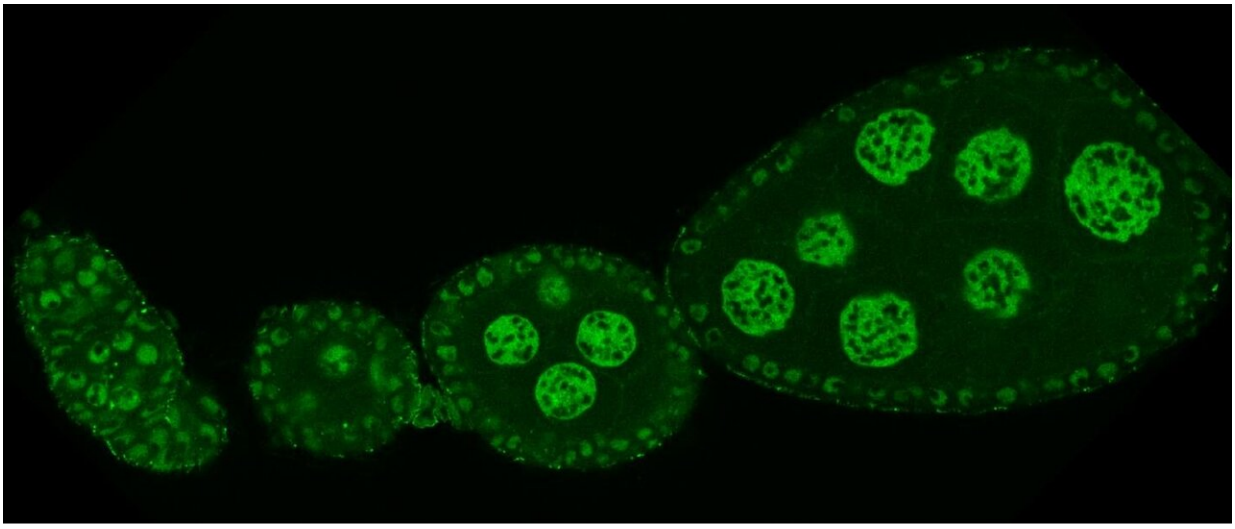
Gene silencing by crosstalk

February 16 2021



Confocal microscopy image of Cut up (Ctp/LC8), tagged with Green Fluorescent Protein (GFP), in a Drosophila ovary. Credit: IMBA/Jakob Schnabl

Researchers at IMBA—Institute of Molecular Biotechnology of the Austrian Academy of Sciences–unveil functional and mechanistic details in small RNA-mediated co-transcriptional gene silencing. The results are published in the journal *Genes & Development*.

Why do genes need to be silenced? The 'genes' in question are in fact transposons, selfish genetic elements that seek to self-multiply at the host's expense and that need to be controlled. Julius Brennecke's group at IMBA focuses on lifting the mysteries of a specific type of transposon silencing, namely the piRNA pathway in animal gonads. Understanding

this ancient silencing system promises to reveal general mechanistic principles of gene expression and chromatin biology.

Gene silencing: either before they 'speak," or right as they attempt to

Heterochromatin, a tightly packed form of DNA, plays an essential role in transposon silencing and in safeguarding eukaryotes' genomic integrity. Distinct strategies are set in motion to guarantee that heterochromatin formation be sequence specific against undesirable genetic elements, or target loci. One such strategy uses so called nuclear Argonaute proteins, which are complexed with small RNAs that guide them to nascent transposon transcripts in chromatin (i.e., transposon mRNAs still associated with the transcribing polymerase). This strategy is therefore dependent on target locus transcription and is referred to as 'co-transcriptional gene silencing.'

One specific Argonaute protein associated with co-transcriptional gene silencing in Drosophila ovaries is Piwi, bound to short piRNAs that are encoded by genomic master loci called piRNA clusters. Piwi-mediated silencing requires heterochromatin factors (silencing effectors) to be recruited, a step that is mediated by the SFiNX complex (silencing factor interacting nuclear export variant), a complex formed of the heterodimeric nuclear RNA export variant Nxf2-Nxt1 and the Piwi-associated orphan protein Panoramix (Panx). The SFiNX complex had been previously identified by four groups in the field independently, including the Brennecke lab. For more on this topic, have a look at a previous press release on how flies repeatedly coopted nuclear export factors for genome defense: www.imba.oeaw.ac.at/research-h … -dna-from-parasites/ .

Cut up and the two SFiNXes

The current publication in the journal *Genes & Development* was spearheaded by the Ph.D. student Jakob Schnabl and unravels the molecular mechanism and function of the SFiNX complex. The researchers first identified Cut up (Ctp), the Drosophila ortholog of the highly conserved Dynein Light Chain 8 (LC8), to be a functional member of the SFiNX complex. Intriguingly, Cut up mediates SFiNX dimerization, which turns out to be essential for its molecular functionality. "In order for Piwi to be able to silence transposons, the SFiNX complex needs to be dimerized by Cut up," says IMBA group leader Julius Brennecke. Just as one hand alone does not clap, it takes two SFiNXes, brought together by Cut up, to silence!

The least expected player and a myriad of other functions yet to be dissected

Jakob Schnabl has already been involved in the 2019 publication from the Brennecke lab describing SFiNX. This earlier work was guided by Ph.D. student Julia Batki, who received award recognitions for her thesis and who is also an author on the current SFiNX publication. When asked about Julia's contribution to the Cut up paper, Jakob's eyes light up: "Quite some part of the paper is about this small protein [Ctp/LC8, aka Cut up] that acts as a dimerization mediator for the SFiNX complex, and there I collaborated very closely with Julia."

Jakob elaborates: "Honestly, we originally thought that the Dynein Light Chain [Cut up] is a contaminant in our experiments because if you look at it superficially it just doesn't make sense that a component of a cytoplasmic motor protein complex is involved in nuclear SFiNX biology. But Cut up kept coming up in our interaction screens. At some point we just had to test for it directly with the aim of ruling out a function. That experiment, however, strongly pointed to Cut up being a functional SFiNX subunit!" Jakob goes on to say, "In fact, when you

look more closely into the literature, you notice that this protein [Cut up] is implicated in many, many functions! It is sort of a dimerization hub protein, yet somewhat overlooked in general."

A DNA-RNA crosstalk leading to gene silencing

Finding that SFiNX is a dimer set the stage for Jakob to characterize SFiNX biochemically. Jakob could show that dimeric SFiNX is able to interact with either the DNA target locus or the nascent RNA. These two distinct interactions ultimately lead to the same goal, namely transposon silencing. The authors also showed that the SFiNX complex can form condensates in vitro in the presence of nucleic acids and used this property to assay for SFiNX's ability to form multivalent interactions. In light of the new findings, the authors propose a model to explain the SFiNX complex's function and molecular mechanism.

This model of multivalent interactions would involve a SFiNX-mediated DNA-RNA crosstalk to enable other domains within SFiNX or co-recruited silencing effectors to establish heterochromatin. "In the end, a nascent transcript is a transient molecule on chromatin, and SFiNX multivalency and its ability to bind DNA and RNA seems to be a key activity to keep the nascent RNA on chromatin. This might well be a more general mechanism underlying co-transcriptional or RNA-mediated heterochromatin formation," concludes Julius Brennecke.

More information: Jakob Schnabl et al, Molecular principles of Piwi-mediated cotranscriptional silencing through the dimeric SFiNX complex, *Genes & Development* (2021). DOI: 10.1101/gad.347989.120

Provided by Austrian Academy of Sciences