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PERSPECTIVE

Elective affinities: a Tudor–Aubergine tale of germline partnership

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In *Drosophila melanogaster* and many other metazoans, the specification of germ cells requires cytoplasmic inheritance of maternally synthesized RNA and protein determinants, which are assembled in electron-dense cytoplasmic structures known as germ or polar granules, found at the posterior end of the oocytes. Recent studies have shown that the formation of germ granules is dependent on the interaction of proteins containing tudor domains with the piwi-interacting RNA (piRNA)-binding Piwi proteins, and such interactions are dependent on symmetrically dimethylated arginines (sDMAs) of Piwi proteins. Tudor–Piwi interactions are crucial and are conserved in the germ cells of sexually reproducing animals, including mammals. In the September 1, 2010, issue of *Genes & Development*, Liu and colleagues (pp. 1876–1881) use a combination of genetics, biochemistry, and crystallography to uncover the molecular and structural details of how Tudor recognizes and binds the sDMAs of the Piwi protein Aubergine.

It has long been known that, in *Drosophila* as well as many other species with morphologically asymmetrical oocytes, primordial germ cell (PGC) specification and maintenance in the progeny is dictated by maternally deposited determinants accumulated in the cytoplasm of the posterior end of the oocyte, which is known as germ or pole plasm (Ephrussi and Lehmann 1992; Mahowald 2001). In a series of classical experiments, the germ plasm was shown to be necessary and sufficient to induce the formation of a germline (Ephrussi and Lehmann 1992; Mahowald 2001). Many of the components of the germ plasm (RNA and proteins) are synthesized by the nurse cells, and accumulate during oogenesis in a stepwise fashion (Dansereau and Lasko 2008). Among these are the products of the genes *vas* (Vasa, a DEAD-box RNA helicase) (Hay et al. 1988; Lasko and Ashburner 1988), *tud* (Tudor, the founding member of the homonymous family) (Boswell and Mahowald 1985), *aub* (Aubergine, a Piwi protein) (Schupbach and Wieschaus 1991; Harris and Macdonald 2001), *csul* (Capsuleen, a methyltransferase)

(Gonsalvez et al. 2006; Anne et al. 2007), and *valois* (cofactor of csul) (Anne and Mechler 2005)—most of which were identified by genetic screens for maternal-effect sterility. The progeny carrying such mutations is viable but does not form a germline, and in some cases the embryos show posterior defects; hence, these genes are called *posterior-grandchildless* (Strome and Lehmann 2007). Recent studies uncovered the relation that ties these genes together and also provided molecular explanations for the mutant phenotypes: Csul and its cofactor, Valois, symmetrically dimethylate arginines on Aubergine that are recognized and bound by Tudor (Kirino et al. 2009, 2010b; Nishida et al. 2009). The formation of the Tud–Aub complex is required for the two proteins to localize and contribute to the germ plasm formation (Nishida et al. 2009; Kirino et al. 2010b). PGC specification in mammals follows a different mechanism, but the functional network of these genes is conserved and is essential in males for proper spermatogenesis (Chuma et al. 2006; Reuter et al. 2009; Vagin et al. 2009; Vasileva et al. 2009; Wang et al. 2009; Arkov and Ramos 2010; Siomi et al. 2010).

Piwi proteins constitute a subclade of the Argonaute family, and bind to the extremely diverse class of piwi-interacting RNAs (piRNAs) (Klattenhoff and Theurkauf 2008; Ghildiyal and Zamore 2009). All Piwi mutants studied cause sterility due to the developmental arrest of gametogenesis or regeneration defects in planarians, where they are essential for neoblast maintenance (Thomson and Lin 2009). The most studied Piwi proteins are *Drosophila* Aub, Piwi, and Ago3, and mouse Mili, Miwi, and Miwi2 (Thomson and Lin 2009). Piwi proteins colocalize with tudor domain-containing proteins in characteristic electron-dense, nonmembranous, cytoplasmic structures called germ or polar granules, which often contact mitochondria and/or assemble perinuclearly (Arkov and Ramos 2010). In *Drosophila* ovaries, a germ cell-specific organelle called the nuage is formed around the nucleus of the nurse cells, and germ granules containing Aub are located at the posterior pole of the oocyte. In mouse spermatocytes, Piwi proteins associate with various Tdrds (Tudor domain-containing proteins) during the formation of intermitochondrial cement (Mili/Tdrd1) (Wang et al. 2009) and chromatoid bodies (Miwi–Mili/Tdrd6) (Vagin et al. 2009; Vasileva et al. 2009).

piRNAs and Piwi proteins also have a well-documented role in retrotransposon silencing (Vagin et al. 2006; Aravin et al. 2007; Brennecke et al. 2007; Gunawardane et al. 2007; Shoji et al. 2009). Many Piwi/piRNA ribonucleoproteins (piRNPs) target complementary, active mobile elements and cleave them. The transposon remnants are processed as secondary piRNAs, which in turn are used for the feedback processing of primary, anti-transposon piRNAs. This adaptive mechanism has been termed the ping-pong amplification loop (Brennecke et al. 2007; Gunawardane et al. 2007). Moreover, Piwi proteins are required for transposon taming at the DNA level by facilitating suppressive DNA methylation during epigenetic reprogramming of the developing germline (Aravin et al. 2007; Kuramochi-Miyagawa et al. 2008). However, large subpopulations of piRNAs are not related to repeat elements, and their function remains unknown. Despite recent progress, the biogenesis of primary piRNAs, their exact mode of function, and their full RNA target spectrum remain largely unclear. Also, a dynamic variation in the protein complement, form, and localization of germ granules is observed, which implies that protein–protein and RNA–protein interactions can be modulated during RNP restructuring (Aravin et al. 2009; Chen et al. 2009; Wang et al. 2009; Vagin et al. 2009; Vasileva et al. 2009). To proceed from observation to comprehension, a mechanistic understanding of Piwi–Tudor associations in the variety of germ granules and their functional repercussions is necessary. The study by Liu et al. (2010) in the September 1, 2010, issue of *Genes & Development* is a decisive step toward this goal.

Tudor is a 285-kDa protein harboring 11 homonymous domains (Thomson and Lasko 2005). Previous work (Arkov et al. 2006) established that a truncation mutant carrying only domains 7–11 is able to rescue Aub localization at the germ plasm and germline formation in a *tud*-null background. Those domains were found to interact with Aub (Kirino et al. 2010b). Liu et al. (2010) now focus on the tud7–11 transgene and perform an elegant mutation study in an effort to identify a single tud domain within the transgene responsible for Aub association. They sequentially mutate a pair of conserved aromatic amino acid residues in each one of the predicted tud domains of tud7–11, and probe Aub localization and germ cell formation. These mutations are expected to precisely disrupt symmetrically dimethylated arginine (sDMA) binding (Sprangers et al. 2003; Friberg et al. 2009) without significantly affecting overall structure. Strikingly, only domain 8 appears to be unnecessary for these functions, with the rest being indispensable. This result suggests Tudor–Aubergine binding of complex stoichiometry, and it will be interesting to see in subsequent studies whether tud domains bind their targets independently or synergistically.

Liu et al. (2010) proceeded to the crystallization of a polypeptide (amino acids 2344–2515) encompassing tudor domain 11—thus termed extended (eTud11)—in the absence and presence of Aub peptides (amino acids 6–18) carrying a single sDMA (R13 or R15) (Fig. 3 in Liu et al. 2010). The canonical tud domain spans residues 2390–2446, (one-third of the overall size of eTud11). It is most

interesting that the resolved structures depict eTud11 folding into a single compact structure, with the flanking N and C termini interlocked in an oligonucleotide-binding (OB) fold at an off-center position and the tud domain to the side, connected to the OB fold via a helix at its N terminus and a small helix–linker loop at its C terminus (Fig. 3 in Liu et al. 2010). This structure is surprisingly similar (root-mean-square deviation: 1.84) to the extended tudor domain of the Tudor SN/p100 protein fold reported by Sattler's team (Friberg et al. 2009), which was also shown to preferentially bind sDMAs. The tud11 domain forms a barrel-like structure consisting of four β strands, and can be superimposed to Tud SN and the tud domain of the Survival of Motor Neurons (SMN) protein (Selenko et al. 2001; Sprangers et al. 2003), also with minimal deviation (Fig. 3 in Liu et al. 2010).

The structure within the tud domain that actually contacts the sDMA has been fittingly described as an aromatic cage. In eTud11, this cage is composed of four aromatic residues (Phe2403, Tyr2410, Phe2427, and Phe2430), with their benzene rings arranged as the right, back, upper, and left faces of an hexahedron. The sDMA enters from the front and lower side, and its guanidine group is stacked in parallel to the right and left faces of the cage, stabilized by both hydrophobic and cation-π interactions. The modified residue is secured in place by what looks like a latch: An asparagine residue (Asn2432) protruding from the upper rim of the cage-like structure makes a hydrogen bond with its amide carbonyl and the outward amino group of the sDMA. Mutating Asn2432 to alanine results into a dramatic decrease in the affinity of the tud domain for the methylated peptide. Given its "gatekeeper" role in sDMA binding, it is interesting to note that this residue displays various degrees of conservation, even within tudor proteins with germline functions, such as *Drosophila* Tudor (present in eight out of 11 domains), Krimper (one out of two) (Lim and Kai 2007), and Tejas (zero out of one) (Patil and Kai 2010), and mouse Tdrd6 (six out of seven), Tdrd1 (four out of four), and RNF17/Tdrd4 (two out of five) (Pan et al. 2005).

The study by Liu et al. (2010) also provides important evidence on the role of secondary sites of interaction involving Aub residues of the RG/RA motif, and structural elements other than the tud domain. Such interactions stabilize the peptide backbone that is positioned in parallel to the N-terminal helix (αA) connecting the tud domain to the OB fold, and facilitate the exposure of the methylated side chain to the primary site of interaction: the aromatic cage. Glycine at position −1 relative to the sDMA and arginine at position −2 contact two residues at the αA helix. The latter is a glutamic acid (E2374) and forms a hydrogen bond with a side chain amino group of the −2 arginine. Moreover, in the resolved structure of the Aub peptide methylated at R15, R11 (position −4) makes multiple contacts with OB-fold residues, two of which are hydrogen bonds, through its two amino groups. It is assumed that these contacts can "sense" the methylation state of the Arg11. Unfortunately, no evidence is provided for interactions that are formed with Aub residues outside the RG motif due to crystal disorder.

Such evidence would help us understand the interactions that the tudor module uses to distinguish its cognate partner between proteins carrying similar RG/RA motifs.

The two most conserved residues in >1000 tudor domains catalogued in the pfam protein family database (Finn et al. 2010) are also present in eTud11. These are an arginine (R2411) and an aspartate (D2429) located on the external surface of the tud β barrel that form charged interactions with each other through their side chains. Asp2429 lies between two of the aromatic residues forming the sDMA-binding cage, but faces in an opposite direction. In addition to the charged interaction, the R–D pair makes multiple contacts with the C-terminal small helix–linker loop that connects the tud domain with the OB fold. These interactions form a network that consolidates the tud domain with the rest of the module and stabilizes the overall structure. Intriguingly, both residues are substituted in the tud domain of SMN from several species (Battle et al. 2006). The importance of this interaction is depicted in mutants tud^{A36} and tud^{B42} (Arkov et al. 2006), both of which carry a mutation of the respective arginine (of the R–D pair described above) in domains tud1 and tud10, respectively. tud^{A36} has a severe defect in germ granule morphology, but, perhaps, not surprisingly, can still form a germline, as the tud1 domain was shown to be dispensable for this function (Arkov et al. 2006). tud^{B42} shows a complete loss of germ cells, underlining the importance of this tud domain for Aub binding and subsequent germline formation (Arkov et al. 2006).

Liu et al. (2010) conclude by making an intriguing observation: The sequence motif YR-D(F/Y)GN appears to be conserved in the Piwi-binding extended Tudor domain, making it a good starting point for designing subsequent experiments. Remarkably, this motif occurs in all five tudor domains of the tud7–11 truncation mutant, which was shown repeatedly to mediate Aub binding, but is found in only two of the other six tudor domains, which are dispensable for Aub binding.

In summary, this study uncovers discrete roles of specific amino acid residues for the formation of the aromatic cage within the tudor domain responsible for the binding of sDMA: as auxiliary attaching sites for the Aub backbone chain that possibly also play a role in recognizing the sDMA, and thereby drive it toward the tud domain and the sDMA-binding cage; and for the stabilization of the overall fold of the tud domain and surrounding structures. Furthermore, a signature motif for germline tudor domains has been put forward that provides a testable prediction for consideration in subsequent studies. Moreover, as illustrated in the examples described above, we can now integrate structural and genetic data, and draw several fascinating conclusions on the structure and function relationships of the multiple tudor domains.

There are still many important questions to be answered. Tudor has a role in abdominal patterning of the developing embryo, and, strikingly, different tudor domains can rescue a strong loss-of-function mutant in a similar way (Arkov et al. 2006). This redundancy is also implied in mice, where many Tdrds harbor multiple tudor domains. Is this an adaptation important for struc-turing the large RNPs of the germ plasm? Piwi proteins are the only examples of proteins that have been shown to interact via their sDMAs with germline Tdrds. Are there other sDMA-modified proteins that interact with specific germline Tdrds? For example, it was shown recently that Vasa carries both sDMAs and aDMAs (asymmetrically dimethylated arginines), the former of which are produced by Capsuleen (Kirino et al. 2010a). Although the sDMAs of Vasa appear dispensable for its interaction with Tudor (Kirino et al. 2010a), it is unknown whether they are required for interaction with other Tdrds. Additional *Drosophila* proteins with important germline functions carry tudor domains (Tejas, Spindle-E, and Krimper), but no methylated binding partner has been specified for them yet. Finally, the exact molecular function of the Piwi–Tudor interactions is unclear. It looks like there will be more interesting stories on tudors and their binding partners in the near future.

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