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# KDM5B regulates embryonic stem cell self-renewal by repressing cryptic intragenic transcription

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**Review timeline:**

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| Submission date: | 03 November 2010 |
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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 04 December 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has been now been evaluated by three referees and I enclose their reports below, as you will from the comments the referees find the role of KDM5B in the co-transcriptional repression of cryptic transcripts in ESCs to be interesting and require some further experimental analysis to make the manuscript suitable for The EMBO Journal.

The main questions that arise involve the cryptic transcripts and the link with the co-transcriptional recruitment of KDM5B. The issues raised by referee #1 and #3 are important to the main conclusions and should be addressed. Referee #2 raises an interesting issue about the specificity of the mechanism for ESC cells and asks for experiments to be added in somatic cells, I would very much appreciate your opinion on this issue before making a decision if they are necessary, do you have data that the same mechanism occurs in other cell types? Once the main issues are satisfactorily addressed I believe the study will make a nice contribution to the Journal and given the interest from the referees should you be able to address these issues, we would be happy to consider a revised manuscript.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments 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

 

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

Yours sincerely,

Editor
The EMBO Journal

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REFEREE COMMENTS

Referee #1 (Remarks to the Author):

Xie et al.
KDM5B regulates embryonic stem cell self-renewal by repressing cryptic intragenic transcription
EMBO J

In this manuscript, Xie and colleagues demonstrated that the H3K4me3 demethylase KDM5B, an Oct4 and Nanog target gene, functions as an activator in gene expression. By ChIP-seq analysis, they found that KDM5B mainly localizes in intragenic region and is highly correlated with the localization of Ser2P PolII, K36me3 and MRG15. They showed that KDM5B is recruited to intragenic region through the interaction with MRG15. Knockdown of Set2 or MRG15 results in decreased KDM5B recruitment to the intragenic region. Knockdown of KDM5B causes increased cryptic transcripts and decreased full length transcripts of KDM5B target genes. They proposed that KDM5B functions in inhibition of cryptic transcription by erasing H3K4me3 deposited by elongating PolII-associated HMTs.

Overall, their data are solid and convincing. Here are a few comments:

1. In figure 6C, they show that knockdown of KDM5B increases the expression of cryptic transcripts by RT-PCR. However, there is no sufficient evidence showing that these transcripts are caused by transcription originating from cryptic promoters within the gene. The detail of the methods they used for detecting cryptic transcription should be provided. Assuming they used primers against intronic region in the RT-PCR to detect cryptic transcription, the possibility of KDM5B causing splicing defects cannot be ruled out. Northern blot analysis would be a better way to detect cryptic transcription.

2. In figure 6E, they show that there is no change in Ser5P PolII recruitment at promoter while recruitment of Ser2P decreases in intragenic region when knocking down KDM5B. Does the occupancy of Ser5P increase in intragenic region? It would provide evidence that intronic transcripts they detected in Fig6C are caused by transcription originating from cryptic promoters within the gene. Also, the description of Fig.6E in the figure legend is not clear about where they ChIP Ser5P and Ser2P respectively.

3. In figure 6B, they show that the enrichment of H3K4me3 is decreased at KDM5B chip-seq peaks after 1hr treatment of inhibitor of PolII elongation, suggesting that intragenic H3K4me3 deposition depends on the elongating PolII-associated HMTs. In Fig. S7C, they observed that the recruitment of KDM5B is decreased after 6 hr treatment of inhibitor of PolII elongation. Does K4me3 stay decreased? It would support the model in which KDM5B erases the K4me3 mark deposited by elongating PolII-associated HMTs.

Referee #2 (Remarks to the Author):

Comments on Xie et al., KDM5B regulates embryonic stem cell self-renewal by repressing cryptic intragenic transcription

This manuscript shows that histone 3 trimethyl lysine 4 (H3K4me3) demethylase, KDM5B is a

downstream target of Nanog and implicates it in embryonic stem cell (ESC) self-renewal. The authors present data showing KDM5B as an activator of gene network associated with self-renewal, contrary to the previous notion of it being a promoter bound repressor. Using Chip-Seq, they also convincingly show that KDM5B primarily resides in intragenic regions of its target genes and is likely recruited to these sites by H3K36me3 via an interaction with the chromodomain protein MRG15. Finally they hypothesized and demonstrated that KDM5B represses intragenic transcription by removing H3K4me3, in a process that safeguards transcription elongation. The mirror images of H3K4me3 and KDM5B support this notion.

This is an interesting study, which sheds light on different pathways that are downstream to pluripotency factors in maintaining self-renewal of ESCs. The authors show a previously known epigenetic regulator to be important for the ESC self-renewal. This opens up the possibility that several other known chromatin regulators might have important yet unknown functions in maintaining the stem cell state.

Overall, the paper is convincing and the data is strong. However, the major weakness of this work is the lack of comparison with differentiated cells. Many of the reported experiments could have been conducted on somatic cells with perhaps similar outcome. The specificity to ESC biology is missing. The authors don't even show whether KDM5B is down-regulated during ESC differentiation. I believe that adding a few key experiments (knockdown, for example) in differentiated cells and comparing the results with ESCs would substantially improve the manuscript.

Additional issues:
The authors state on page 4 that "Prolonged knockdown of KDM5B triggered morphological differentiation, loss of alkaline phosphatase activity, and a reduction in pluripotency-associated gene expression (Figure 1F; data not shown)". However, in Figure 6D the authors show that Oct4 and Nanog full length mRNAs are not affected by KDM5B knockdown. It is therefore important to specifically refer to which pluripotency genes are reduced by KDM5B knockdown, show the data, and comment on / explain the apparent discrepancy between these results.

Minor comments:

Fig. 2B&J. It would be helpful to have a heat map scale.
Fig. 5I&J representative gene names should be indicated
Fig. 6 B- Labeling not clear.

Referee #3 (Remarks to the Author):

In this manuscript, Xie et al uncovered a potential role of histone H3K4me3 demethylase KDM5B in regulating stem cell self-renewal. They showed that Nanog and Oct3/4 directly regulate KDM5B transcription, and knock-down of KDM5B leads to differentiation. Transcription profiling analysis suggested that KDM5B mostly functions as a transcriptional activator. To find out underlying mechanisms, they performed ChIP-seq of KDM5B and, surprisingly, found that KDM5B is enriched at coding regions and co-localizes with Pol II Ser2P and H3K36me3. They went on and demonstrated that K36me3 and the mammalian Rpd3S ortholog (to a less extent) contribute to the recruitment of KDM5B to coding regions and subsequent H3K4me3 removal. Finally they showed that depletion of KDM5B causes increased cryptic transcription and decreased full length mRNA at target genes. Although the association of KDM5B homolog-Lid with Rpd3 has been reported by the Yi Zhang lab, this manuscript extended this physical association and presented an interesting model that H3K4 demethylase cross talk with H3K36me via Rpd3S to regulate transcribed chromatin. Overall, the data are solid and fairly interpreted. However, several critical issues detailed below should be addressed before warranting publication in EMBO J.

Specific Comments:

1. The title and the model in Fig6G are misleading.
(1). Although they demonstrated that KDM5B is downstream target of Nanog/Oct, the model in Fig6G implied that KDM5B is a somewhat secondary master gene for maintaining stem cells. To

support this hypothesis, they should test if ectopically overexpressed KDM5B in Nango/Oct knock down cells could rescue the stem cell defects.
(2). There is no evidence suggesting that increased cryptic transcription is the direct cause of the loss of stemness in KDM5B knock down cells. In Fig1E, they observed increased level of differentiated genes. However, for those genes that display cryptic transcription phenotype, FL transcripts actually are reduced, implying that this rather global function of KDM5B may not relate to the stem cell defect at all. Although a decisive conclusion requires more rigorous testing which may be beyond the scope of current manuscript, the authors should consider change the title to "KDM5B regulates stem cells AND cryptic transcription" instead of "by".

2. The authors should examine the states of acetylation and K36me at KDM5B targets in KDM5B knock down cells. As for yeast studies, K36me3-Rpd3S controls histone acetylation level, which presumably leads to increased fluidity of nucleosomes or histone exchange rates, which in turn exposes cryptic transcription start sites. Although K4me3 can attract TFIID, there is no evidence in literatures suggesting that K4me3 is sufficient to recruit TFIID/ PIC. Based on authors' model, KDM5B is downstream of K36me3/Rpd3, therefore one would assume that KDM5B knock down would not influence acetylation and K36me3. If these are the cases, it will represent a novel finding that K4me3 alone can lead to cryptic initiation.

3. In Fig6, authors showed H3K4 methyltransferase subunits associate with both Ser2 and Ser5 phosphorylated CTD. This contradicts to the report by the Struhl lab (Mol cell 2003) that Set1 only associates with Ser5. ChIP analysis of those two subunits at middle or 3'end of ORF would provide strong supports.

4. It was not clear how authors performed cryptic transcription analysis using RT-PCR. Do they compare 5' primer set with 3' primer set? How do they subtract the FL population from cryptic ones, since FL should be picked up by all primer sets. A northern blotting should help clarify this issue a bit.

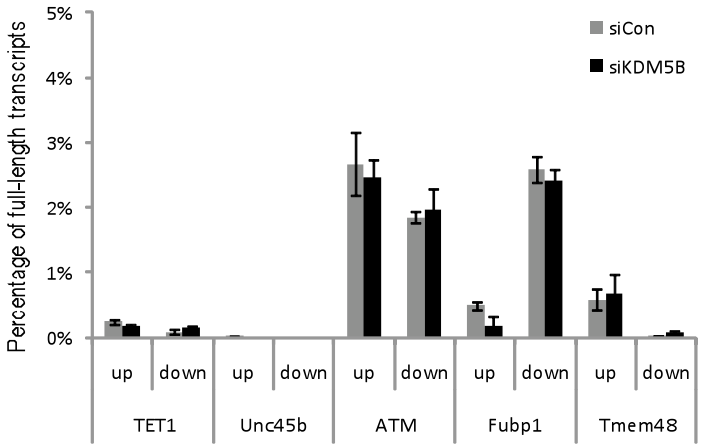
5. The manuscript requires extended editing to increase clarity. Descriptions for genome wide analysis are not very clear in many cases (Figure legends). A few examples/typos:
1). It should read "Histone H3", not "histone 3"
2). "where the transcription-assoicated H3K4me3 mark accumulates repressive H3K27me3".
3).Page8-subtitle, it should read "Rpd3S", not rpds

1st Revision - authors' response 10 February 2011

"*However, there is no sufficient evidence showing that these transcripts are caused by transcription originating from cryptic promoters within the gene."*

We believe that these transcripts do not originate from the canonical promoter because: 1) PolII recruitment to promoter regions was not regulated by KDM5B depletion (Figure 6E); 2) the spliced transcript shows distinct regulation from the intronic transcript (Figure 6C and 6D); 3) strand-specific RT-PCR shows that some cryptic transcripts are antisense to the canonical transcript (Supplementary Figure S10). We have included new PCR data showing that cryptic transcription is not detected in upstream or downstream regions (see Figure R1 below). We also provide data showing that PolII is recruited to intragenic H4K4me3 peaks following KDM5B depletion (Figure 6F).




**Reviewer Figure 1**. Real time PCR analysis of cryptic transcripts in J1 ESCs following KDM5B knockdown. PCR primers were designed upstream and downstream of representative KDM5B target genes as shown in Figure 6C and 6D. Data is shown as percentage of detected transcripts from corresponding full-length transcripts.

*"Assuming they used primers against intronic region in the RT-PCR to detect cryptic transcription, the possibility of KDM5B causing splicing defects cannot be ruled out."*

Our data reveals specific regulation of elongating PolII occupancy in KDM5B target genes. We designed primers spanning multiple exons and did not detect a "specific" splicing defect. Nevertheless, it is conceivable that regulation of splicing also contributes to the KDM5B phenotype. Indeed, work from a number of labs has shown that PoIII elongation and histone acetylation regulate splicing events. Interestingly, the KDM5B-interacting protein MRG15 was also recently shown to regulate PTB-dependent alternative splicing (Luco et al, 2010). We have added discussion of this point to the text.

*"Does the occupancy of Ser5P increase in intragenic region? It would provide evidence that intronic transcripts they detected in Figure6C are caused by transcription originating from cryptic promoters within the gene."*

We have added new data showing that intragenic Ser5P was depleted from target genes following KDM5B knockdown (Figure 6E). This is not surprising because Ser5 phosphorylated (and Ser2/5 biphosphorylated Pol II) contributes to transcriptional elongation (Egloff & Murphy, 2008) and has been detected throughout transcribed chromatin in mammalian cells (Govind et al, 2010; Hargreaves et al, 2009). Unphosphorylated PolII is tethered to sites of initiation via its interaction with TFIID/TBP(Nikolov et al, 1995; Usheva et al, 1992). Because TFIID components also interact with H3K4me3, we examined whether unphosphorylated PolII is recruited to KDM5B-induced H3K4me3 ChIP-Seq peaks. Interestingly, KDM5B knockdown markedly stimulated recruitment of unphosphorylated PolII to intragenic H3K4me3 peaks strongly suggesting that these sites represent sites of cryptic initiation (Figure 6F).

*"The detail of the methods they used for detecting cryptic transcription should be provided."*

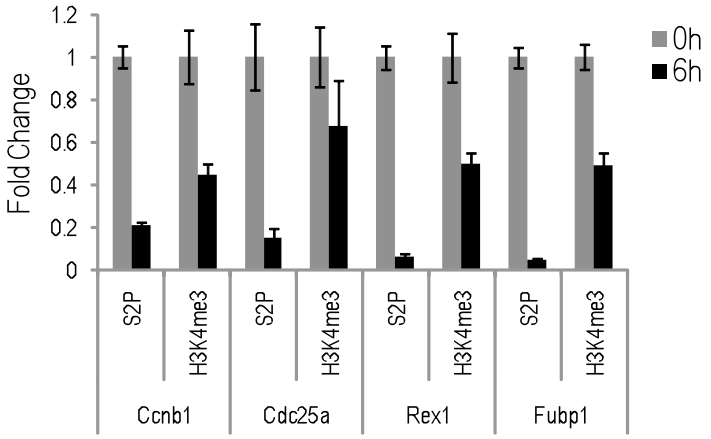
We now provide detailed description of methods used to detect cryptic transcription in the methods section.

*"Also, the description of Figure.6E in the Figure legend is not clear about where they ChIP Ser5P and Ser2P respectively."*

We have added additional data and modified the text accordingly.

"*In Figure. S7C, they observed that the recruitment of KDM5B is decreased after 6 hr treatment of inhibitor of PolII elongation. Does K4me3 stay decreased? It would support the model in which KDM5B erases the K4me3 mark deposited by elongating PolII-associated HMTs."*
We provide data showing that continued depletion of H3K4me3 following DRB 6h treatment (see Figure R2) and made note of this in the text.




**Reviewer Figure 2.** ChIP analyses of phosphorylated Ser2 PolII (S2P) and H3K4me3 at intragenic regions following 6h DRB treatment in J1 ESCs.

Referee #2

*"The specificity to ESC biology is missing. The authors don't even show whether KDM5B is down-regulated during ESC differentiation."*

This experiment is included in another manuscript that specifically addresses the epistatic role of KDM5B in Nanog-dependent self-renewal. We have reproduced this data for the reviewer (figure not included in this peer review process file). We also find that KDM5B expression in non-malignant cells is restricted to pluripotent cells and the testes (Figure not included in this peer review process file). Indeed, preliminary data suggests that other Kdm5 family members are upregulated during ESC differentiation.

*"It is therefore important to specifically refer to which pluripotency genes are reduced by KDM5B knockdown, show the data, and comment on / explain the apparent discrepancy between these results."*

Data showing the reduction in pluripotency genes following knockdown of KDM5B are now included (Supplemental Figure 1B). Importantly, all of the genes showing a decreased expression are direct targets of KDM5B. Pluripotency-associated genes that are not direct targets of KDM5B (e.g. Oct4, Nanog, UTF1, etc) were unaffected by KDM5B knockdown. This observation implies that the reduction of a siKDM5B-responsive pluripotent gene expression at mRNA level may reflect a distinct cell state of loss of pluripotency.

Referee #3

*"To support this hypothesis, they should test if ectopically overexpressed KDM5B in Nango/Oct knock down cells could rescue the stem cell defects."*

We believe that this question is beyond the scope of the current study. Another manuscript in preparation directly addresses the role of KDM5B in self-renewal and its epistatic relationship with

Nanog.

*"the authors should consider change the title to "KDM5B regulates stem cells AND cryptic transcription" instead of "by".*

We agree and have changed the title accordingly.

*"The authors should examine the states of acetylation and K36me at KDM5B targets in KDM5B knock down cells."*

We now include data showing that KDM5B knockdown does not affect intragenic H4 acetylation and H3K36me3 (Supplementary Figure S8).

*"it will represent a novel finding that K4me3 alone can lead to cryptic initiation."*

We provide data showing recruitment of non-phosphorylated PolII to KDM5B-regulated H3K4me3 Chip-Seq peaks induced by knockdown of KDM5B (Figure 6F).

*"In Figure6, authors showed H3K4 methyltransferase subunits associate with both Ser2 and Ser5 phosphorylated CTD. This contradicts to the report by the Struhl lab (Mol cell 2003) that Set1 only associates with Ser5.*"

Our data is consistent with work form the Shilatifard and Dehe labs showing a weak interaction with Ser5P (Krogan et al, 2003) (Dehe et al, 2006). We have amended our discussion of this issue in the text.

*"It was not clear how authors performed cryptic transcription analysis using RT-PCR."*

We utilized the methodology described by Hargreaves et al. (Hargreaves et al, 2009). For RT-PCR analysis of cryptic transcripts we used gene- and strand-specific primers directed to intronic regions. For full-length transcripts we used intron-spanning primers directed to the 3'-most exons. Because several cryptic transcripts were antisense to the parental gene they by definition cannot originate from canonical promoters. The methods section has been expanded to make this clear.

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2nd Editorial Decision 25 February 2011

Thank you for submitting the revised version of your manuscript to The EMBO Journal, it has now been evaluated by two of the original referees who find that you have addressed all their concerns and both now recommend publication. I am therefore happy to accept your manuscript for publication in The EMBO Journal. You will receive an official acceptance letter in the next day or so.

Best wishes,

Editor
The EMBO Journal

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REFEREE COMMENTS

Referee #1 (Remarks to the Author):

In this revised manuscript, Xie and colleagues answered most of the comments raised by the reviewers. They carried out new experiments including Ser5 and unphosphorylated Pol II ChIP showing that the level of unphosphorylated PolII is increased at intragenic H3K4me3 peak in KDM5B knocked down cells. It provides evidence for the cryptic transcription originated within a gene. Overall, this reviewer is satisfied with the revised manuscript.

Referee #3 (Remarks to the Author):

The authors adequately addressed all my comments.

 