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A kinesin-1 binding motif in vaccinia virus that is widespread throughout the human genome

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Review timeline:

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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 08 August 2011

Many thanks for transferring your manuscript to the EMBO Journal. I have now read it and the associated transferred referee reports carefully, and have sought additional input from an arbitrating referee, whose comments are enclosed below. As a result, I am pleased to say that - pending minor revision - we would be able to offer publication of your work in EMBOJ.

As you will see, the referee finds the work to be of a high standard and well suited to publication here. His/her only concern echoes the one we have already discussed: that is, the false positive rate from your bioinformatic screen. It would be important to state clearly how many candidates you tested that did not give a positive result in your validation assays, and to discuss this explicitly.

A couple of points from my side as well:
- In Figure 3A, left panel, the histograms show identical values for WE/AA and WD/AA (which is rather surprising), and yet it looks to me that the averages are slightly different in the inset. I wonder whether something has been plotted wrongly here - perhaps you can look into this and clarify?
- Given the concerns expressed by the referees in the initial submission elsewhere as to whether - in the light of the previous literature - your analysis of the A36 KLC binding site genuinely represents the 'identification' of a novel binding site, I would be inclined to change the title. It seems to me that, while the text is very accurate in representing what was already known and what you have added, the title might give the impression that this is a completely new motif. I'm happy to discuss alternatives if you like...

I would therefore ask you to revise the text accordingly, and to submit the revision as soon as possible. We should then be able to proceed rapidly to acceptance and production of the work.

Please let me know if you have any further questions; otherwise I look forward to receiving your revision.

With best wishes,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

In this manuscript Dodding et al demonstrate carefully that a bipartite tryptophan-based motif found in the Vaccinia virus protein A36 mediates its binding to kinesin light chain and kinesin-1 dependent motility of the virus toward the cell periphery.
As a similar motif exists in the KLC binding region of Calsyntenin the authors then test whether substituting the cytoplasmic region of A36 (containing the KLC binding motif) by the region of Calsyntenin containing the WD motifs may restore the motility of the virus to the cell periphery. They find that this is indeed the case, setting up a very efficient assay to test for kinesin-1 recruitment of any candidate protein.
Based on these data the authors use a bioinformatics approach to look for human KLC binding proteins based on the presence of a bipartite WE/D motif in their sequence. This approach generates a list of 460 putative KLC binding proteins including known KLC binding partners like DYNC1I1), Huntingtin associated protein (HAP1) and SKIP.
The authors go on into validating the prediction using their assay for kinesin-1 dependent motility of virus particles with the predicted KLC binding motifs of several of these proteins fused to the transmembrane domain of A36. Interestingly, while all of them are indeed able to restore motility they show different preferences for KLC1 or KLC2.
This work is carefully done and convincing. Despite the many functions of kinesin-1 in intracellular motility, few cargoes of this motor have been identified. Although it is well possible that not all the proteins identified through the bioinformatics approach are bonafide KLC binding partners, it is a resource that will be extremely useful for a number of researchers in various fields. Moreover the possibility to use the assay developed by the authors to test efficiently for KLC binding and kinesin-1 motility will provide an efficient way to validate efficiently any candidate of interest.
One minor comment:
It seems that any candidate that the authors used for validation were positive. Is this the case or did they find some that did not restore A36 motility to the cell periphery? In this case what was the percentage of success?

1st Revision - authors' response 08 August 2011

Reviewer

One minor comment:

It seems that any candidate that the authors used for validation were positive. Is this the case or did they find some that did not restore A36 motility to the cell periphery? In this case what was the percentage of success?

As you will see, the referee finds the work to be of a high standard and well suited to publication here. His/her only concern echoes the one we have already discussed: that is, the false positive rate from your bioinformatic screen. It would be important to state clearly how many candidates you tested that did not give a positive result in your validation assays, and to discuss this explicitly.

As we have discussed by email the false positive rate in our transport assay is an issue that is hard to judge. Given this we have changed the text and added additional lines into pages 11-12 when we discuss the results using our transport assay. We think this additional text states our position clearly

and alerts readers to the limitations of our assay.

“Our search pattern identified 460 proteins that have the potential to bind KLC1/2. This list will undoubtedly contain false positives and is unlikely to be exhaustive. However, it did identify bi-partite tryptophan motifs in the kinesin-1 binding regions of SKIP, HAP1 and the Dynein intermediate chain (Boucrot et al, 2005; Dumont et al, 2010; McGuire et al, 2006). Using our virus transport assay, we confirmed that the bi-partite tryptophan based motifs of SKIP are capable of recruiting kinesin-1 and conveying IEV to the cell periphery. We were also able to show that bi-partite tryptophan motifs from ATF6, BSDC1, FAM63B, LDLRAP1, PARC, PRKAG3, RASSF8 and RIC3 could rescue IEV transport to the cell periphery in the absence of A36. In contrast, we found that single clones containing the bi-partite tryptophan motifs of DAB2, EMP2AIP, GRP, HYLS1, Nostrin and PCM1 did not rescue transport of the virus to the cell periphery in the absence of A36. However, it is hard to draw an unambiguous conclusion from this negative data, as the ability to recruit kinesin-1 is likely to be highly dependent on how the protein is presented on the surface of the virus, which will vary depending the position of the fusion to the transmembrane domain of A36. Consistent with this notion, pull down assays demonstrated that Nostrin can interact with KLC1. We were also able to confirm that full-length ATF6, BSDC1, FAM63B, LDLRAP1, PARC, PRKAG3, RASSF8 and RIC3 could interact with KLC.:

A couple of points from my side as well:
In Figure 3A, left panel, the histograms show identical values for WE/AA and WD/AA (which is rather surprising), and yet it looks to me that the averages are slightly different in the inset. I wonder whether something has been plotted wrongly here - perhaps you can look into this and clarify?

Good spot…Mark had accidently duplicated the data used to plot the graph. The correct data has now been used to generate the figure so the graphs now look quite different.

Given the concerns expressed by the referees in the initial submission elsewhere as to whether - in the light of the previous literature - your analysis of the A36 KLC binding site genuinely represents the 'identification' of a novel binding site, I would be inclined to change the title. It seems to me that, while the text is very accurate in representing what was already known and what you have added, the title might give the impression that this is a completely new motif. I'm happy to discuss alternatives if you like...

As we stated before we do not feel the title implies we found a new motif. Nevertheless we are happy to change it to make sure we do not give this impression. We have changed the title to

“A KLC binding motif in vaccinia virus that is wide spread throughout the human genome”

We have also changed the abstract to make it clear we’re not suggesting we have found a new motif.

New abstract
“Transport of cargoes by kinesin-1 is essential for many cellular processes. Nevertheless, the number of proteins known to recruit Kinesin-1 via its cargo binding light chain (KLC) is still quite small. We also know relatively little about the molecular features that define Kinesin-1 binding. We now show that a bipartite tryptophan-based motif, originally identified in Calsyntenin is present in A36, an integral vaccinia membrane protein. This bipartite motif in A36 is required for kinesin-1-dependent transport of the virus to the cell periphery. Bioinformatic analysis reveals that related bipartite tryptophan-based motifs are present in over 450 human proteins. Using vaccinia as a surrogate cargo we show that regions of proteins containing this motif can function to recruit KLC and promote virus transport in the absence of A36. These proteins interact with the kinesin light chain outside the context of infection and have distinct preferences for KLC1 and KLC2. Our observations demonstrate KLC binding depends on a common set of features that are found in a wide range of proteins associated with diverse cellular functions and human diseases.”

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