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piRNAs Meet Mitochondria

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zuc/MitoPLD encodes a conserved enzyme that localizes to mitochondria and hydrolyzes the mitochondria-specific lipid cardiolipin. Surprisingly, zuc/MitoPLD activity is required for Piwi-interacting RNA (piRNA)-mediated silencing of transposable elements in fly and mouse germlines, suggesting that signaling from mitochondria influences the piRNA pathway.

The germlines of all metazoa contain electron-dense cytoplasmic structures known by a variety of names, in different species and developmental stages, including germline granules, nuage, and pole plasm. The nuage is not simply a convenient marker of germ cells, but in fact is required for germline specification in many species, such as *Drosophila* and *C. elegans*. Moreover, the nuage prove critical for germline genomic stability by suppressing the expression of selfish genetic elements such as transposons. Nuage are nonmembranous structures, but are often associated with mitochondria; in fact, one of the names for germline granules in mammals is the intermitochondrial cement. What could be the reason for this curious association between mitochondria and germline granules in germ cells? Several studies suggested that some mitochondrial components, such as mitochondrial ribosomal RNAs, can be exported for inclusion in the nuage structure (Amikura et al., 2001); however, these data remain controversial (Kloc et al., 2001). Now, the studies by Huang and coauthors (Huang et al., 2011) and Watanabe and coauthors (Watanabe et al., 2011) published in this issue of *Developmental Cell* reinforce the functional link between mitochondria and nuage and suggest that signaling from mitochondria might regulate assembly and function of nuage components.

The molecular function of the nuage is not well understood, but research in the last few years revealed that it hosts components of the piRNA machinery that are responsible for silencing of genomic parasites: transposable elements. If not repressed, the activation of transposable elements leads to genomic damage through insertional mutagenesis and generation of double-stranded DNA breaks. A diverse population of small RNA molecules, the piRNAs, targets transposon transcripts. piRNAs are tightly associated with Piwi proteins that have endonuclease activity that cleaves transposon mRNAs, preventing their expression. Genetic and biochemical studies in several model organisms revealed a number of other proteins required for the biogenesis and function of piRNAs, and microscopic studies showed that almost all these proteins, including Piwis, are localized in the nuage. Furthermore, mutations that interfere with the piRNA pathway often influence morphology of germline granules; conversely, mutations that disrupt nuage formation lead to transposon activation, indicating that proper formation of nuage is a prerequisite for function of the piRNA pathway.

One of the proteins required for piRNA biogenesis, zucchini (zuc), was identified through a genetic screen in *Drosophila* and was proposed, based on sequence similarity, to be a nuclease that processes piRNA molecules from longer RNA precursors (Pane et al., 2007). The existence of such a nuclease had been suspected based on the fact that piRNA biogenesis is independent of Dicer activity and seems to proceed from single-stranded precursors. (Dicer enzymes process double-stranded RNA to generate other classes of small RNAs, such as siRNA and miRNA.) Indeed, zuc is required for generation of piRNA in the *Drosophila* ovary (Olivieri et al., 2010). However, in contrast to other components of the piRNA pathway, which are localized in nuage, zuc is localized to mitochondria (Saito et al., 2010). The studies by Huang et al. (2011) and Watanabe et al. (2011) showed that function of zuc in the piRNA pathway is conserved in mammals: knockout of the mammalian homolog of zuc, MitoPLD, eliminates the majority of piRNA in male germ cells and leads to transposon activation and arrest of spermatogenesis, characteristic phenotypes of piRNA pathway mutants. Furthermore, these studies demonstrated that MitoPLD localizes to mitochondria and has phospholipase activity that hydrolyzes cardiolipin, a mitochondria-specific lipid, to generate phosphatidic acid (PA). PA works as a signaling molecule that affects mitochondrial fusion and morphology.

These studies provide the first direct functional link between mitochondria and the piRNA pathway, but pose another question: How does zuc/MitoPLD localized on the mitochondrial surface affect the piRNA machinery in the nuage? It seems that the phospholipase activity of zuc/MitoPLD is important, as a point mutation in a catalytic residue of *Drosophila* zuc disrupts biogenesis of piRNAs. The authors propose that PA produced by zuc/MitoPLD might regulate recruitment of nuage components to the surface of mitochondria. Indeed, Huang and coauthors report that knockout of Lipin1, an enzyme that hydrolyses mitochondrial PA, has an effect opposite to that of zuc/MitoPLD deficiency and further increases the density of nuage in mouse germ cells. According to this model, a balance between the opposing activities of zuc/MitoPLD and Lipin1 might control the concentration of PA to regulate the assembly of nuage components around mitochondria. PA binding has been shown in other systems to facilitate assembly of protein signaling complexes. For example, Raf-1 kinase has a PA-binding domain that is involved in its recruitment to the plasma membrane upon activation of the ERK signaling pathway (Ghosh et al., 2003; Kraft et al., 2008). Alternatively, a high local density of PA, with its negatively charged headgroup, may change the biophysical properties of the mitochondrial outer membrane to favor nuage assembly. Interestingly, zuc function is essential for the piRNA pathway in *Drosophila* even in cells that do not have typical nuage, such as the ovarian follicular cells, which have a somatic origin. Recent studies revealed that follicular cells have different granular cytoplasmic structures, Yb-bodies, that host piRNA components and seem to be closely associated with mitochondria (Olivieri et al., 2010; Saito et al., 2010), further extending the link between mitochondria and piRNA pathway.

These studies also raise the issue of whether other features of mitochondrial function might be related to the formation of nuage. Given the opposing effects of mitoPLD and Lipin1 mutations on nuage formation, PA on the mitochondrial surface certainly seems to be the prime candidate. However, MitoPLD has been implicated in a late step of mitochondrial outer membrane fusion (Choi et al., 2006). Conversely, Huang et al. provide evidence that Lipin1 is involved in mitochondrial fission. Therefore, it will be interesting to explore whether mitochondrial dynamics is important for nuage formation. Moreover, respiratory function or some other aspect of mitochondrial metabolism may be necessary for nuage formation. It would be important to assess mitochondrial function in the germline of MitoPLD- and Lipin1-deficient mice.

Overall, the studies published in this issue of *Developmental Cell* underline the relationship between piRNA, nuage, and mitochondria and suggest that lipid metabolism on the mitochondrial surface might orchestrate assembly of nuage components and operation of the piRNA pathway. Future studies should reveal the molecular mechanism of this process and identify how signaling from mitochondria affects nuage formation. It would also be interesting to investigate if there is feedback from nuage and piRNA components to regulate the function of mitochondria. Although primarily known for its bioenergetic function, mitochondria continue to surprise by coordinating diverse cellular functions, including programmed cell death, innate immunity, and now germline maintenance.

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