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Jmjd3 contributes to the control of gene expression in LPS-activated macrophages

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(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 19 June 2009

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by two referees whose comments to the authors are shown below. As you will see the referees are both very positive and would support publication here if you could revise the manuscript along the lines suggested. I would thus like to invite you to prepare a revised manuscript in which you need to address the referees' criticisms in an adequate manner. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

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:

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Referee #1 (Remarks to the Author):

The manuscript by De Santa et al. reports the large-scale ChIP sequencing results of Jmjd3, a histone demethylase, complemented with profiles of RNA polymerase II and the histone modifications H3K4me3 and H3K27me3 in macrophages before and after induction of an inflammatory response. The authors find that Jmjd3 binds at promoters of genes that are already active, or that are activated after LPS stimulation, as measured by co occupancy of Jmjd3with H3K4me3 and RNA polymerase II. Surprisingly, no correlation was detected between Jmjd3 binding and localization of H3K27me3, the reported substrate of Jmjd3, raising the question on how Jmjd3 is recruited and what its function is at these promoters. The authors have gone a long way to find a connection between Jmjd3 recruitment and H3K27me3. The data they provide are extensive and convincing. Unfortunately, the issue of release of repression and/or its involvement in general transcriptional activity are not completely resolved by the authors and hence the exact biological role of the Jmjd3 demethylase in the inflammation process remains somewhat unclear. The authors suggest that Jmjd3 fine-tunes the transcriptional output of LPS activated macrophages based on a rather small number of directed ChIP experiments.
Nevertheless these are interesting findings that in contrast to the current thinking/dogma suggest that Jmjd3 and maybe other Jmj proteins may be involved in other pathways in addition to or instead of releasing repressive marks from chromatin.

Specific points
H3K27me3 is often deposited in broad regions also referred to as blocks (Pauler et al. Genome Res. 2009) and not as peaks. Therefore its levels can be elevated throughout specific genomic regions such as the HOX clusters but also present at low but statistically elevated levels at promoter and/or gene coding regions. It seems therefore not correct to merely call peaks. It would be better if the authors count tag reads in the specific regions they address.

The strong increase in the number of H3K27me3 peaks (from 60.000 to 90.000 peaks) after stimulation of macrophages is not explained. Are these peaks or regions that are further enriched?

The authors used knockout cells of Jmjd3 and claim that changes in H3K27me3 are not evident/measurable at early timepoints following LPS induction. When referring to their published data they state that the effects on K27me3 observed previously are not seen in this study because of a slow response. To convince the reader that this holds true, the authors should show the level and pattern of H3K27me3 at late time points for example at 8 and 24 hours. If Jmjd3 has a function beyond or in addition to fine tuning transcription, the pattern of H3K27me3 should be altered as compared to wt cells.

Referee #2 (Remarks to the Author):

De Santa and colleagues present an extremely interesting and well written manuscript detailing their analyses investigating the role of Jmjd3 in LPS-activated macrophages. Using a ChIP-Seq approach they present compelling evidence that in activated macrophages Jmjd3 is recruited to TSSs harbouring relatively high levels of H3K4me3 and RNAPII. Furthermore, their data indicate that Jmjd3 target sites are not associated with H3K27me3, a previuosly reported substrate for the Jmjd3 demethylase. Somewhat surprisingly (at least to me) deletion of Jmjd3 had no effect on the expression of the vast majority of its target genes. Those genes that were affected were only moderately so, and they were genes linked to the biological consequences of LPS-induction of macrophages. The overall take-home message is that "Jmjd3 fine-tunes the transcriptional output of LPS-activated macrophages in an H3K27 demethylation-independent manner".

Throughout the manuscript the data are clearly presented, logically and coherently described, and appropriate and valid conclusions are drawn. The findings will of of interest to a wide readership.

Specific points

1. In figures 1e and 1f why are the values for JmJD3 binding the Gbp6 promoter at 2 hours different in the two graphs? Error bars are given for the data dispalyed in figure 1e but the amount of Jmjd3

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(from wt cells) binding to Gbp6 in 1f lies well outside of the error range.

2. The authors state in the text "a simple possibility is that Jmjd3 is preferentially recruited to sites of active H3K4me3 deposition or turnover by association with H3K4 histone methyltransferases". This is a most interesting idea. It is a pity that the authors did not explore this possibility futher by experimentation. It would be rather trivial to perform a co-immunoprecipitation of Jmjd3 from stimulated macrophages and then test for associated H3K4 methyltransferase activity.

Minor point

The figure legends are minimally described. A little more information would be useful.

1st Revision - authors' response 16 July 2009

Referee #1
We thank this reviewer for the objective evaluation provided, and we completely agree on the observation that at this stage the exact biological role of Jmjd3 in inflammation is still unclear.

-This reviewer suggested us to reanalyze the distribution of H3K27me3 in the light of the recent demonstration (Pauler et al 2009) that this modification is often deposited in broad regions (as opposed to the sharp peaks characterizing other histone modifications like H3K4me3), a finding that appears clear when its distribution is analyzed at low resolution along chromosomes. We carried out this analysis using the publicly available program described by Pauler and colleagues. The data of these analyses are now described in both the results (p. 8) and discussion (p. 15) sections, and a detailed description of both the procedure and the findings has been added in the supplementary material. This analysis confirmed that also in this system, when analyzed at low resolution, H3K27me3 is largely organized in broad regions that include about 70% of all peaks in both unstimulated and LPS-stimulated mecrophages. We identified more than 5000 blocks of H3K27me3 that are listed in the new Suppl. Table 7. However, peaks inside and outside these blocks behaved very similarly or identically in terms of response to LPS stimulation. We therefore retained the original description in peaks (rather than blocks) as it conforms to the standards in the literature and allows a more precise description of the events occurring at the level of individual genes and regulatory regions.
-As regards the changes in H3K27me3 in response to stimulation, the increased number of peaks observed in response to stimulation occurs both inside (3,87%) and outside (2,20%) of blocks. Moreover, analysis of these blocks confirmed that increases in H3K27me3 are more common than reductions (113 increased vs. 3 decreased blocks).
-Changes in H3K27me3 levels at late time points: what we show in this study is that when peak levels of Jmjd3 are reached in LPS-stimulated macrophages, we can detect transcriptional effects that are apparently dissociated from any detectable effect on H3K27me3 demethylation. This study was carried out in a window of time of 4 hours after LPS stimulation because protein levels of Jmjd3 peak at 2hr and are strongly downregulated thereafter, reaching levels lower than 10% of peak values between 4 and 8 hrs after LPS. Therefore, all we claim here is that most if not all Jmjd3 transcriptional effects detected around the peak of its expression, which coincides with the initial burst of inflammatory gene expression, are not associated with H3K27me3 demethylation. At later time points we could identify by microarray analysis (De Santa et al 2007) only a single polycomb target gene whose expression was affected by Jmjd3 depletion, indicating that H3K27me3 demethylation is an overall very uncommon event in response to Jmjd3 induction in this system, irrespective of the time point considered.

Referee #2
We thank this referee for the very positive evaluation of our work.
Specific points:
1. The values obtained in Fig. 1e were obtained in bone marrow-derived macrophages, while those in Fig. 1f came from fetal liver-derived macrophages. Still, considering the intrinsic variablity of ChIP assays, data are remarkably similar.
2. This experiment was in fact already carried out in De Santa et al 2007 (Fig. 6) where we showed

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that Jmjd3 is incorporated in H3K4 methyltransferase complexes.
3. The legends to figures have now been made more exhaustive.

2nd Editorial Decision 29 July 2009

Thank you for sending us your revised manuscript. Our original referee 1 has now seen it again, and you will be pleased to learn that in his/her view you have addressed all criticisms in a satisfactory manner. The paper will now be publishable in The EMBO Journal and you will receive a formal acceptance letter shortly.

Thank you very much again for considering our journal for publication of your work.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORT

Referee 1

The revised version of the manuscript 'Jmjd3 contributes to the control of gene expression in LPS-activated macrophages' includes the block analysis for H3K27me3 we suggested and sufficiently responds to our other concerns. Although the exact role of the Jmjd3 protein in the inflammation process is still not completely resolved, the data showing this role is not linked to H3K27me3 regulation are clear and convincing. Therefore we can recommend the manuscript for publication in EMBO Journal.

Additional Correspondence 29 July 2009

Further to my recent e-mail and according to our policies I would like to ask you whether you could submit your sequence and processed data to one of the public databases and include the accession details in a modified version of the manuscript text. You could send us such an amended manuscript text file via e-mail, and we will upload it for you into the system prior to acceptance.

Thank you very much.

Yours sincerely,

Editor
The EMBO Journal

Authors' Response 17 August 2009

Thank you for your positive reply. I am sending you in attachment the final version of the manuscript in which the public repository of all the ChIP-Seq data described in the manuscript and the temporary accession number are indicated (highlighted in yellow in the materials and methods section).

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