GENE SILENCING

# An RNA exporter that enforces a no-export policy

Small RNAs guide nuclear Argonaute proteins to silence genomic target loci via recruitment of factors that lead to formation of repressive heterochromatin. Animal gonads use this pathway to repress transposable elements with PIWI-clade Argonaute proteins and their associated small RNAs called PIWI-interacting RNAs (piRNAs). Four research groups now identify a protein complex that acts as a molecular bridge between the piRNA pathway and the epigenetic silencing machinery.

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Small RNAs guide their associated nuclear Argonaute proteins to genomic loci for transcriptional silencing[1–3]. *Drosophila melanogaster* ovaries express Piwi, a nuclear member of the PIWI-clade Argonautes that is responsible for transposon repression. Guided to nascent transposon transcripts by its associated piRNAs, Piwi recruits the cellular machinery that establishes histone H3 lysine 9 trimethylation (H3K9me3), a repressive chromatin mark, leading to co-transcriptional gene silencing. This suppression of transposable elements is essential to ensure genome integrity and fertility. In this issue, Batki et al.[4] report the identification of a protein complex, termed SFiNX (for silencing factor-interacting nuclear export variant), which links the piRNA pathway to the general heterochromatin machinery. It is intriguing that this complex includes a variant RNA exporter, nuclear export factor 2 (Nxf2) that no longer functions in RNA export. Instead, it collaborates with its interaction partner Panoramix (Panx) to promote co-transcriptional silencing (Fig. 1). Together with related work from Hannon[5], Siomi[6] and Yang[7] laboratories, this finding represents a substantial leap in our knowledge of piRNA-mediated co-transcriptional silencing.

Gene repression by *Drosophila* Piwi acts through the deposition of H3K9me3 on target loci[8–10]. Histone modification is mediated by members of the general heterochromatin machinery, such as the H3K9me3 methyltransferase Eggless/SetDB1, the H3K9me2/3 reader protein HP1 (Su(var)205) and the H3K4 demethylase Lsd1 (Su(var)3-3) (refs. [11,12]). However, how piRNA-Piwi recognition of the nascent RNA is molecularly linked to recruitment of the heterochromatin machinery is poorly understood. Classical genetics and whole-genome screens using a transposon repression assay[13–15] identified Maelstrom[8], GTSF1 (ref. [16]) and Panx[11,12] as factors that are required for transcriptional silencing by Piwi. In a continuing quest for the recruitment mechanism, the four research groups sought to identify interaction partners of Panx. This led to the discovery of Nxf2 (ref. [17]), a paralog of the general mRNA export factor Nxf1 (ref. [18]), and its co-factor Nxt1 (Fig. 1). As previously shown for *Panx*, loss of

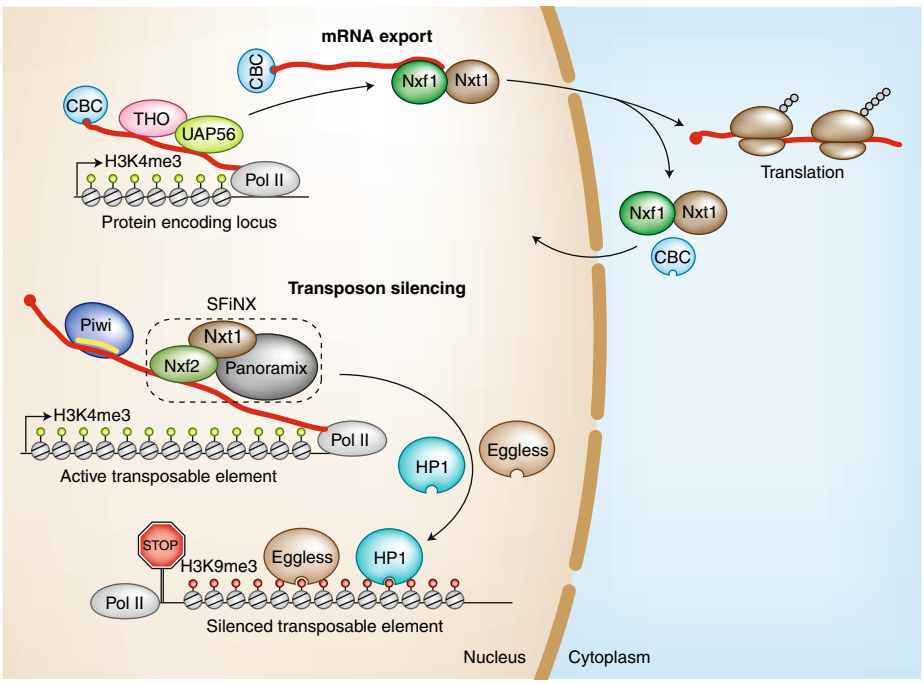


**Fig. 1 | Nxf2 promotes co-transcriptional silencing of transposon loci in the fly germ line.** Canonical RNA export (top) mediated by the export receptor Nxf1 and its co-factor Nxt1. The 5′ cap of the nascent RNA that is still attached to the transcribing RNA polymerase II (Pol II) is bound by the cap-binding complex (CBC). Together with subsequent splicing of the pre-mRNA, this leads to recruitment of the transcription/export (TREX) complex composed of a multiprotein complex called THO and additional factors including UAP56. In transposon silencing (bottom), targeting of nascent transposon transcripts by the Piwi/piRNA complex results in recruitment of SFiNX. Within the SFiNX complex, the variant RNA exporter Nxf2, which is defective in export, uses its RNA-binding domain to bind the nascent RNA, and Panoramix recruits the general heterochromatin machinery (Eggless and HP1). This results in accumulation of silent H3K9me3 heterochromatin marks on transposon loci, shutting down transcription.

*Nxf2* leads to infertility and de-repression of transposable elements in fly ovaries. This is accompanied by increased RNA polymerase II occupancy and a decrease in H3K9me3 marks on transposon loci.

The SFiNX complex (Panx-Nxf2-Nxt1) has a direct role in silencing. Artificial tethering of Nxf2 or Panx to the nascent RNA of a reporter gene, or direct tethering to DNA upstream of this reporter, shows strong repression with a concomitant increase of H3K9me3 marks. There is a clear division of labor within the complex, which the authors reveal by a combination of domain mapping, crystal structure analyses and tethering assays with interaction-interface mutants. Complex formation is obligate, as Panx and Nxf2 depend on each other for protein stability. Furthermore, nuclear accumulation of Nxf2 depends on the nuclear localization signal of Panx. Thus, it is a preformed complex that enters the nucleus. Using structural information from a subcomplex comprising a fragment of Nxf2 (the UBA domain) and Panx (a C-terminal helix), Batki et al.[4] created mutants of Nxf2 and Panx that no longer interact, but are still stable. Individual tethering of these mutants to a reporter RNA lead to notable results. Only the interaction-deficient Panx can function in target repression by deposition of heterochromatin. In a related study, Fabry et al.[5] demonstrate that this repressive activity resides in the N-terminal unstructured region of Panx. Although Panx alone could recruit the heterochromatin machinery in the artificial tethering assay, the interaction-deficient version was inactive in rescue experiments, showing that Panx requires Nxf2 to function in vivo.

Which role does Nxf2 play within the complex? The N-terminal part of Nxf2 has a putative RNA-binding region consisting of an RNA recognition motif (RRM) and a leucine-rich repeat (LRR), which the authors demonstrate can bind RNA in vitro. Nxf2 lacking this RNA-binding domain is inactive in rescue experiments, demonstrating a critical role for RNA binding in vivo as well. Consistent with an RNA-binding role in vivo, the domain becomes dispensable for repression activity when the deletion mutant is directly tethered to a reporter RNA. Using RNA cross-linking and immunoprecipitation (CLIP) experiments with tagged versions of Nxf2, Murano et al.[6] and Zhao et al.[7] identify transposon transcripts as in vivo substrates. The authors also propose a role for Piwi in directing access to such transcripts[5]. The presence of Nxf2 within the SFiNX complex might allow additional control over its RNA-binding activity. In fact, crosslinking mass spectrometry of the recombinant complex reveals extensive intramolecular and intermolecular interactions with the RRM-LRR domain, indicating a potential 'closed' state of the protein. It is likely that interaction with other factors and/or RNA might convert this into an 'open' state to allow RNA binding[4]. Thus, Nxf2 has two distinct functions: to bind the nascent RNA from Piwi-targeted loci to stabilize the complex, and to interact with Panx to mediate repression.

Co-transcriptional repression can be viewed as a dynamic hierarchical process ultimately resulting in deposition of silent histone marks. Murano et al.[6] examined Nxf2-tethered-reporter silencing in a time-resolved manner to arrive at the conclusion that silencing is a two-step process. At early time points, reporter silencing is achieved through reduced RNA polymerase II occupancy, without deposition of H3K9me3 marks[6]. Only long-term silencing is correlated with the presence of silent histone marks. Additional experiments from Batki et al.[4] show that the co-transcriptional silencing process requires a hierarchical assembly of factors. Direct tethering of heterochromatin factors like Eggless, Lsd1 and HP1 to a reporter RNA fails to induce repression, but tethering of these factors to the DNA upstream of the reporter leads to silencing. The authors also find that tethering of Nxf2 to reporter RNA induces stronger repression than tethering to DNA. The results point to a very specific hierarchical assembly of factors when nascent RNA is used as an anchor for co-transcriptional repression.

The involvement of Nxf2 is interesting, as a previous study could not attribute any export function to it[17]. The general mRNA exporter Nxf1 transits through the nuclear pore complex interacting with the phenylalanine-glycine (FG) repeat nucleoporin meshwork within the pore channel. Examining crystal structures of the Nxf2-Panx and Nxf2-Nxt1 fragments, Batki et al.[4] find that the two putative FG-repeat binding pockets in Nxf2 are occluded and might be unavailable for interaction with FG nucleoporins, a fact Zhao et al.[7] confirmed experimentally. Zhao et al.[7] report an additional mechanism by which Nxf2 might operate—a direct interaction between Nxf2 and Nxf1 that prevents interaction of Nxf1 with FG-repeat nucleoporins[7]. Thus, Nxf2 sabotages RNA export: it snatches the nascent RNA away from Nxf1 to retain it at the site of transcription and actively allows co-transcriptional repression via the SFiNX complex components. Co-transcriptional repression of transposon loci offers a unique challenge to the RNA export machinery. Under conditions of transposon derepression, for example, in piRNA pathway mutants, the nascent transposon RNA is a perfectly good cargo for the general RNA export machinery. However, when used as an anchor for co-transcriptional silencing, it should be detained at the site of transcription as long as possible. It is worth mentioning that studies in fission yeast show that mutation of factors involved in transcriptional elongation[19] and general mRNA export[20] favor small RNA-mediated silencing, possibly because these interventions increase retention of the nascent RNA at the site of transcription.

In conclusion, the four reports identify a molecular link between the nascent RNA and the general silencing machinery. However, events that lead from initial recognition of nascent RNA by the piRNA-Piwi complex to downstream recruitment of the SFiNX complex remain to be determined. The molecular role of other factors implicated in the silencing process also needs to be clarified. Finally, the existence of testis-specific Nxf paralogs in mammals, with some being required for male fertility[21], offers the exciting possibility that similar mechanisms might be operational in the mammalian male germ line where piRNAs guide DNA methylation. ❐

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## Competing interests

The authors declare no competing interests.