JCB

Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation

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March 27, 2020

Re: JCB manuscript #202003038

Prof. Martin Humphries
University of Manchester
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United Kingdom

Dear Martin,

Thank you for submitting your manuscript entitled "Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

As you can see, the reviews from these three expert reviewers - all of them acknowledged leaders in the fields related to your study - are all enthusiastic about your study and support acceptance after considering the specific points they raise.

Please consider carefully each point they raise, using your judgment concerning length limitations for added text. We look forward to a resubmitted manuscript that provides further discussion about the choice of biological system (cell type) and baits, speculation on timing and alternative substrates, and especially comparisons with previous publications in the field.

Thanks very much for submitting this well-documented, valuable advance to JCB.

With kind regards,

Ken

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:

- Please provide a short eTOC statement
- Provide the main and supplementary texts as separate, editable .doc or .docx files
- Provide main and supplementary figures as separate, editable files according to the instructions for authors on JCB's website *paying particular attention to the guidelines for preparing images and blots at sufficient resolution for screening and production* (N.B. text sizes may be too small on some figures)
- Supplementary figures must fit on one page, S1 currently spans three. Although we have a limit of three supplementary figures, it would be fine to get these down to four.
- Provide tables as excel files

- Format references for JCB
- Add paragraph after the Materials and Methods section briefly summarizing all "Online Supplementary Materials"
- Add author contributions

GENERAL GUIDELINES:

Text limits: Character count for an Tools is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Toolss may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Toolss may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

The typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

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,

Kenneth Yamada, M.D., Ph.D.
Editor

Marie Anne O'Donnell, Ph.D.
Scientific Editor

Journal of Cell Biology

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

This manuscript from the Humphries laboratory describes a massive, commendable, effort to use proximity biotinylation to define the topology and interaction hierarchy of integrin adhesion complexes. The field has benefitted enormously from previous experiments using mass-spec to define adhesion components with variable purification and enrichment methods. These 7 studies have defined the so-called "meta adhesome". In the past, the Humphries laboratory has curated the consensus adhesomes (60 components), based on the overlap of proteins identified in the meta-adhesome. This has become a highly cited and valued resource. The data in this manuscript, provides a huge new leap in our understanding of IAC composition and interconnectivity. Chastney et al. have multiplexed BioID data from a set of 16 IAC component baits to generate a proximity-dependent adhesome, representing both the core adhesome machinery and proximal interactors more peripheral to IACs. This carefully prepared and analysed dataset has many novel observations that will be seminal to launching new investigation into IAC biology. In addition to highlighting currently under appreciated components and nodes in IAC, these data are also provide important experimental validation in support of earlier studies aiming to define IAC architecture. The protein proximity data in this manuscript broadly correlates with the stratified architecture of IACs determined by super-resolution microscopy and supports recent work in Drosophila. In addition, the authors have taken advantage of the BirA approach to determine protein stratification based on talin biotinylation profiles by specific baits. This seems like a novel extension of the BioID technique and significantly increases the granularity of these data in defining IAC component localization with respect to each other.
These data are of immediate value and broad utility to the cell biology community and the data with using talin biotinylation as an "intra IAC ruler" provide proof-of-principle and show the potential cell biological value of this approach.

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

In this manuscript entitled "Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation", Chastney and collaborators mapped experimentally the interactome of 16 proteins known to be part of integrin adhesion complexes (IACs) using proximity biotinylation coupled to label-free quantitative mass-spectrometry. First, using computational biology, their analysis revealed the precise cartography of 350+ interactions between 147 distinct proteins, most of these interactions being reported experimentally for the first time. Then, by precisely locating the sites of biotinylation detected by mass spectrometry and prior knowledge on proteins' structural features, the authors demonstrated that IACs present a stratified organization using the compelling example of talin (Supp Fig 3) to illustrate this. Further analysis of the data led to the categorization of the interactors into 5 clusters of baits and 16 clusters of bait interactors (preys) underlying possible functional relevance. Last, the authors highlight some of the proteins found in this study but whose roles in IAC formation or regulation have, until now, not extensively been studied.

This is an elegant study that uses state-of-the-art biochemical and computational approaches to tackle a fundamental biological problem: how do cells build structures permitting their adhesion to the ECM? The manuscript is beautifully written and the datasets generated (shared as supplementary tables as well as raw data for mass-spec aficionados) provide a useful resource to the scientific community.

I have a few minor comments that are aimed at clarifying a few technical and conceptual points, mostly for non-adhesome specialists:

- Could the authors provide more details about the selection of the 16 baits. They refer to the fact that they cover the 4 signaling modules identified by the analysis of the computationally-predicted adhesome, but Figure 1A suggests that they are all activated downstream of the same integrins (a5/b1 or av/b3). Could the authors expand on the concept of signaling modules downstream of integrins for non-integrin experts?

- Could the authors present in a figure or table the overlap between the computationally-predicted adhesome or the the consensus adhesome (60 proteins), and the adhesome experimentally-defined here.

- Could the authors justify the choice of the model system used (mouse pancreatic fibroblasts). Which integrins are expressed by these cells? Why having decided not to grow them on any ECMs for the assay?

- Related to that, could the authors briefly comment on how the molecular composition of IACs may change when cells are plated on different ECM proteins in vitro or encounter different microenvironments in vivo.

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

In this manuscript, the proximity biotinylation approach was applied together with mass spectrometry to identify proteins presence within 10-15 nm of several bait proteins within the integrin adhesions. This approach enabled the inference of protein-protein interactions and spatial organization within the adhesions. Bioinformatic analysis of the results for pancreatic fibroblasts reveal distinct modular organizations that appear to be in line with current model of adhesion structural organization.
Overall, this appears to be the most comprehensive protein-protein interaction mapping studies of integrin adhesions, to date. The manuscript is well-written and the authors are well established investigators in this area. This will likely be a useful resource to the community and would be highly appropriate for JCB. A few comments below:
1. One of the potential limitations of the BioID approach would seem to be the relatively low temporal resolution as the cells are incubated with biotin for 24 hr. There are a number of implications that perhaps may merit some discussion:
1.1 The integrin adhesions are dynamic structures that form and mature in minutes. With 24 h incubation time, the analysis performed here would likely be over-weighting the contribution of the mature focal adhesions or even fibrillary adhesions as these are likely more massive/abundant. Would a shorter time course be possible, as in Dong et al., Sci Signaling 2014, 5 hr. incubation time was used?
1.2 Similarly, with the long incubation time, proteins may translocate through different compartments of the cells and the results would reflect not just the adhesion-resident proteins, but possibly other cellular compartments.
1.3 Perhaps a control whereby the cells are on non-specific surfaces such as polylysine would be useful? If this is feasible, this would help differentiate whether the protein-protein interactions are adhesion-specific or constitutive or cytoplasmic-only.
2. Perhaps some biological context on why pancreatic fibroblasts was used in this study can be

provided. In particular, cell-type specificity are increasingly appreciated in many aspects of adhesions organization and functions and not just adhesome protein-protein interactions (for example: the variation in protein organization in embryonic stem cells was also shown in Xia et al., ACS Biomaterials Sci & Eng, 2019, which the authors may not be aware of) but thus far, data from different studies tend to come from different cell types, which makes it difficult to separate different sources of variability.

3. How are the modular organizations observed here compared to information obtained from previous studies such as Hoffmann.. eLife 2014 or in Zamir et al. PLOS ONE 2008 (which the authors may not be aware of)?

4. A previous BioID study using Paxillin and Kindlin2 as baits (Dong et al., Sci. Signaling 2014) also provided quite a comprehensive analysis of protein-protein interaction. To make these resources more useful to the community, it would be worthwhile to compare the results of the current study with such previous study (e.g. in terms of coverage, interaction topology, any cell-type dependent variability), as the average cell biologists to whom this could be useful but who are not specialists would probably not be able to do these comparisons easily on their own.

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April 9th, 2020

Dr. K.M. Yamada
Journal of Cell Biology

Dear Ken:

Many thanks for your email of April 3rd. We were delighted that our manuscript "Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation" received such favourable reviews and that you have invited a resubmission.

We have made the editorial changes that you requested in the uploaded files, and below we present a response to each of the points raised by the reviewers (verbatim reviewer comments in italics).

Reviewer 1

There were no points raised.

Reviewer 2

1. Could the authors provide more details about the selection of the 16 baits. They refer to the fact that they cover the 4 signaling modules identified by the analysis of the computationally-predicted adhesome, but Figure 1A suggests that they are all activated downstream of the same integrins (a5/b1 or av/b3). Could the authors expand on the concept of signaling modules downstream of integrins for non-integrin experts?

Text has been inserted in the first paragraph of the Results section (page 6):
"There is evidence for all four of these axes contributing to integrin-dependent signalling and mechanotransduction but, as yet, no systems-level understanding of their integration has been generated. The extent to which these axes assemble independently or, conversely, whether they are all interconnected is also unclear."

2. Could the authors present in a figure or table the overlap between the computationally-predicted adhesome or the the consensus adhesome (60 proteins), and the adhesome

experimentally-defined here.

Such a table was present in the original manuscript (Table S1).

3. Could the authors justify the choice of the model system used (mouse pancreatic fibroblasts). Which integrins are expressed by these cells? Why having decided not to grow them on any ECMs for the assay?

The actual reason that pancreatic fibroblasts were chosen for this study is that the work was funded by Cancer Research UK and has the aim of elucidating mechanotransduction pathways in pancreatic cancer. However, as mechanotransduction is not part of the current manuscript (such manuscripts will follow), it is not a relevant justification. Instead, we hope it is sufficient to confirm that these cells are morphologically similar, and adhere in a related manner to, other standard primary fibroblasts, such as those from the foreskin or other dermal tissues.

Although we have yet to test the full range of integrin alpha- and beta-subunits, we have flow cytometry data demonstrating that the pancreatic fibroblasts express integrin β1, β4, α5 and α6 in long-term culture, with low levels of β3 and α3. Integrin αv was not tested by flow cytometry, but has been detected by mass spectrometry in ventral membrane preparations of IACs (short-term culture on fibronectin), alongside integrin β1 and α5 (the three most commonly-detected integrin subunits in the consensus/meta adhesome). These cells therefore have an integrin profile that is common to most fibroblasts.

We did not plate the cells on an ECM ligand because, over the timeframe of a BioID experiment, there would be sufficient time for the cells to assemble their own ECM. It would therefore be unsafe to conclude that the original ligand was being used.

4. Related to that, could the authors briefly comment on how the molecular composition of IACs may change when cells are plated on different ECM proteins in vitro or encounter different microenvironments in vivo.

Unfortunately, we don't as yet know the answer to this question and it is impossible to speculate. However, a clause has been added to the final paragraph (page 19) to recognise the importance of this issue, and the fact that BioID is a relevant technique to explore it:
"Future studies that focus on how this network is altered <u>when the composition of the extracellular matrix varies and</u> under disease-relevant conditions"

Reviewer 3

1. One of the potential limitations of the BioID approach would seem to be the relatively low temporal resolution as the cells are incubated with biotin for 24 hr. There are a number of implications that perhaps may merit some discussion:
1.1 The integrin adhesions are dynamic structures that form and mature in minutes. With 24 h incubation time, the analysis performed here would likely be over-weighting the contribution of the mature focal adhesions or even fibrillary adhesions as these are likely more massive/abundant. Would a shorter time course be possible, as in Dong et al., Sci Signaling 2014, 5 hr. incubation time was used?

When cells adhere from suspension, the types of adhesion complex that assemble vary

dramatically over the first few hours of culture. Understanding this somewhat unnatural evolution is of course a very important question for the adhesion field because it is thought to be relevant for cell movement. However, for this study, we wanted to generate a steady-state view of the composition of IACs and for this we used cells that had been adherent for 32 hours. BioID is perfect for such a study because of the need to label for approximately 24 hours. When we initially established the BioID technique, we found that shorter labelling times were sub-optimal for protein detection. Shorter analyses are certainly possible using alternative labelling methods (e.g. TurboID, miniTurbo and APEX can deliver data from 10 minutes labelling), but this would address a different set of questions and may be less relevant for understanding the mature state of an adherent cell.

1.2 Similarly, with the long incubation time, proteins may translocate through different compartments of the cells and the results would reflect not just the adhesion-resident proteins, but possibly other cellular compartments.

This issue is already included at the start of the discussion.

1.3 Perhaps a control whereby the cells are on non-specific surfaces such as polylysine would be useful? If this is feasible, this would help differentiate whether the protein-protein interactions are adhesion-specific or constitutive or cytoplasmic-only.

There is certainly a need to validate many of the putative associations that we have identified; however, we do not believe inclusion of this specific negative control in a BioID experiment would be particularly useful. This is primarily because cells will still be able to make their own ECM over the course of the biotin labelling period and it is unlikely that all adhesion would be via polylysine.

2. Perhaps some biological context on why pancreatic fibroblasts was used in this study can be provided. In particular, cell-type specificity are increasingly appreciated in many aspects of adhesions organization and functions and not just adhesome protein-protein interactions (for example: the variation in protein organization in embryonic stem cells was also shown in Xia et al., ACS Biomaterials Sci & Eng, 2019, which the authors may not be aware of) but thus far, data from different studies tend to come from different cell types, which makes it difficult to separate different sources of variability.

See above for comments on the choice of pancreatic fibroblasts. Unfortunately, there is no way to control which cell lines different laboratories use for their experiments and therefore the comparative analyses that we have performed have always included the caveat of cell type-specific differences. In due course, we intend to investigate changes in the composition of the BioID-generated network under different microenvironmental conditions and for these studies we will use the same cell line.

3. How are the modular organizations observed here compared to information obtained from previous studies such as Hoffmann.. eLife 2014 or in Zamir et al. PLOS ONE 2008 (which the authors may not be aware of)?

We thank the reviewer for making this excellent point. We were remiss in not citing, and including a discussion of, these seminal studies in the original manuscript. We have now cited both papers and included the following text in the first paragraph of the Results & Discussion

section ‘Functional modules within IACs’ (page 8):
“These five bait clusters broadly correlated with theoretical interaction networks in the literature (Horton et al., 2015; Green and Brown, 2019) and with pre-assembled ternary complexes of ILK-PINCH-parvin and FAK-p130Cas-paxillin previously identified by fluorescence cross-correlation spectroscopy and fluorescence recovery”.

4. A previous BioID study using Paxillin and Kindlin2 as baits (Dong et al., Sci. Signaling 2014) also provided quite a comprehensive analysis of protein-protein interaction. To make these resources more useful to the community, it would be worthwhile to compare the results of the current study with such previous study (e.g. in terms of coverage, interaction topology, any cell-type dependent variability), as the average cell biologists to whom this could be useful but who are not specialists would probably not be able to do these comparisons easily on their own.

A comparison with the data in Dong *et al.* was included in the original manuscript; however, this has been expanded. First, an extra column has been added to Table S1 to indicate the presence or absence of preys from Dong *et al.* Second, the text has been supplemented as follows in the final paragraph of the section entitled ‘Generation of a proximity-dependent adhesome’ (pages 7-8):
“In the study by Dong et al., 14 prey were identified that associated with both BirA*-tagged kindlin-2 and paxillin. In our study, this was true for six of these proteins (talin-1, KANK2, PEAK1, tensin-1, tensin-3 and lamellipodin/Raph1). ILK, α-parvin, EphA2 and RN-Tre (Usp6nl) associated with one of the baits, while RASnGAP (Rasal2), liprin-α1 (Ppfia1), PINCH and Bcar3 were not found (although some did associate with other baits). It is conceivable that the absence of some prey may be due to cell type-specific differences.” It is not really possible to compare further our network topology with that of Dong *et al.* because they only used two BioID baits and therefore a network as such wasn’t generated.

We hope these responses are sufficient, but if you require any further changes or information, do not hesitate to contact me.

Best wishes,

Martin