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Negative, proline-rich and positive disordered patches drive KDM5A 'bubbles' in the nucleus

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Abstract

KDM5A is a histone lysine demethylase which catalyzes the removal of methyl groups from mono-, di-, and tri-methylated lysine 4 on histone H3. The catalytic activity of the KDM5A N-terminal is well characterized biochemically, but like the majority of chromatin modifying enzymes the mechanisms underpinning its behavior in vivo are poorly understood. In studying the contribution of PHD3 to H3K4me3 demethylation, we discovered a greater impact on KDM5A distribution within the nucleus. PHD3 appears to inhibit the formation of discrete KDM5A puncta, in opposition to negative, proline-rich and positive patches in the C-terminal region. These patches appear to be responsible for the novel biophysical behavior we have observed and may play an important role in the regulation of gene expression.

KDM5A is a histone lysine demethylase

KDM5A is a demethylase specific for mono-, di- , tri-methylated lysine 4 of histone H3 (H3K4me3). H3K4me3 is highly associated with transcriptional start sites of actively transcribed genes.

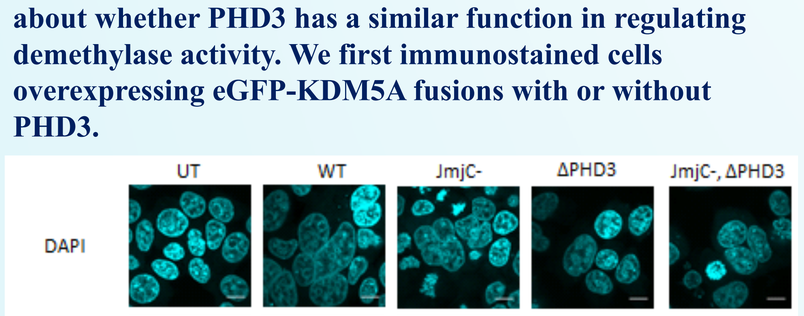

Figure1. Cartoon of KDM5A at a Core Promoter.

KDM5A has been shown to repress inappropriate expression of genes encoding developmental regulators by removing H3K4me3 from their promoters. Removal of this mark is catalyzed by conserved JmjN and JmjC domains, while Plant Homeodomain Domain (PHD)1, and PHD3 regulate catalysis and recruit KDM5A to H3K4me3-rich sites respectively.

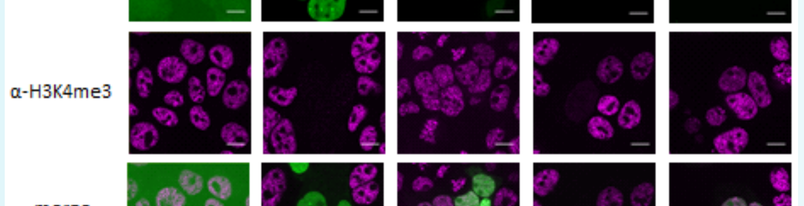

Figure2. KDM5A Domain Architecture. Torres, I. O. *et al. Nat Comm.* (2015).

The effect of PHD3 on demethylase activity

Since KDM5A is a histone demethylase, and PHD1 is reported to affect the catalytic function, we were curious about whether PHD3 has a similar function in regulating demethylase activity. We first immunostained cells overexpressing eGFP-KDM5A fusions with or without PHD3.

Figure3. Immunostaining of HEK293T cells overexpressing with KDM5A truncations with or without PHD3. UT, untransfected cells. JmjC-, catalytically dead mutant.

Overexpression of active KDM5A results in a clear depletion of H3K4me3 even when PHD3 is removed. As expected, the catalytically dead mutants show no significant change in H3K4me3 compared to untransfected cells. Interestingly, our imaging experiments revealed several bright KDM5A puncta in many cell nuclei. As PHD3 appeared to be dispensable for this observation, we wondered which regions of KDM5A might be involved.

PHD3 restricts puncta formation by KDM5A

To dissect the molecular basis for puncta formation we designed a panel of systematic truncations to be expressed as GFP fusions (Fig4). As the C-terminal region (CTR) is largely unstructured, we focused on computationally predicted intrinsically disordered regions (IDRs).

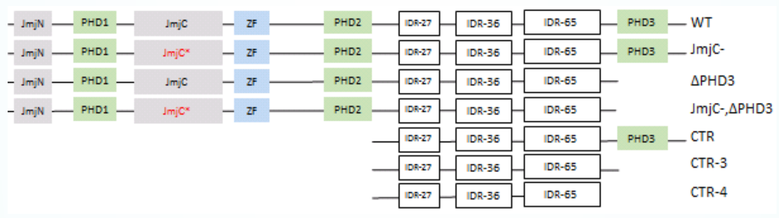

Figure4. KDM5A truncations. PHD, plant homeodomain. IDR, intrinsically disordered region, predicted through D2P2, threshold 75%. Red text, catalytically dead mutant.

Live cell confocal imaging of our truncation series revealed that certain truncations can promote the formation of puncta. Loss of PHD3 drives the appearance of a great number of puncta, suggesting that PHD3 restricts puncta formation by KDM5A. Critically, expression of CTR4 containing the predicted IDRs is sufficient for puncta formation.

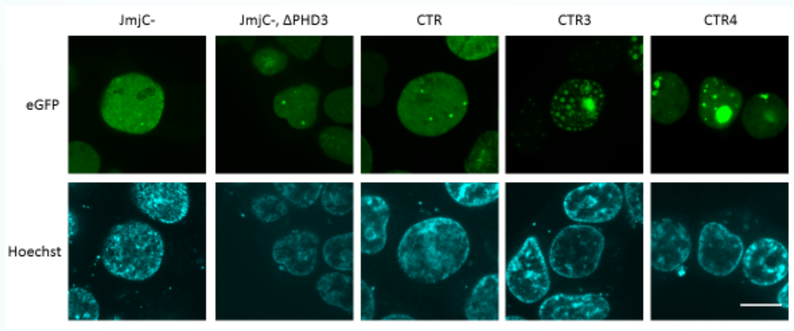

Figure5. Live cell confocal imaging of HEK293T overexpressing KDM5A truncations. JmjC-, catalytically dead mutant; PHD, plant homeodomain; CTR, C- terminal region. Scale bar, 10um.

To investigate intermolecular interactions, cells were co-transfected with mCherry and eGFP fusions. We first validated that mCherry-CTR and eGFP-CTR show the same behavior, ruling out the effect of different fluorescence tags on puncta formation.

eGFP-CTR and mCherry-CTR form mixed puncta, suggesting the two species are able to interact. Using eGFP-CTR3 as a bait, we observe an increase in puncta formation by the prey mCherry-CTR, despite the prey retaining a functional PHD3. This suggests that increased nucleation of puncta by CTR3 can drive further recruitment of CTRs in trans, perhaps explaining the tendency of full length KDM5A to form fewer but larger puncta than the truncations.

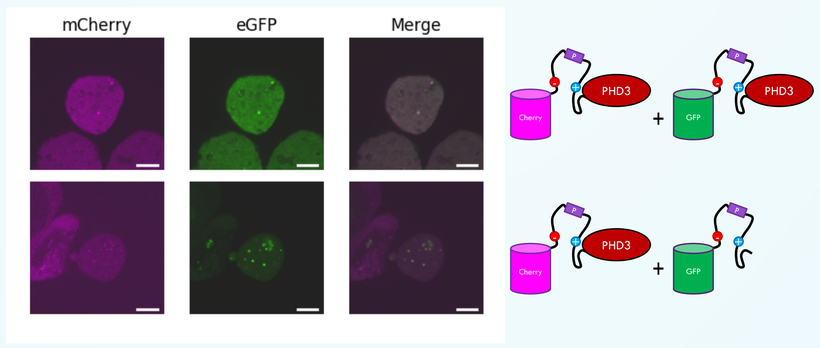

Figure6. Live cell confocal imaging of co-transfection with mCherry-CTR and either eGFP-CTR or eGFP-CTR3. Scale bar, 5um. The right cartoon panel shows the diagram.

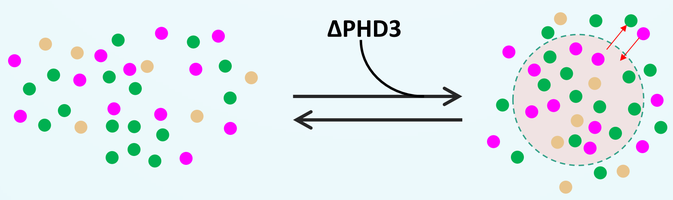

Figure7. A Phase Separation model. KDM5A exists in diffuse and dense (punctate) phases within the nucleus. Deletion of PHD3 shifts the equilibrium towards the dense phase forming an increased number of puncta. mCherry-CTR (pink), eGFP-CTR (green), other nuclear proteins (yellow).

Acknowledgements

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KDM5A puncta are protein 'bubbles'

Live cell confocal imaging of eGFP-CTR3 (Fig5) revealed that many of larger puncta we observed appeared hollow, resembling 'bubbles' rather than droplets. Although less common, we have also observed these properties using full length eGFP-KDM5A.

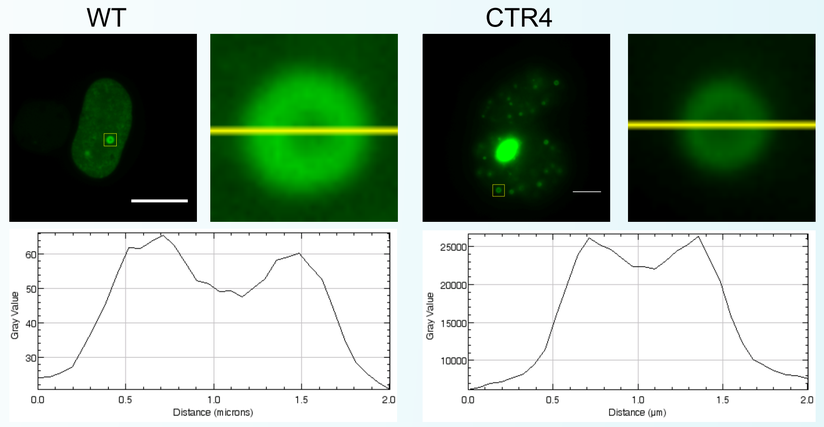

Figure8. Live cell confocal imaging suggests the existence of hollow puncta in HEK293T cells overexpressing KDM5A. Scale bar, 10um and 5um, left and right respectively.

As their small size approaches the limits of conventional light microscopy, we employed super resolution STORM imaging of fixed samples to investigate their 3D structure (Fig9). STORM microscopy confirmed our observation of hollow puncta and extends it to smaller puncta which fall below the diffraction limit. This structural information has significant implications for the biophysical properties of KDM5A.

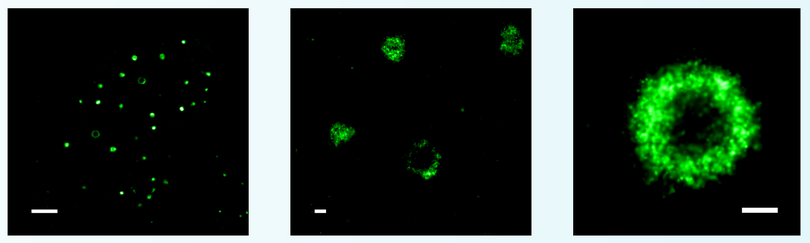

Figure9. STORM imaging of immunostained HEK293T cells with GFPnanobody647 transfected eGFP-CTR4 reveals that even small puncta are hollow. Scale bar, 2um for the whole image and 200nm for zoom-in image.

IDRs predictions correspond to negative, proline-rich and positive patches

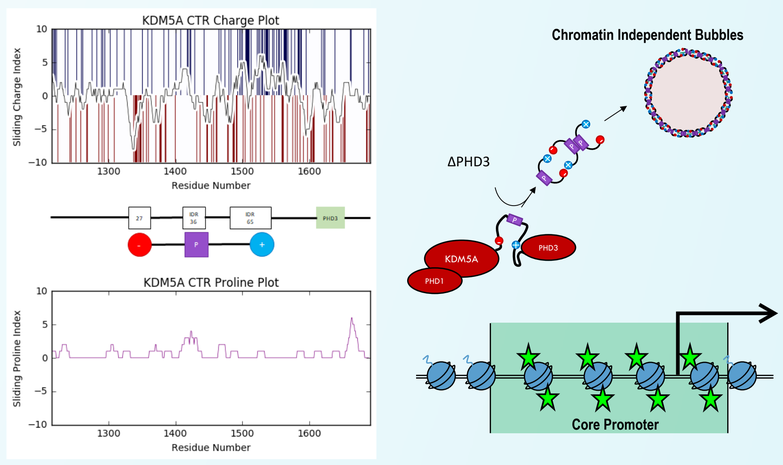

Figure10. The net charge over a 10 amino acid sliding window is shown for the KDM5A C-terminal Region. The positions of positive residues are shown in blue while negative residues are indicated in red. Schematics indicate the positions of predicted intrinsically disordered regions (IDRs), relative to PHD3, and to peaks in negative charge, proline residues and positive charge. Lower plots shows proline count over a 10 amino acid sliding window.

Discussion

Our observations suggest that KDM5A separates into diffuse and dense phases in vivo, and that regions in the CTR mediate the formation of hollow protein bubbles. We hypothesize that the rigidity of a proline rich region between two regions of strong opposite charge may resist intramolecular neutralization favoring higher order interactions between KDM5A molecules.

Further Work

We are very excited to test;

1) How charge scrambled CTR variants behave in vivo
2) How PHD3 inhibits dense phase nucleation, and…
3) How bubble formation affects KDM5A function.