A Nuclear Licence to Silence Transposons

Poppy A Gould and Helen M Rowe

Division of Infection and Immunity, Cruciform Building, University College London, WC1E 6BT, UK
Correspondence should be addressed to: h.rowe@ucl.ac.uk

Abstract
Transposon silencing requires the histone methyltransferase, SETDB1. In this issue of *EMBO Reports*, Tsusaka et al.¹ and Osumi et al.² illustrate how the cofactor ATF7IP and its fly homolog Windei (Wde) regulate the methyltransferase function of SETDB1 through its nuclear licensing. The new insight gained from these two articles will shift how we think about epigenetic regulation and its multiple layers of control.

Chromatin structure and function is moulded by histone modifying enzymes such as SETDB1. SETDB1 catalyses histone 3 lysine 9 trimethylation (H3K9me3), a repressive histone mark, which leads to heterochromatin formation and silencing of endogenous retroviruses (ERVs) and genes and is thus essential for early development³⁵. Eggless (Egg) is the fly homolog of SETDB1 and is indispensable for H3K9me3 deposition in the ovaries⁶ and PIWI-interacting RNA (piRNA)-mediated transposon silencing⁷. Previously, SETDB1/Egg has been proposed to be regulated by post-translational modifications⁸ and proteasomal degradation⁹ and pivotal recent work has highlighted that the catalytic activity of SETDB1 depends on its licencing by ubiquitination⁸. Here, Tsusaka et al.¹ and Osumi et al.² provide a detailed mechanistic description of how ATF7IP and fly homolog Wde¹⁰ ensnare SETDB1 within the nucleus to facilitate its ubiquitination and H3K9-methyltransferase activity.

Nuclear import and export of SETDB1
Using ATF7IP knockout (KO) mouse embryonic stem cells (mESCs), Tsusaka et al.¹, demonstrate that ATF7IP is required for nuclear retention of SETDB1 and that the nuclear export inhibitor leptomycin B (LMB) can partially rescue SETDB1 nuclear localisation. Using deletion mutants, the authors map ATF7IP binding to the N-terminal domain of SETDB1 overlapping its two nuclear export signals (NES) and propose that ATF7IP may trap SETDB1 in the nucleus by occluding its export signal and preventing its export. Interestingly, in ATF7IP
KO human HEK293T cells, LMB treatment can only marginally rescue nuclear localisation, suggesting that in this system, ATF7IP is required for nuclear import as well as retention of SETDB1 and this need can be overcome by tagging SETDB1 with a stronger nuclear localisation signal (NLS). Notably, inhibition of proteasomal degradation in ATF7IP KO 293T cells enhances nuclear accumulation of SETDB1, supporting previous evidence for a role of proteasomal degradation as a regulator of this histone methyltransferase. Similarly, Osumi et al., show that Egg is located in both the cytoplasm and nucleus of cultured drosophila ovarian somatic cells (OSC) and that its nuclear retention is dependent on its interaction with a single coiled-coil domain of Wde. In this system, nuclear entry of Egg is independent of Wde, relying instead on its two pre-SET domain NLS sites, loss of either one of which impedes nuclear localisation.

**SETDB1 post-translational modifications and activity**

It was previously shown that monoubiquitination of SETDB1 at Lys885 is required for its histone methyltransferase activity; Tsusaka et al. re-examine the post-translational modifications of SETDB1 and show that ATF7IP is not directly required for monoubiquitination, but that its nuclear entrapment of SETDB1 enhances its ubiquitination. Osumi et al. delve into the previously less explored post-translational modifications of Egg, employing an in vitro histone methyltransferase assay and in vivo rescue experiments to show that monoubiquitination of Egg, (but not phosphorylation or SUMOylation), is required for H3K9me3 deposition at PIWI-targeted transposons. Egg monoubiquitination occurs in the nucleus and is governed by Egg nuclear retention by Wde. Tsusaka et al. demonstrate that loss of ATF7IP in mESCs phenocopies SETDB1 depletion leading to a de-repression of the endogenous retroviruses, IAP, MERVK10C and MusD, which are usually epigenetically silenced by the SETDB1/KAP1/KRAB-zinc finger protein (KZFP) pathway. Likewise, loss of Egg in drosophila OSCs is accompanied by the de-repression of retroviral-like elements Stalker, Mdg1 and Gypsy, all of which are subject to piRNA/SETDB1 regulation (see Figure 1 for a summary model).

**Outlook**

In sum, Tsusaka et al. and Osumi et al. illustrate how SETDB1/Egg is both chauffeured to the nucleus and detained there by ATF7IP/Wde, where it becomes licenced to silence transposons through its monoubiquitination. The key remaining question is - does ATF7IP/Wde regulate the biological function of SETDB1/Egg beyond controlling its nuclear
localization? Osumi et al.\textsuperscript{2} use chromatin-immunoprecipitation to gracefully demonstrate that Egg binds directly to PIWI-targeted transposons and that this binding is dependent on the presence of Wde. However, this may relate to the decrease in nuclear Egg in the absence of Wde rather than suggesting a role for Wde in chromatin targeting. Future work aimed at understanding SETDB1 chromatin targeting and function will shed light on how histone modifying enzymes can fine-tune gene expression programmes and tame transposons.

**Conflict of interest statement**
The authors declare no conflict of interest.

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**Figure 1 legend**

**Proposed model of SETDB1/Egg nuclear retention by cofactors ATF7IP/Wde**
Tsusaka et al.\textsuperscript{1} show that in the mouse, SETDB1 nuclear entry depends on its nuclear localization signal (NLS) and interaction with its cofactor, ATF7IP. SETDB1 is then retained in the nucleus by ATF7IP, which docks at and masks its nuclear export signal, allowing it to become mono-ubiquitinatib (essential for its catalytic function\textsuperscript{8}). ATF7IP also protects SETDB1 from proteasomal degradation\textsuperscript{9}. Osumi et al.\textsuperscript{2} show that in the fly, Egg relies on its NLS for entry into the nucleus, in this case independently of Wde. Similarly to the mouse, Wde binds and ensures that Egg is retained in the nucleus, permitting its mono-ubiquitination and preventing nuclear exit. Mono-ubiquitinatib and entrapped in the nucleus, ATF7IP-bound SETDB1 and Wde-bound Egg are now licenced for H3K9-trimethylation and silencing of SETDB1/KAP1/KZFP- and piRNA-regulated ERVs, respectively.
References


