A new twist to Sin3 complexes in pluripotent cells

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Raphaël Pantier, Nicholas P. Mullin and Ian Chambers

MRC Centre for Regenerative Medicine, Institute for Stem Cell Research, School of Biological Sciences, University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU, Scotland

Correspondence: ichambers@ed.ac.uk

Running header: A new twist to Sin3 complexes in pluripotent cells

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Summary

Sin3a is a central component of a class of histone deacetylase containing transcriptional co-regulatory complexes. In this issue of *The EMBO Journal*, Streubel et al. purify Sin3a and identify a variant Sin3a complex containing Fam60a in undifferentiated embryonic stem cells (ESCs). Fam60a is a critical component of the ESC Sin3a complex since Fam60a knock-down leads to an extended G1 cell cycle phase and reduced ESC self-renewal. These exciting results open up new questions about how biochemical differences between variant Sin3a complexes may facilitate alterations in cell-specific function.

Text

Sin3a is the central scaffolding component of a class of histone deacetylase (HDAC) containing complexes that were first described as transcriptional repressors but whose association with actively transcribed loci genome-wide suggests a role in "fine-tuning" ongoing transcription (Reynolds et al, 2013).

In this work, Streubel and colleagues used a label-free quantitative mass spectrometry approach (Smits et al, 2013) to analyse and compare the composition of Sin3a complexes immunoprecipitated with two separate Sin3a antibodies (Streubel et al, 2017). This analysis was performed on undifferentiated ESCs, differentiating embryoid bodies and differentiated fibroblasts to search for cell-specific differences in Sin3a complex composition. An important aspect of the Streubel et al. approach is that they assessed the stoichiometry of the endogenous Sin3a complex. In addition to eliminating potentially confounding effects of overexpression, this identified Fam60a as a component of a variant Sin3a complex present in ESCs at a 1:1 stoichiometry with Sin3a, suggestive of a strong, stable interaction. Although Fam60a had previously been found in Sin3a complexes from the U2OS osteosarcoma cell line by qualitative co-immunoprecipitation (Munoz et al, 2012) or by overexpression of Sin3a complex components in 293T cells (Smith et al, 2012) or ESCs (McDonel et al, 2012), quantitative analysis of endogenous Sin3a was required to demonstrate that Fam60a is a stoichiometric component of the ESC Sin3a complex.

Reciprocal purifications of Fam60a (Streubel et al, 2017) identified Sin3a complex components as the major Fam60a interacting partners, suggesting that the main function of Fam60a in ESCs is within the Sin3a complex. The results from both Sin3a and Fam60a

purifications indicate that the ESC Sin3a core complex is composed of Sin3a, Fam60a, Sap30, Rbbp4 and Hdac1 (**Figure 1**).

The ESC Fam60a/Sin3a complex also contains Tet1 and Ogt which are not associated with Sin3a in differentiated cells. This raises the possibility that Fam60a may bridge Tet1 and Ogt to Sin3a (**Figure 1**). Interestingly, Tet1 and Ogt were detected in the ESC Sin3a complex at a stoichiometry of 0.1 relative to Sin3a. Notably, this low stoichiometry is similar to that of Ing1/2, which are thought to target Sin3a complexes to promoters through binding to the trimethylated lysine of H3K4me3 (Shi et al, 2006). It will be important to determine if these sub-stoichiometric interactions reflect association of Tet1 and Ogt with only a subclass of Fam60a/Sin3a complexes. Alternatively, sub-stoichiometry may reflect lower affinity interactions between Tet1/Ogt and the core Fam60a/Sin3a complex, which would suggest that interactions of sub-stoichiometric components with the core complex may be highly dynamic.

These are interesting and important findings because they show that the predominant Sin3a complex in ESCs contains a protein that is not an essential component of Sin3a complexes in less versatile cell types. Adding to the sense that Fam60a is a critical, integral component of the ESC Sin3a complex, knock-down of Fam60a leads to a reduction in the Sin3a protein level (**Figure 1**). Destabilisation of Sin3a by Fam60a depletion appears to be a cell specific effect, since Fam60a knock-down did not destabilise Sin3a in either U2OS cells (Munoz et al, 2012) or in 293T cells (Smith et al, 2012). Therefore, despite being a non-obligatory Sin3a sub-unit in differentiated cells, Fam60a is nevertheless required for the functional integrity of the ESC Sin3a complex. This raises the possibility that a non-Fam60a component of the Sin3a complex is responsible for Sin3a stabilisation in non-ESCs (**Figure 1**). Alternatively,

the Sin3a complex may be post-translationally modified in ESCs in a way that makes it susceptible to degradation when Fam60a levels are reduced.

As Sin3a is thought to scaffold the Sin3a complex and Fam60a is required to stabilise Sin3a, this implies that other Sin3a complex components would be lost from chromatin when Fam60a is depleted. While formally, this remains to be shown, an exciting consequence may be that cycles of Sin3a complex de-stabilisation and re-formation on chromatin enable dynamic changes in the regulation of Sin3a target genes to occur.

In this regard, Streubel et al. show that the cell cycle regulator E2F1 binds to the *Fam60a* locus, suggesting that Fam60a may be dynamically regulated during the ESC cell cycle. Although this notion is also unconfirmed, Fam60a protein levels are regulated during the cell cycle in U2OS cells with highest levels at G1/S (Munoz et al, 2012). The pluripotency transcription factors Nanog and Oct4 also bind to the *Fam60a* locus with Fam60a expression decreasing as ESCs differentiate. The present work therefore throws up a myriad of questions about connections between Sin3a, pluripotency transcription factors and the ESC cell cycle, which has an unusually short G1 phase (Soufi & Dalton, 2016).

Interestingly, a link to pluripotency transcription factors has already been shown by the detection of Sin3a in the Nanog interactome (Gagliardi et al, 2013). Earlier this year, Sin3a complexes were reported to function in cooperation with Nanog (Saunders et al, 2017), which acts as a concentration dependent self-renewal rheostat (Chambers et al, 2007). Future studies should reveal how Nanog and Sin3a complexes might work together, possibly to fine-tune ongoing transcription.

Phenotypically, depletion of Fam60a in ESCs leads to an extended G1 and aberrant expression of lineage associated genes (Streubel et al, 2017). This effect on cell cycle is the opposite of that seen when Fam60a in knocked down is U2OS cells, where rather than G1 being extended, the G1-S transition is accelerated (Munoz et al, 2012). Further work is needed to resolve this apparent paradox. It will also be important to confirm the effects of Fam60a knock-down in ESCs by conditional genetic deletion. Nevertheless, the similarity in knock-down phenotypes of Fam60a and Sin3a in ESCs is consistent with the idea that Fam60a and Sin3a are both essential components of the ESC Sin3a complex. Therefore, regulating the composition of the ESC Sin3a complex could lead to an extended cell cycle and enable the onset of differentiation. However, further experiments will be required to firmly connect Fam60a/Sin3a function to regulation of the ESC cell cycle.

The new paper changes how we think about Sin3 complexes because it indicates that non-obligatory components may be present in all the Sin3a complexes present in a specific cell type. This may presage the existence of additional 'non-canonical' Sin3a complexes in other cell types and raises the prospect that additional variant transcriptional co-regulatory complexes may be deployed more widely during development for cell-restricted purposes.

Figure Legend

In ESCs, the core Sin3a complex, which is composed of Sin3a, Sap30, Rbbp4 and Hdac1 in many other cell types is joined by Fam60a at a 1:1 stoichiometry relative to Sin3a. Sub-stoichiometric associations of Sin3a with Ing1/2 and Tet1/Ogt are indicated by looser interactions. These could be due to heterogeneity within Fam60a/Sin3a complexes or could reflect dynamic interactions with sub-stoichiometric components that interact with with core complex subunits at lower affinities. The possibility that Fam60a acts as a molecular bridge to bring Tet1/Ogt to the Sin3a complex is indicated. In addition, Nanog may act to connect Sin3a to the core pluripotency gene regulatory network (Saunders et al, 2017). In ESCs when Fam60a is removed by knock-down, the Sin3a complex is destabilised, indicated by the fading out of Sin3a; the possibility of an additional protein (X) acting to stabilise Sin3a in differentiated cells is indicated. Studies in U2OS cells show that Fam60a protein oscillates during the cell cycle. Whether this also occurs in ESCs is an important missing piece of the jigsaw that could suggest that cycles of Sin3a complex formation and dissolution impinge on the genome in a regulatory way during the cell cycle.

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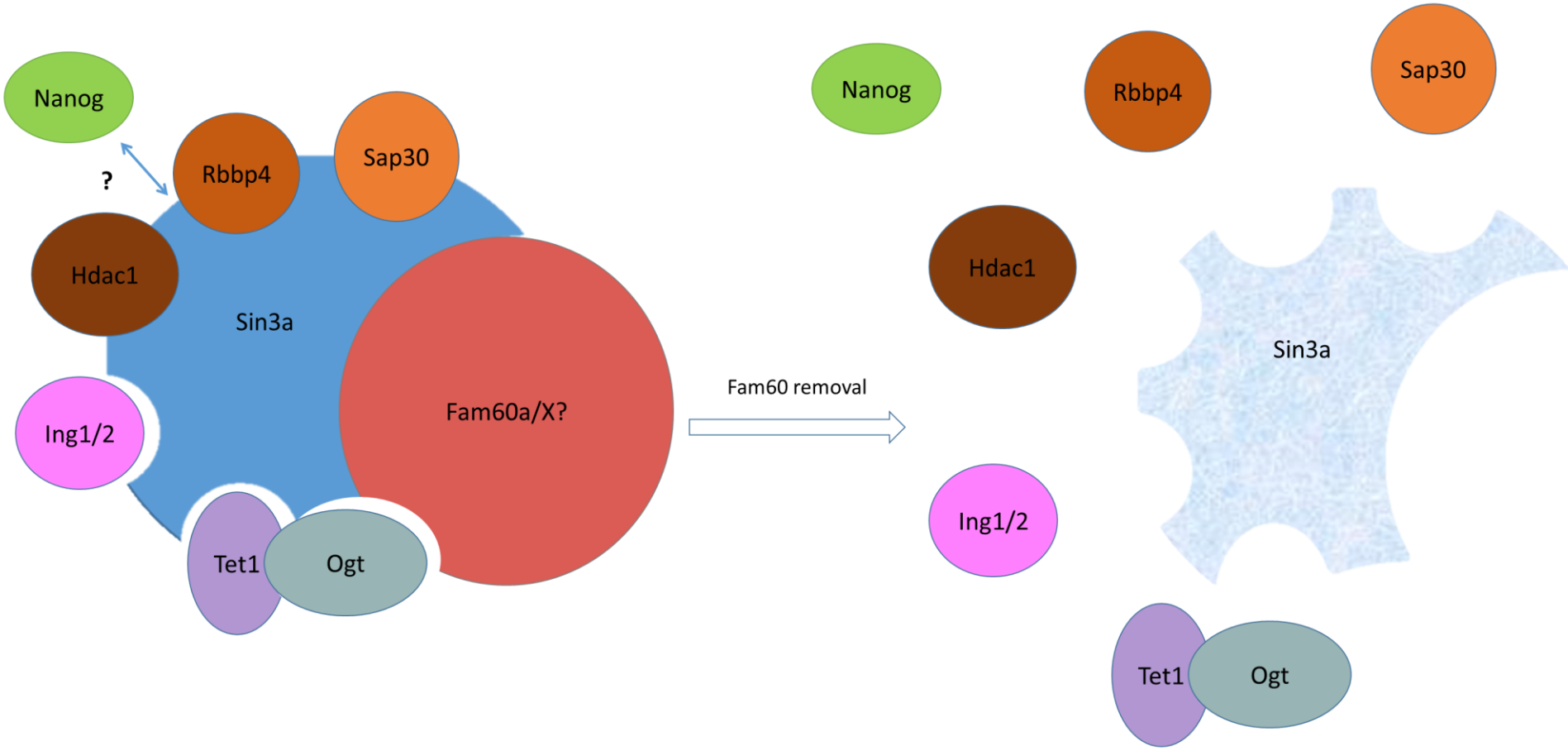
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