

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Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Patrik Verstreken, PhD
Monitoring Editor

Andrea L. Marat, PhD
Senior Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

This is a very nice paper that I believe is suitable for publication in JCB. The authors have addressed my comments in the revised manuscript.

There remain a few examples where where I believe the claim regarding phosphorylation of S1665 overstates the data e.g. Top of page 16: 'we show that phosphorylation of this residue is essential'. I have already set out my thoughts on this, so I'll leave it to the authors to decide how they wish to describe their findings.

Reviewer #2 (Comments to the Authors (Required)):

The ms. is now suitable for publication

Reviewer #3 (Comments to the Authors (Required)):

The manuscript is a structure-function study on the kinesin motor KIF1A,investigating the selectivity and regulation of transport and cargo specificity by different protein domains. It shows that KIF1A-mediated cargo trafficking involves motor dimerization, PTMs as well as cargo adapters.

The study is a nice and interesting piece of work addressing motor-cargo interactions that fits to JCB.

In the revised manuscript the authors have sufficiently addressed my concerns and I can now support publication in its present form.