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Arginine methylation as a molecular signature of the Piwi small RNA pathway

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Almost all eukaryotes have small RNA pathways that regulate expression of protein-coding genes, control the activity of endogenous transposable elements and fight exogenous viral infection. Despite diversity of small RNA pathways functions and mechanisms, their core is conserved throughout evolution: it is an effector complex containing a small RNA that is tightly bound to a member of the Argonaute protein family.[1] The small RNA provides specificity by recognition of complementary RNA targets. The Argonaute protein provides the effector function; it either destroys target RNA directly using its endonuclease activity or inhibits it indirectly, for example by recruiting additional protein factors that cause translational repression (in animals) or inducing changes in chromatin structure (in fission yeast and possibly some plants).

In animals, members of the Argonaute superfamily are subdivided into two clades, Ago and Piwi, based on their sequence and expression pattern. Although Ago family members are ubiquitously expressed throughout development, Piwi expression is largely restricted to the germline lineage where these proteins combat the potentially deleterious activity of transposable elements and possibly play other germline-specific functions.[2] Each clade of Argonautes interacts with separate classes of small RNAs that differ in size and biogenesis: Ago proteins bind small interfering RNAs (siRNAs) and microRNAs (miRNAs) and Piwi proteins bind Piwi-interacting RNAs (piRNAs). Argonaute proteins do not ubiquitously distribute in the cell but instead localize in cytoplasmic granules. Granules that contain Ago family members were identified as P-bodies and represent the sites of storage and degradation of translationally repressed mRNA.[3] Piwi-containing germline-specific granules have been called nuage/germ granules or P-granules in different organisms. Both Ago and Piwi granules contain number of other proteins, and Ago proteins physically interact with a key P-body component, GW182.4

Ago and Piwi family members have identical domain structures and therefore it is not obvious why Agos and Piwis are involved in different pathways or how localization to distinct cytoplasmic granules is achieved. Although information on protein partners of Ago family members was accumulating, partners for Piwi were unknown. We recently comprehensively characterized Piwi-associated proteins in mouse germline cells and found that Piwi associates with the PRMT5/WDR77 complex.[5] This enzyme is known to methylate arginine residues in numerous proteins. Piwi complexes also contain several proteins with Tudor domains;[5–8] these domains bind to symmetrically methylated arginine (Fig. 1A). We then showed that Piwi proteins harbor several methylated arginines and these residues are critical for interaction with Tudor proteins. Methylated arginines are clustered at N-termini of Piwi proteins and are absent in Ago family members, providing a molecular mechanism that differentiates between members of the two clades. Indeed, we found that

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Piwi, but not Ago, members faithfully interact with their specific Tudor protein partners in a heterologous cell culture system. Mourelatos and colleagues showed that methylation of arginines in the N-terminal domains of Piwi proteins by a homolog of PRMT5 is seen in Drosophila[9] and corresponding residues present in Piwi members in other animals suggest that arginine methylation and interaction with Tudors is conserved feature of Piwi pathway that distinguishes it from the somatic Ago pathway.

What is the function of arginine methylation of Piwis and the interaction with Tudor proteins? Knock-outs of several Tudor proteins cause male-specific sterility in mouse, a phenotype similar to that observed in Piwi mutants. It is possible that arginine methylation and subsequent interaction with Tudors is required for Piwi recruitment to nuage granules. Indeed, in germ cells, several Tudor-domain proteins localize to nuage granules together with Piwi.[5] Piwis do not form cytoplasmic granules when expressed in a heterologous cell culture system, but are recruited to these granules if co-expressed with TDRD1.[5] Tudor proteins might, therefore, provide the platform necessary for assembly of numerous proteins that function as a complex in the Piwi pathway. Several Tudor-domain proteins have multiple Tudor domains and may simultaneously interact with several proteins harboring methylated arginines (Fig. 1A and B). For example, TDRD1 harbors four Tudor domains and interacts with three Piwi family members as well as other proteins such as mouse vasa homolog (MVH).

The assembly of a multiprotein complex that includes two Piwi proteins might be particularly important for the piRNA pathway. Recent studies showed that a subset of piRNAs function in feed-forward loops, so called ping-pong cycles, that require a tight physical interaction of two Piwi proteins. It is attractive to propose that TDRD1 is required to bring two Piwi proteins together (Fig. 1B). Indeed, we found that while piRNAs are not eliminated in Tdrd1 knock-out mice, the efficiency of the ping-pong cycle is greatly reduced. Interestingly, this defect is also coincides with delocalization of MIWI2 from nuage granules, suggesting that proper localization is important for the pathway function. The role of multiple Tudor-domain proteins that have specific temporal expression patterns and Piwi-association properties remains unclear (Fig. 1C). Future studies should reveal the precise molecular roles that Tudor-domain proteins play in the assembly of Piwi complexes.

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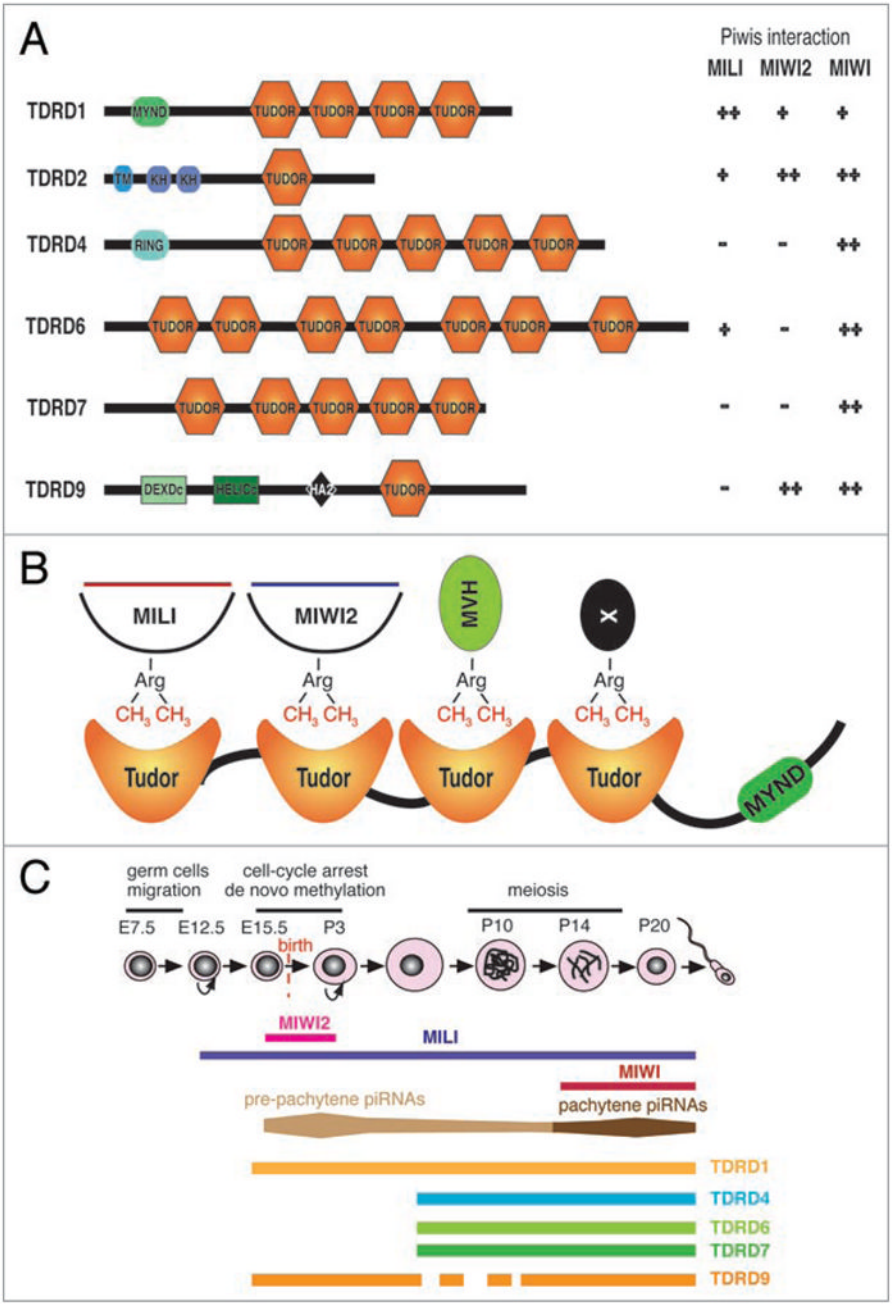

Figure 1.

(A) The structure of Tudor proteins and their interaction with PIWIs. KH is RNA binding motif; TM is transmembrane motif; DEXDC, HELICc and HA2 are RNA helicase motifs; MYND and RING are motifs with unknown functions. (B) The proposed model for TDRD1 function in assembly of nuage and association of Piwi proteins for the ping-pong cycle. Methylated arginines of Piwi proteins (MILI and MIWI2), as well as those of other proteins such as MVH, interact with different Tudor domains of TDRD1. (C) Expression of PIWI and Tudor family members and piRNA during male germline development in mouse.