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Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres

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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 01 February 2011

Thank you for submitting your manuscript for consideration by the EMBO Journal. Please let me first apologise for the very long delay in getting back to you with a decision. We have been waiting for the final report since I last wrote to you, and despite promises to the contrary, this has still not been delivered. Given the length of time that has now elapsed, and the fact that it is not clear whether the report will ever be returned, we are therefore making a decision based on the two reports we have to hand.

As you will see from the reports enclosed below, both referees appreciate the high technical quality of your work. However, they disagree somewhat as to the potential suitability of the study for EMBOJ: referee 1 argues that your data do not really help to resolve the question of whether there is any pre-patterning of the mouse embryo, whereas referee 2 finds that the strong evidence that there is no global transcriptome asymmetry at the 2-3 cell stage does add significantly to the debate. Having discussed the manuscript and associated reports extensively within the editorial team, we have come to the conclusion that we agree with referee 2 - that your data do provide valuable information about the earliest events in mammalian embryogenesis - and we would therefore like to invite you to submit a revised version of the manuscript. However, there are a number of important issues that would need to be addressed - primarily by changes to the text rather than additional experimental work. These are clearly laid out by referee 2 and I see no need to repeat them here. I would just stress that it is important that the data are not 'oversold' in terms of the scope of the conclusions that can be drawn from them.

I should add that it is EMBO Journal policy to allow only a single round of revision. Acceptance of your manuscript will thus depend on the completeness of your responses included in the next, final version of the manuscript. When preparing your letter of response to the referees' comments, please 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://www.nature.com/emboj/about/process.html

We generally allow three months as a standard revision time, and as a matter of policy, we do not consider any competing manuscripts published during this period as negatively impacting on the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. I don't see this as being a problem, but should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

The authors have carried out a very careful analysis of mRNA distribution between the zygote and the polar body and between the first two and the next 3-4 blastomeres in mouse development. While they do find transcript differences related to spindle formation and polar body extrusion, they do not find any significant differences between 2-cell blastomeres or among 3-4 cell blastomeres. While the first observation is interesting, it is not followed up mechanistically. Are any of the segregated transcripts actually necessary for polar body formation or later development? The absence of significant differences in transcripts among early blastomeres, while useful baseline data, does not help resolve whether there is any prepatterning in the mouse embryo. It excludes the most extreme model whereby specific mRNAs are segregated to different cells at early cleavage stages. However, no-one has really suggested this is a likely mechanism. If there really is an early determinant stage it is quite likely that the segregation is specific to maternal mRNAs, or that mRNAs are segregated within, not between cells, or that the determinants are segregated at the protein, not mRNA level. The current study does not address any of these possibilities and so does not provide major new insight into early mammalian development.

Referee #2 (Remarks to the Author):

This manuscript examined transcript profiles in subcellular samples of oocytes and zygotes, as well as cellular samples of 2- or 3-cell stage blastomeres. In general, the results supported two conclusions that could be drawn from their analyses:
1) The spindle in oocytes and zygotes exhibited association with a unique transcriptome different from that of the enucleated cytoplasm.
2) Sister blastomeres in 2- or 3-cell stage embryos express indistinguishable transcriptome profiles.

While it is hardly surprising to find that the spindle is associated with a unique transcriptome, this manuscript is the first experimental report using genome-wide transcript profiling to demonstrate this. The authors should make an effort to discuss the biological significance of the observed transcriptome differences. The region around MII spindle will be and the 2nd polar body has been discarded and will play no role in the further development. Thus spindle associated mRNAs can only play a role as a basis for "spindle specific" localized translation in MII oocyte and zygote. What is the possible purpose/consequence of such localized translation? The authors should also make clear that the localizations of specific mRNAs they describe is, in terms of developmental mechanisms, completely different from regional localization of specific mRNAs observed in e. g. fruit fly or frog oocyte, where their protein products will regulate developmental fate of the cells containing them. Spindle localized mRNAs in mouse oocyte will not regulate anything once meiosis is completed. This paper is also the first report of a direct single cell transcriptome microarray

comparison of 2-cell stage sister blastomeres. This report thus contributes additional information to the debate on the absence or presence of prepatterning mechanisms in early mouse embryos. Hence, there is definitely some merit to the publication of this work.

Additional comments:
1. Figures 1D-F: cortical samples that do not contain the spindle exhibited completely different transcript profiles as the spindle-containing samples (Fig 1B). The simplest interpretation is that the spindle samples are specifically enriched/depleted of some transcripts, independent of cortical associations (which should be the same in all cortical region samplings). The complex conclusion drawn by the authors (page 8, lines 11-14) is an over-interpretation not fully supported by the limited data, and should be removed.

2. 25/30 genes tested by qPCR fit the microarray trend but what the authors failed to mention was that fold-changes in the qPCR did not correlate very well with fold-changes in the microarray. For example, the microarray data showed a logFC of ~ -0.8 for both Rsu1 and Atp1a1, but fold-difference for Rsu1 was ~ 1 (i.e. no difference), while Atp1a1 had a -6.8 fold-difference. These data cast doubts on the 83.3% microarray-qPCR correlation stated by the authors (page 7, line 19). Could the authors discuss this issue?

3. The subcellular analyses of spindle sample vs. enucleated cytoplasm in oocytes and zygotes are essentially different from the whole cell transcriptome analyses of sister blastomeres. There are no grounds for comparison between these two sets of experiments. The title, which implied such a comparative study, is somewhat misleading.

1st Revision - authors' response 23 February 2011

Responses to comments
We are extremely grateful to those who have reviewed our work for their time and insightful comments.

Editor
As you will see from the reports enclosed below, both referees appreciate the high technical quality of your work. However, they disagree somewhat as to the potential suitability of the study for EMBOJ: referee 1 argues that your data do not really help to resolve the question of whether there is any pre-patterning of the mouse embryo, whereas referee 2 finds that the strong evidence that there is no global transcriptome asymmetry at the 2-3 cell stage does add significantly to the debate. Having discussed the manuscript and associated reports extensively within the editorial team, we have come to the conclusion that we agree with referee 2 - that your data do provide valuable information about the earliest events in mammalian embryogenesis - and we would therefore like to invite you to submit a revised version of the manuscript.

Thank you. **We address both reviews point-by-point below**.

However, there are a number of important issues that would need to be addressed - primarily by changes to the text rather than additional experimental work. These are clearly laid out by referee 2 and I see no need to repeat them here. I would just stress that it is important that the data are not 'oversold' in terms of the scope of the conclusions that can be drawn from them.

Thank you. We would appreciate any and all guidance on this point and in this new version have taken our cue from **Referee #2, whose comments are addressed in full below and via revisions to the manuscript text as indicated**. In particular, we have now added the caveat in response to Referee #2, that our data are "entirely consistent with such [polar body-zygote] sorting exerting non-absolute effects for normal rates of healthy mammalian development." (Discussion, p. 16, l. 9). In addition, we have sought to tone down the language of the text including the following changes:

Abstract, p.2, l.16: **Deletion**. "...and provides the first documentation..."
(Results, p.7, l. 16): **Deletion** of the entire exposition corresponding to the final paragraph.

(Results, p.12, l. 2): **Deletion** of the final conclusion "As such, developmentally important inter-cellular transcriptome asymmetry does not exist." .
VerMilyea et al, responses to comments, page 2
Discussion, p.14, l. 2: **Substitution** of the opening words, "This is the first We here report...".
Discussion, p.14, l. 3: **Substitution**. "It is also believed to be This is perhaps the..."

Referee #1
The authors have carried out a very careful analysis of mRNA distribution between the zygote and the polar body and between the first two and the next 3-4 blastomeres in mouse development. While they do find transcript differences related to spindle formation and polar body extrusion, they do not find any significant differences between 2-cell blastomeres or among 3-4 cell blastomeres. While the first observation is interesting, it is not followed up mechanistically. Are any of the segregated transcripts actually necessary for polar body formation or later development?
Thank you. We have not conducted a systematic search for spindle-localized mRNAs known to be required for (second) polar body formation and are unclear what this would show at this stage. We previously commented that second polar body mRNAs do not appear of themselves to completely abrogate subsequent development, and **now elaborate upon this to clarify the point in the Discussion** (p. 16, l. 4-7):

"However, the elimination of Pb2 components is not critical in the mouse: replacing the zygotic female pronucleus with a nucleus derived from the Pb2 following electrofusion of the entire Pb2 supports full development to term..."

The absence of significant differences in transcripts among early blastomeres, while useful baseline data, does not help resolve whether there is any prepatterning in the mouse embryo.
With respect, we disagree with this view. An obvious mechanism by which to achieve pre-patterning would be *via* asymmetric RNA sorting; this is what occurs in many metazoans, including *Drosophila* and *Xenopus*. We regard the finding that it does not in the mouse to be a major blow to the argument that pre-patterning occurs in mammals. It does not in and of itself show that there is no pre-patterning in mammalian early embryos, but substantially constrains the scope of mechanisms by which such pre-patterning occurs if it occurs at all. This is a contribution to resolving whether there is any pre-patterning in the mouse embryo.

It excludes the most extreme model whereby specific mRNAs are segregated to different cells at early cleavage stages. However, no-one has really suggested this is a likely mechanism.
This point is conjecture, but in as much as it is correct, perhaps it is because it has been widely *assumed* that the extensive corpus of evidence effectively showing transcriptome polarity in *Drosophila* and *Xenopus* oocytes and embryos also applies to mammals. But this has never been tested, and as we report here, it does *not* also apply to mammals.

If there really is an early determinant stage it is quite likely that the segregation is specific to maternal mRNAs, or that mRNAs are segregated within, not between cells, or that the determinants are segregated at the protein, not mRNA level. The current study does not address any of these possibilities and so does not provide major new insight into early mammalian development.
We apologize for being rather uncertain of what is meant here. What, for example, is meant by "an early development stage"? All of the RNAs under investigation in oocytes are maternally-derived, and probably the majority in zygotes given the time at which major embryonic transcription initiates in the mouse. We analyzed cleavage products immediately after cytokinesis at all stages, to try to eliminate the effects of *de novo* transcription, but our conclusions do not depend upon the provenance of the transcripts we are studying for any of them. So we are unsure what is meant. Clearly, the study addresses RNA, not protein, and there are many elegant and welldocumented studies of protein asymmetries in mouse oocytes and early embryos. So we were unclear here, too.
We disagree with the concluding comment:
demonstrating (*inter alia*) for the first time that a cardinal feature of other metazoan development is not conserved in mice is evidently a new insight into early mammalian development.

Referee #2

This manuscript examined transcript profiles in subcellular samples of oocytes and zygotes, as well as cellular samples of 2- or 3-cell stage blastomeres. In general, the results supported two conclusions that could be drawn from their analyses:
1) The spindle in oocytes and zygotes exhibited association with a unique transcriptome different from that of the enucleated cytoplasm.
2) Sister blastomeres in 2- or 3-cell stage embryos express indistinguishable transcriptome profiles. While it is hardly surprising to find that the spindle is associated with a unique transcriptome, this manuscript is the first experimental report using genome-wide transcript profiling to demonstrate this.

Thank you.

The authors should make an effort to discuss the biological significance of the observed transcriptome differences. The region around MII spindle will be and the 2nd polar body has been discarded and will play no role in the further development. Thus spindle associated mRNAs can only play a role as a basis for "spindle specific" localized translation in MII oocyte and zygote. What is the possible purpose/consequence of such localized translation?
Thank you. We completely agree with this point which is related to the next one. Accordingly, **we have extensively remodeled the relevant part of the Discussion and added a detailed consideration of spindle localization, including the possible purpose/consequence of such localized translation, as requested**. Our consideration includes the possibility that polar body extrusion is a mechanism for eliminating mRNAs that would be deleterious to subsequent development. We also consider the possibility that spindle targeting is, *ipso facto*, a means to target protein expression, and as the reviewer requests, have now discussed this and suggested candidate processes and proteins.

The authors should also make clear that the localizations of specific mRNAs they describe is, in terms of developmental mechanisms, completely different from regional localization of specific mRNAs observed in e. g. fruit fly or frog oocyte, where their protein products will regulate developmental fate of the cells containing them. Spindle localized mRNAs in mouse oocyte will not regulate anything once meiosis is completed.
Thank you. We again completely concur and were aghast that having described the developmental roles of fly and frog oocyte mRNA polarity in the Introduction, we had neglected to comment on them in the light of our results. **We have now added a section to the Discussion as suggested by the Reviewer, explicitly stating that mouse mRNA localization is completely different in terms of developmental mechanisms from *Xenopus* and *Drosophila*. We also now consider the possibility that mRNA expulsion *via* the polar body is a critical developmental mechanism in the mouse**.

This paper is also the first report of a direct single cell transcriptome microarray comparison of 2-cell stage sister blastomeres. This report thus contributes additional information to the debate on the absence or presence of prepatterning mechanisms in early mouse embryos. Hence, there is definitely some merit to the publication of this work.

Thank you.

Additional comments:
1. Figures 1D-F: cortical samples that do not contain the spindle exhibited completely different transcript profiles as the spindle-containing samples (Fig 1B). The simplest interpretation is that the spindle samples are specifically enriched/depleted of some transcripts, independent of cortical associations (which should be the same in all cortical region samplings). The complex conclusion drawn by the authors (page 8, lines 11-14) is an over-interpretation not fully supported by the limited data, and should be removed.
Thank you. This text has **now been deleted** as requested and **the remainder of the paragraph altered so that there is now a simple statement of our results (p. 8. l. 7-9).**

2. 25/30 genes tested by qPCR fit the microarray trend but what the authors failed to mention was that fold-changes in the qPCR did not correlate very well with foldchanges in the microarray. For example, the microarray data showed a logFC of ~ - 0.8 for both Rsu1 and Atp1a1, but fold-difference for Rsu1 was ~ 1 (i.e. no difference), while Atp1a1 had a -6.8 fold-difference. These data

cast doubts on the 83.3% microarray-qPCR correlation stated by the authors (page 7, line 19). Could the authors discuss this issue?
Thank you. This is an important point that we had not covered, and which is explained in greater detail below. To address this in the manuscript, **we have now amended the text** in two places; we have also added minor corrections to the qPCR data of Figures 1 and 2. In the main text (p. 6, l. 23) we have **added the term "directional"** so that it is clear that we are not referring to the magnitudes of change. **Also, we have now explained this**, as requested, in a section added to the Methods Section on qPCR (p. 19, l. 33-35) and **added a supporting reference**:

"Note that the dynamic range of qPCR is known to be greater than that of microarrays; they produce similar directions, but not necessarily magnitudes, of transcript level differences between samples (Park *et al*, 2004; Hartmann and Klein, 2006)."

These changes to the text reflect a summary of the answers to the point raised by Referee #2. A full explanation might include the following points. None of the methods directly measures absolute transcript numbers, with qPCR giving an estimate relative to the reference(s) - not identical in every cell - and microarrays being globally normalized (quantile normalization) and its measurements combining transcript numbers and labeling efficiencies (which are sequence-dependent) for signal generation. Consequently, values produced by the methods correlate but are not expected necessarily to be identical. Also, the dynamic range of qPCR and microarrays is different and we cite references from our own work (Hartmann and Klein, 2006) and that of others (Park *et al*, 2004) that establishes and characterizes this difference. Finally, qPCR is typically used to confirm/validate findings by microarray analysis and is the more precise measurement. In this sense, microarray analysis can be regarded as a primary technology employed to detect qualitative differences between groups ahead of secondary corroboration by qPCR.

3. The subcellular analyses of spindle sample vs. enucleated cytoplasm in oocytes and zygotes are essentially different from the whole cell transcriptome analyses of sister blastomeres. There are no grounds for comparison between these two sets of experiments. The title, which implied such a comparative study, is somewhat misleading.
Thank you. **We have now changed the title to "Transcriptome distributions in mouse metaphase II oocytes and early embryos".**

However, we respectfully request that we be allowed to retain the original title ("Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres"). Firstly, it clearly states which parts of the analysis are intracellular ("within zygotes") and which are intercellular ("between blastomeres"). Secondly, we respectfully disagree with the Reviewer: the zygotic analysis of the title is not essentially different to that between blastomeres. All are comparisons of cytokinetic products: the second polar body is as much a cytokinetic product as is a blastomere from a 2-cell embryo, for example. In all cases referred to in the title, we compared sister cytokinetic products - the second polar body with its sister zygote, and a blastomere with its sister blastomere. So the title is accurate. Thirdly, note that the title does not refer to enucleated cytoplasm or spindles, even though the reviewer does refer to them and indeed, they are subjects of part of the work. Fourthly, the title comprises two clauses that state the findings of our work: (i) Transcriptome asymmetry within zygotes, (ii) No transcriptome asymmetry between sister blastomeres. They are free-standing and not comparative. We briefly considered titles such as "Transcriptome asymmetry in mouse zygotes is not transmitted to produce asymmetries between sister blastomeres", but agree with the reviewer that the link is too much and duly avoided it. Fifthly, a comparison is indeed made *within* the second clause, but again, we are clear and explicit in the title as to what is being compared ("between blastomeres"). Sixthly, the original title conveys important information that the new title omits, and we feel that the omission does the reader and the scientific record a disservice.

2nd Editorial Decision 02 March 2011

Many thanks for submitting the revised version of your manuscript EMBOJ-2010-76647R. It has now been seen again by referee 2, whose comments are appended below. He/she is satisfied with the changes you have made and now supports publication. As you will see, he/she is also happy for you to use the original title from the first submission, rather than the new one you propose here.

I am therefore pleased to be able to tell you that we can now accept your manuscript for publication here at EMBOJ. If you are happy for us to do so, we can just change the title at this end, so that you don't need to do anything else. Can you just confirm that you would like the title to be:

"Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres".

Once I have your confirmation, we will make that change, and can then go ahead and accept the paper.

Many thanks for choosing EMBOJ for publication of this study!

Best wishes,

REFEREE REPORT

Referee 2:

The authors addressed my comments and concerns and in my opinion the manuscript is now suitable for publication. The authors changed the title upon our request but also made an impassioned plea for the old title. I tend to agree with them especially since the old title is definitely better than the new one.

