

# Nxf3: a middleman with the right connections for unspliced piRNA precursor export

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**RNA export is tightly coupled to splicing in metazoans. In the *Drosophila* germline, precursors for the majority of Piwi-interacting RNAs (piRNAs) are unspliced. In this issue of *Genes & Development*, Kneuss and colleagues (pp. 1208–1220) identify Nxf3 as a novel germline-specific export adapter for such unspliced transcripts. Their findings reveal the sequence of events leading from its role at the site of transcription to delivery of the cargo to cytoplasmic piRNA biogenesis sites.**

RNA polymerase II (Pol II) transcripts are cotranscriptionally processed by 5' capping, splicing, and 3' polyadenylation. RNA nuclear export is tightly coupled to processing: Only mature transcripts are exported to the cytoplasm, whereas unprocessed RNA is targeted for degradation. In addition to recognition of the 5' cap by the cap-binding complex (CBC), splicing deposits a transcription and export complex (TREX) on the nascent RNA. Components within the TREX act as adaptors to recruit the export receptor nuclear export factor 1 (Nxf1) and its cofactor, NTF2-related export protein 1 (Nxt1), which escort the ribonucleoprotein particle (RNP) out of the nucleus (Fig. 1). Thus, coupling of RNA processing to assembly of TREX serves to control the quality of the cargo being readied for export.

Despite splicing and polyadenylation being a prerequisite for export of most RNAs, there are a few exceptions for selective export of RNAs. Nonspliced and nonpolyadenylated replication-dependent histone mRNAs end in a conserved stem-loop structure that recruits the stem-loop-binding protein (SLBP/HBP) for their export. Other molecules, such as transfer RNAs (tRNAs) or pre-microRNAs (pre-miRNAs), are directly bound by specific export receptors such as Exportin-t and Exportin5, respectively. Furthermore, viruses such as HIV export their unspliced genomic RNAs using viral adaptor proteins such as Rev via the Crm1 (chromosomal maintenance 1)-dependent

protein export pathway. In this issue of *Genes & Development*, Kneuss et al. (2019) describe yet another novel exception in the context of biogenesis of Piwi-interacting RNAs (piRNAs), specialized small RNAs that repress transposable elements in animal gonads.

Canonical RNA Pol II transcripts arising from large genomic regions called piRNA clusters are precursors for piRNAs in almost all model systems. Such long precursor transcripts have to be exported to cytoplasmic perinuclear granules called nuage, where the biogenesis factors reside. In *Drosophila*, two distinct piRNA biogenesis pathways are operational in the two ovarian compartments (somatic follicle cells and the germline). In the ovarian soma, piRNA precursors are canonical RNA Pol II transcripts that are exported via the Nxf1–Nxt1 pathway (Dennis et al. 2016). In contrast, the majority of piRNAs in the fly ovarian germline arise from noncanonical precursors transcribed from both strands of so-called dual-strand clusters.

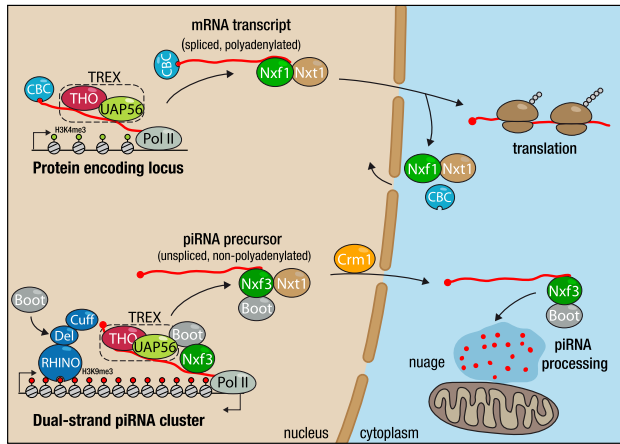
Dual-strand clusters are unique in every aspect of their biology and are found only in Drosophilids. They are embedded in heterochromatic regions containing H3K9me3 modifications, marks linked to transcriptionally silent regions (Fig. 1). To overcome this problem, flies have evolved a unique transcription/processing apparatus that depends on a “reader” protein (Rhino [Rhi]) that recognizes the methyl marks and assembles a complex consisting of a linker protein (Deadlock [Del]) and an inactive nuclease (Cutoff [Cuff]) (Zhang et al. 2014). Del recruits a variant transcription initiation factor (Moonshiner [Moon]) that kick-starts transcription from the heterochromatic locus (Andersen et al. 2017). Interestingly, Cuff ensures that the resulting 5'-capped transcripts remain unspliced and nonpolyadenylated (Zhang et al. 2014; Chen et al. 2016). In fact, artificial tethering of Rhi to a reporter gene can suppress splicing of the resulting reporter transcript and promote its entry into the piRNA biogenesis pathway (Zhang et al. 2014). Thus, in addition to unique requirements for transcription, subversion of key RNA

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**Figure 1.** Canonical RNA Pol II transcripts originating from euchromatic loci (H3K4me3) are exported via the Nxf1 export receptor. Noncanonical Piwi-interacting RNA (piRNA) precursors from the dual-strand piRNA clusters that are located in heterochromatin (H3K9me3) use the germline-specific adaptor Nxf3 and the Crm1 (chromosomal maintenance 1)-dependent export pathway.

processing events is a specialty of dual-strand clusters. So, how are such noncanonical piRNA precursors exported to the cytoplasm?

Nxf3, a germline-specific paralog of Nxf1, was originally unearthed by the Hannon laboratory (Czech et al. 2013) in a genome-wide RNAi screen for factors essential for transposon control in the fly germline. The same screen also identified another factor, CG13741. Kneuss et al. (2019) now demonstrate that fly mutants of *Nxf3* and *CG13741* (renamed Bootlegger [Boot]) derepress germline-expressed transposons, leading to infertility. They determined that transposon derepression is due to a piRNA biogenesis defect specifically affecting dual-strand clusters. Hinting at a direct role for Nxf3 and Boot, both factors are localized at Rhi-dependent transcription foci in the germline nucleus. Apart from the relatedness of Nxf3 to Nxf1, the presence of Nxf3 (and Boot) in the cytoplasmic nuage led the investigators to explore the possibility that the factors might in fact facilitate export of the dual-strand cluster transcripts. Consistently, endogenous Nxf3 is associated with dual-strand cluster precursor transcripts, and the transcripts are detected in both the nuclear foci and the nuage. Loss of *Nxf3* renders the precursor undetectable in the nuage while still being present in nuclear foci, demonstrating its importance for RNA export. Very similar findings are described in a complementary study by the Brennecke laboratory (ElMaghraby et al. 2019).

How is the specificity of Nxf3 for dual-strand cluster precursors determined? Direct RNA-binding preference is unlikely to play a role, as even artificial reporter sequences can enter the Rhi-dependent piRNA biogenesis pathway (Zhang et al. 2014). A transcription-dependent loading of an RNP on nascent RNA arising from Rhi-dependent loci seems to be the mechanism used. Interestingly, TREX components such as UAP56 (Zhang et al. 2012) and the THO subcomplex (Hur et al. 2016; Zhang

et al. 2018) that are normally associated with spliced RNAs are also found to bind unspliced dual-strand cluster transcripts generated from Rhi-dependent loci. So, what distinguishes the cluster transcripts from spliced RNAs? Kneuss et al. (2019) and ElMaghraby et al. (2019) now place Nxf3 and Boot as novel germline-specific components within this noncanonical RNA export cargo. Using knockdown experiments, they provide evidence for a sequence of events in which Rhi (via Del) first recruits Boot, which in turn recruits Nxf3 and UAP56 to the transcription loci to bind the nascent RNA. Subsequently, Nxt1, the common cofactor of the Nxf family members, is also recruited to stabilize association of Nxf3 with the RNA cargo.

All commerce with the cytoplasm is conducted through the nuclear pore complexes (NPCs). The central channel of the NPC presents a sieve-like permeability barrier in the form of phenylalanine-glycine (FG) repeats from the FG-nucleoporins that line the channel. Nxf1 directly interacts with the FG repeats to facilitate passage of the mRNP cargo. To function as an RNA export receptor, Nxf3 would also have to interact with FG-nucleoporins. The C-terminal ubiquitin-associated domain (UBA) of Nxf1 is implicated in this activity, but this region in the human Nxf3 ortholog seems to be diverged (Yang et al. 2001). Instead, the human Nxf3 is shown to depend on the Crm1-mediated protein export pathway to transport an Nxf3-tethered RNA to the cytoplasm (Yang et al. 2001). Kneuss et al. (2019) reach a similar conclusion by demonstrating that nucleo-cytoplasmic shuttling of fly Nxf3 protein is also Crm1-dependent. Crm1 recognizes nuclear export signals (NESs) on cargo proteins to facilitate their passage into the cytoplasm. The related study by ElMaghraby et al. (2019) identifies such an NES in fly Nxf3, with point mutations in this signal trapping the protein in the nucleus.

Once in the cytoplasm, the export cargo is likely disassembled by the action of RNA helicases that remove the export receptors, ensuring directionality of the transport. Localization of Boot and Nxf3 in the nuage suggests an export pathway that accompanies the precursor all the way from the site of transcription to the cytoplasmic nuage, ensuring a safe passage to the processing centers. A direct juxtaposition of UAP56 signals in the fly germline nucleus across from cytosolic nuage marked by Vasa was noted previously (Zhang et al. 2012), leading us to wonder whether export may take place through specialized nuclear pores. Taken together, these two studies identify Nxf3 as a trusted middleman in a specialized RNA export pathway for unspliced piRNA precursors to ensure silencing of mobile elements in the fly germline.

## References

- Andersen PR, Tirian L, Vunjak M, Brennecke J. 2017. A heterochromatin-dependent transcription machinery drives piRNA expression. *Nature* 549: 54–59. doi:10.1038/nature23482
- Chen YA, Stuwe E, Luo Y, Ninova M, Le Thomas A, Rozhavskaia E, Li S, Vempati S, Laver JD, Patel DJ, et al. 2016. Cutoff suppresses RNA polymerase II termination to ensure expression

- of piRNA precursors. *Mol Cell* **63**: 97–109. doi:10.1016/j.molcel.2016.05.010
- Czech B, Preall JB, McGinn J, Hannon GJ. 2013. A transcriptome-wide RNAi screen in the *Drosophila* ovary reveals factors of the germline piRNA pathway. *Mol Cell* **50**: 749–761. doi:10.1016/j.molcel.2013.04.007
- Dennis C, Brasslet E, Sarkar A, Vaury C. 2016. Export of piRNA precursors by EJC triggers assembly of cytoplasmic Yb-body in *Drosophila*. *Nat Commun* **7**: 13739. doi:10.1038/ncomms13739
- ElMaghraby MF, Andersen PR, Pühringer F, Hohmann U, Meixner K, Lendl T, Tirian L, Brennecke J. 2019. A heterochromatin-specific RNA export pathway facilitates piRNA production. *Cell* **178**: 964–979. doi:10.1016/j.cell.2019.07.007
- Hur JK, Luo Y, Moon S, Ninova M, Marinov GK, Chung YD, Aravin AA. 2016. Splicing-independent loading of TREX on nascent RNA is required for efficient expression of dual-strand piRNA clusters in *Drosophila*. *Genes Dev* **30**: 840–855. doi:10.1101/gad.276030.115
- Kneuss E, Munafò M, Eastwood EL, Deumer U-S, Preall JB, Hannon GJ, Czech B. 2019. Specialization of the *Drosophila* nuclear export family protein Nxf3 for piRNA precursor export. *Genes Dev* (this issue). doi:10.1101/gad.328690.119
- Yang J, Bogerd HP, Wang PJ, Page DC, Cullen BR. 2001. Two closely related human nuclear export factors utilize entirely distinct export pathways. *Mol Cell* **8**: 397–406. doi:10.1016/S1097-2765(01)00303-3
- Zhang F, Wang J, Xu J, Zhang Z, Koppetsch BS, Schultz N, Vreven T, Meignin C, Davis I, Zamore PD, et al. 2012. UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery. *Cell* **151**: 871–884. doi:10.1016/j.cell.2012.09.040
- Zhang Z, Wang J, Schultz N, Zhang F, Parhad SS, Tu S, Vreven T, Zamore PD, Weng Z, Theurkauf WE. 2014. The HP1 homolog rhino anchors a nuclear complex that suppresses piRNA precursor splicing. *Cell* **157**: 1353–1363. doi:10.1016/j.cell.2014.04.030
- Zhang G, Tu S, Yu T, Zhang XO, Parhad SS, Weng Z, Theurkauf WE. 2018. Co-dependent assembly of *Drosophila* piRNA precursor complexes and piRNA cluster heterochromatin. *Cell Rep* **24**: 3413–3422.e4. doi:10.1016/j.celrep.2018.08.081