Review

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LOTUS-domain proteins - developmental effectors from a molecular perspective

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Abstract: The LOTUS domain (also known as OST-HTH) is a highly conserved protein domain found in a variety of bacteria and eukaryotes. In animals, the LOTUS domain is present in the proteins Oskar, TDRD5/Tejas, TDRD7/TRAP/Tapas, and MARF1/Limkain B1, all of which play essential roles in animal development, in particular during oogenesis and/or spermatogenesis. This review summarizes the diverse biological as well as molecular functions of LOTUS-domain proteins and discusses their roles as helix-case effectors, post-transcriptional regulators, and critical cofactors of piRNA-mediated transcript silencing.

Keywords: DEAD-box RNA helicase Vasa; MARF1; Oskar; OST-HTH; piRNA pathway; TDRD.

Introduction

In 2010, the LOTUS/OST-HTH domain has been identified through two independent bioinformatic studies and named after the animal proteins in which it resides: LOTUS after Limkain, Oskar and Tudor domain-containing proteins 5 and 7, or OST-HTH after Oskar-TDRD5/TDRD7 – helix-turn-helix, respectively (Anantharaman et al. 2010; Callebaut and Mornon 2010). For convenience, throughout this review the domain is termed LOTUS. The LOTUS

domain is widely conserved in eukaryotes and bacteria, but LOTUS domain-containing proteins have so far been studied experimentally only in animals; their role in bacteria, fungi, and plants is not known. In animals, four LOTUS domain proteins have been identified, namely Oskar, Tudor domain-containing protein 5 (TDRD5), TDRD7, and Meiosis arrest female 1 (MARF1), recently renamed meiosis regulator and mRNA stability factor 1 (MARF1), all of which are essential developmental effectors with a prominent function in gametogenesis (Figure 1; Table 1). The LOTUS domain comprises approximately 80–100 amino acid residues and its fold resembles a winged helix-turn-helix (wHTH) domain (Anantharaman et al. 2010; Callebaut and Mornon 2010; Jeske et al. 2015; Yang et al. 2015). Based on structural and functional differences, LOTUS domains have been further classified into extended LOTUS (eLOTUS) and minimal LOTUS (mLOTUS) domains (Jeske et al. 2017) (Figure 2). Recently, it has been discovered that eLOTUS and mLOTUS domains, including those of bacteria and plants, specifically bind to G-rich RNAs and RNA G-quadruplex (G4) secondary structures *in vitro* (Ding et al. 2020), a seemingly common and distinctive feature of LOTUS domains, the biological function of which remains to be explored.

eLOTUS domains are C-terminally extended and are present in a single copy in Oskar, TDRD5 and TDRD7, but absent from MARF1 and non-animal proteins. The eLOTUS domains from various species have been demonstrated to bind the germline-specific ATP-dependent DEAD-box RNA helicase Vasa and to stimulate its ATPase activity, suggesting that Vasa stimulation is a conserved function of the eLOTUS domain (Jeske et al. 2017). The molecular mechanism of how the eLOTUS domain stimulates Vasa is currently unknown. Structural and functional analyses of the eLOTUS domain of Oskar revealed that for Vasa binding the eLOTUS domain requires the C-terminal extension, which appears unstructured in an unbound form but folds into an α -helix upon Vasa interaction (Jeske et al. 2017) (Figure 2). Oskar does not interact with Belle, the DEAD-box helicase most closely related to Vasa, suggesting that the Oskar eLOTUS domain recognizes Vasa specifically (Jeske et al. 2017). Whether the eLOTUS domains of

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Figure 1: LOTUS-domain proteins. Domain organization of *Drosophila* and mouse LOTUS-domain proteins. eLOT, eLOTUS; mL, mLOTUS.

Table 1: Phenotypes and subcellular localization of LOTUS-domain proteins.

Protein	Organism	Phenotype	Subcellular localization	Reference
MARF1	<i>Drosophila</i>	Female sterility	Ovaries: Ubiquitous in nurse cells and oocytes	Kawaguchi et al. 2020; Zhu et al. 2018
Short Oskar	Mouse	Female sterility	Oocytes: n.d.	Su et al. 2012
	<i>Drosophila</i>	Female sterility; patterning defects in embryos	Oocytes and early embryos: polar granules	Breitwieser et al. 1996; Lehmann and Nüsslein-Volhard 1986; Markussen et al. 1995
Long Oskar	<i>Drosophila</i>	Low frequency of female sterility and embryo patterning defects; defects in germ plasm anchoring	Oocytes: endocytic membranes	Tsai et al. 2014; Vanzo and Ephrussi 2002; Vanzo et al. 2007
TDRD5	<i>Drosophila</i> (Tejas)	Female sterility	Ovaries: peri-nuclear nuage of nurse cells	Patil and Kai 2010
		Upregulation of <i>Stellate</i> transcripts in testes; male sterility of <i>tejas/tapas</i> double mutants	Testes: n.d.	Patil and Kai 2010; Patil et al. 2014
TDRD7	Mouse	Male sterility	Spermatogonia and spermatocytes: IMC and CB	Yabuta et al. 2011
	<i>Drosophila</i> (Tapas)	Slight upregulation of transposons in ovaries	Ovaries: peri-nuclear nuage of nurse cells	Patil et al. 2014
		Upregulation of <i>Stellate</i> transcripts in testes	Testis: n.d.	Patil et al. 2014
	Mouse	Male sterility	Spermatocytes and spermatids: IMC and CB	Hosokawa et al. 2007; Lachke et al. 2011; Tanaka et al. 2011b
	Mouse, human, chicken	Cataract and glaucoma	Lens cells of eyes: cytoplasmic RNA granules	Lachke et al. 2011

TDRD5 and/or TDRD7 regulate other DEAD-box helicases in addition to Vasa is currently not known. In addition to Vasa binding and stimulation, the eLOTUS domain of Oskar from *Drosophila* and a few other insects forms high-affinity dimers (Jeske 2015; Yang 2015), but the function of Oskar dimerization has so far not been studied. Whether eLOTUS domains are capable of binding to Vasa and G-rich/G4 RNAs simultaneously and whether RNA binding by eLOTUS domains affects Vasa’s enzymatic and/or biological function remain interesting open questions.

In contrast to eLOTUS domains, mLOTUS domains lack a C-terminal extension and do not interact with Vasa or related DEAD-box proteins (Jeske et al. 2017; Zhu et al. 2018) (Figure 2). mLOTUS domains are monomeric and widely conserved in animals, bacteria, fungi, and plants.

In animals, mLOTUS domains are present in multiple copies in MARF1 and are also found in most TDRD5 and TDRD7 proteins, where one or more mLOTUS domains follow a single N-terminal eLOTUS domain. mLOTUS domains exhibit various functions, some of which differ between species. mLOTUS domains not only bind to RNA (Brothers et al. 2020; Ding et al. 2020; Yao et al. 2018), but in specific cases are capable of recruiting the CCR4-NOT deadenylase complex to mRNAs resulting in shortening of their poly(A) tails and diminished translation (Zhu et al. 2018).

In the following sections we will discuss the biological as well as the molecular functions of LOTUS-domain containing proteins. These proteins exhibit distinct functions, which differ even across species, such as mouse and *Drosophila*,

Figure 2: Structure models of LOTUS-domain proteins.

Structure models of mLOTUS, eLOTUS, Short Oskar, mammalian MARF1, and *Drosophila* MARF1 were drawn with the help of solved crystal/NMR structures of the following protein domains (Protein Data Bank [PDB] codes are given in parentheses): mLOTUS of mouse MARF1 (5YAD), eLOTUS of *Drosophila* Oskar (5NT7), *Drosophila* Oskar eLOTUS-Vasa-CTD complex (5NT7), *Drosophila* Vasa-NTD (2DB3), OSK of *Drosophila* Oskar (5A4A), NYN of mouse MARF1 (5YAA), RRM1 of human MARF1 (2DIU), and RRM2 of human MARF1 (2DGX). In the eLOTUS domain structure, the C-terminal extension required for Vasa binding is indicated by a surrounding dashed line. The Tejas structure model is composed of homology models generated with SWISS-MODEL (Waterhouse et al. 2018) based on Oskar eLOTUS-Vasa-CTD (5NT7) and Tudor eTud-Aub (3NTH) complex crystal structures. An aromatic cage, as shown for the Tudor eTud domain in complex with an sdMA-modified Aub peptide (green), is absent from Tejas.

and thus we will review the properties of mammalian and insect LOTUS-domain containing proteins separately.

MARF1

Mammalian MARF1 - an endoribonuclease critical for oogenesis and brain development

Mouse MARF1 has been first described as an essential regulator of oogenic processes. Mutations in MARF1 cause oocyte meiotic arrest and infertility in females, while males are not

affected (Su et al. 2012a,b) (Figure 3). MARF1-deficient oocytes display upregulation of IAP and LINE1 retrotransposons and an increased number of DNA double-strand breaks (Su et al. 2012a). Furthermore, in MARF1-deficient mouse oocytes, several hundred mRNAs are upregulated (Su et al. 2012a). Of these, key to the meiotic arrest phenotype seems an elevated level of the *Ppp2cb* transcript, which encodes the β -isoform of the catalytic subunit of protein phosphatase 2A (PP2A). High PP2A activity results in failure to activate the oocyte maturation-promoting factor, thus causing block of meiotic resumption (Su et al. 2012a). Interestingly, the function of MARF1 seems not to be limited to the germline. In the

Figure 3: Biological context of selected LOTUS-domain proteins.

Schematic drawing of mouse/*Drosophila* oogenesis, mouse spermatogenesis, a *Drosophila* egg chamber, and the *Drosophila/Bombyx* ping-pong cycle of the piRNA biogenesis pathway with potential connection to Tejas/Tapas. For clarity, nurse cell (NC) membranes were omitted from the *Drosophila* egg chamber drawing.

developing mouse brain, a somatic isoform of MARF1 is expressed as a result of alternative splicing, causing an insertion of 179 amino acid residues within the N-terminal region (Kanemitsu et al. 2017; Su et al. 2012a) (Figure 1). Somatic MARF1 promotes the differentiation of cortical neuronal progenitor cells (Kanemitsu et al. 2017), but exactly how is currently not understood.

Mammalian MARF1 is a multi-domain protein consisting of an N-terminal Nedd4-BP1, YacP Nuclease (NYN) domain, two central RNA-recognition motifs (RRMs) and a C-terminal array of eight mLOTUS domains (Figure 2). Mouse MARF1 acts as a ribonuclease through its NYN domain, which belongs to the superfamily of PIN nucleases (Anantharaman and Aravind 2006; Yao et al. 2018). The crystal structure of the MARF1 NYN domain together with sequence analyses revealed three strictly conserved aspartic acid residues, which create a negatively charged metal-binding pocket required for enzymatic activity (Anantharaman and Aravind 2006; Glavan et al. 2006; Nishimura et al. 2018; Xu et al. 2012; Yao et al. 2018). Mutating the conserved aspartic acid residues leads to loss of RNase activity *in vitro*, and results in the full spectrum of phenotypes

in vivo that have been observed in MARF1-deficient mice, including meiosis arrest, retrotransposon upregulation, enhanced DNA double-strand breaks, and female sterility (Yao et al. 2018). The NYN domain is also required for the function of somatic MARF1 in developing brains (Kanemitsu et al. 2017), suggesting that the underlying molecular mechanisms might be similar to those in oocytes.

The NYN domain is followed by two RRM domains, which display low micromolar affinity for ssRNA *in vitro* (Yao et al. 2018). In addition, the full array of mLOTUS domains binds to ssRNA and dsRNA with nanomolar affinity *in vitro* (Yao et al. 2018). As individual mLOTUS domains of mouse MARF1 have not been tested, it is unclear if all eight or only one or a few mLOTUS domains are critical for RNA binding. The mLOTUS domains of mouse MARF1 are required for full ribonuclease activity *in vitro*, suggesting that RNA recognition by mLOTUS domains contributes to efficient substrate binding of the NYN RNase domain (Yao et al. 2018). Alternatively, a mechanism is conceivable in which mLOTUS domains enhance the RNase activity by directly binding the NYN domain and altering its conformation favorably for RNA cleavage.

How mouse MARF1 recognizes its mRNA targets *in vivo*, whether MARF1 binds to a specific RNA motif or is recruited by another target-specific RNA-binding protein remains to be investigated. In this context, it will be interesting to understand the contribution of the RRM domains to MARF1's *in vivo* function and to determine the RNA-binding interfaces of the RRM as well as of the mLOTUS domains. Finally, whether the molecular properties of oogenic MARF1 are similar to those of the somatically expressed isoform remains to be explored.

Human MARF1 (a.k.a. Limkain B1, LMKB, or LKAP) possesses a domain architecture very similar to the murine protein and experiments using cultured cells that ectopically express human MARF1 have provided further interesting insight into its molecular function. The cell culture experiments allowed identification of several direct mRNA targets, to which MARF1 preferentially bound through their 3' UTRs (Brothers et al. 2020). In tethering assays, MARF1 induced degradation of reporter mRNAs and further experiments revealed the contribution of the individual MARF1 domains to mRNA regulation (Brothers et al. 2020). Firstly, in line with mouse MARF1, the NYN domain of human MARF1 is essential for mRNA degradation. Secondly, full NYN activity requires the RNA-binding capacity of the first RRM domain of human MARF1, potentially to properly position the NYN domain on a target mRNA. This finding appears different from mouse MARF1, where a contribution of the RRM domains to NYN activity has not been observed *in vitro* (Yao et al. 2018). Lastly, recognition of mRNA targets is mediated through the mLOTUS domains of human MARF1, in particular the central mLOTUS domains 3, 4 and 5 (Brothers et al. 2020). However, exactly how MARF1 recognizes its mRNA targets remains unresolved. The cell culture studies suggest that MARF1 does not bind to a specific linear RNA sequence, but instead might recognize structured motifs and/or positional elements within its mRNA targets (Brothers et al. 2020). This idea is supported by the observation that purified human MARF1 mLOTUS domains bind specifically to G4 RNAs *in vitro* (Ding et al. 2020).

In cultured cells, human MARF1 has been shown to interact with mRNA decapping factors, including enhancer of decapping 4 (EDC4) through a C-terminal region downstream of the mLOTUS domain array (Bloch et al. 2014; Nishimura et al. 2018). The decapping complex hydrolyzes the 5' cap structure of mRNAs committing a transcript for exonucleolytic decay (Jonas and Izaurralde 2013; Mugridge et al. 2018). In this pathway, EDC4 functions as scaffolding protein that is required for optimal decapping activity (Chang et al. 2014). However, the C-terminal region of

MARF1, and thus EDC4 recruitment, is not critical for reporter transcript degradation (Nishimura et al. 2018). Instead, EDC4-binding to MARF1 has an opposite effect and impairs reporter mRNA repression, possibly through interfering with binding of the mLOTUS domains to their mRNA target sequences (Brothers et al. 2020). Whether mouse MARF1 also binds to decapping factors is not known. Despite the insight gained from the cell culture experiments ectopically expressing human MARF1, it remains an interesting open question to what extent these findings apply in the *in vivo* context of mammalian MARF1, such as the developing oocyte or brain of a mouse model. Finally, it remains also unclear whether human MARF1 functions similarly to the mouse ortholog and likewise plays a predominant role in the control of oocyte meiotic progression and genomic integrity.

***Drosophila* MARF1 - a post-transcriptional regulator driving oocyte maturation**

Drosophila MARF1 (dMARF1) is maternally provided and exclusively expressed in ovaries and early embryos (Casas-Vila et al. 2017; Kawaguchi et al. 2020; Zhu et al. 2018). Compared to mammalian MARF1, dMARF1 exhibits a simpler protein domain architecture (Figure 2). Instead of two RRM domains, dMARF1 harbors only a single one, which shows highest similarity to the second RRM domain of the mammalian proteins. In databases (such as NCBI), dMARF1 is described as harboring six mLOTUS domains. However, from our analysis using PHYRE2 (Kelley et al. 2015), additional secondary structure is predicted within the C-terminus of dMARF1 that very likely corresponds to two extra mLOTUS domains, which might have been missed due to their low sequence conservation. Moreover, the C-terminal region of dMARF1 is distinct from human MARF1, and dMARF1 does not associate with Ge-1, the *Drosophila* ortholog of the decapping factor EDC4 (Zhu et al. 2018). Finally, the most striking difference to mammalian MARF1 is the lack of a NYN domain in dMARF1, and thus, in contrast to mammalian MARF1, dMARF1 is unlikely to act as a ribonuclease. As the NYN domain is indispensable for MARF1 function in mouse oocytes, it is remarkable that dMARF1 is nevertheless essential for fertility in *Drosophila* females. dMARF1-deficient females display severe defects in late stage oocyte maturation, particularly in meiosis I to II transition (Kawaguchi et al. 2020; Zhu et al. 2018) (Figure 3).

dMARF1 regulates cyclin protein levels during late oogenesis (Kawaguchi et al. 2020; Zhu et al. 2018). Cyclins A, B or B3 bind to cyclin-dependent kinase 1 (CDK1) to regulate its kinase activity and substrate specificity during

mitosis and meiosis (Bourouh et al. 2016). During *Drosophila* oogenesis, the Cyclin A/CDK1 complex triggers the release of the first meiotic arrest, which then leads to upregulation of Cyclin B allowing the formation of the Cyclin B/CDK1 complex and progression of oocyte maturation (Vardy et al. 2009; von Stetina and Orr-Weaver 2011). In dMAREF1-deficient late stage-oocytes, Cyclin protein levels are changed such that Cyclin A is upregulated while Cyclin B and B3 are downregulated (Kawaguchi et al. 2020; Zhu et al. 2018). Zhu and colleagues showed that dMAREF1 specifically associates with *Cyclin A* mRNAs as well as with the CCR4-NOT complex, the main deadenylase in eukaryotes essential for default and regulated mRNA decay (Collart and Panasenko 2017; Temme et al. 2014). This is in contrast to human MARF1, which does not associate with the CCR4-NOT complex (Nishimura et al. 2018). In the absence of functional dMAREF1, *Cyclin A* transcripts exhibit elongated poly(A) tails, suggesting that dMAREF1 recruits the CCR4-NOT complex to *Cyclin A* mRNA. Interestingly, when tethered to a reporter mRNA in oocytes, dMAREF1 causes its deadenylation and translation repression, and the first mLOTUS domain of dMAREF1 was shown to be sufficient for CCR4-NOT complex recruitment. However, a transgene comprising only the RRM and the first mLOTUS domains of dMAREF1 is unable to rescue the dMAREF1 phenotype, suggesting that additional parts of dMAREF1 might be required for mRNA target recognition *in vivo*.

Kawaguchi et al. proposed an additional layer of dMAREF1-mediated regulation of cyclin protein levels during oogenesis. They and Zhu et al. showed that in dMAREF1 mutant female ovaries, Nanos protein levels are slightly upregulated during late-stage oogenesis (Kawaguchi et al. 2020; Zhu et al. 2018). In embryos, Nanos is a known repressor of *Cyclin B* mRNA translation (Asaoka-Taguchi et al. 1999; Kadyrova et al. 2007) letting Kawaguchi et al. to suggest that Nanos plays a similar role during late oogenesis and hence, that the decreased Cyclin B levels might be caused by increased Nanos levels in dMAREF1-deficient late-stage oocytes (Kawaguchi et al. 2020). That Cyclin B protein levels are regulated by dMAREF1 only indirectly is supported by the observation that *nanos* but not *Cyclin B* has been identified as one of very few mRNAs that associate with dMAREF1 (Kawaguchi et al. 2020; Zhu et al. 2018). Similar to dMAREF1-mediated regulation of *cyclin A* mRNA (Zhu et al. 2018), dMAREF1 might also recruit the CCR4-NOT complex to *nanos* mRNA in late-stage oocytes.

Compared to mammalian MARF1, the molecular function of dMAREF1 is less-well studied. dMAREF1 has been identified in a global RNA-bound proteome study as a high-confidence direct RNA-binding protein (Sysoev et al. 2016). However, it is not clear whether RNA binding of dMAREF1

functions similarly to mammalian MARF1 and is also mediated through the RRM and/or the mLOTUS domain(s). So far, dMAREF1 mLOTUS domains have not been subjected to *in vitro* RNA binding analyses and it remains to be investigated whether dMAREF1 indeed physically interacts with *cyclin A* and *nanos* and if so, how these mRNAs are specifically recognized.

Oskar - the *Drosophila* germ plasm organizer

The *oskar* gene was discovered in *Drosophila* in a genetic screen for maternally expressed factors essential for embryonic development (Lehmann and Nüsslein-Volhard 1986). Oskar is an insect-specific eLOTUS-domain protein, and is the only known protein capable of inducing the formation of functional germ cells in an organism. During *Drosophila* oogenesis, Oskar synthesis is restricted to the posterior tip of the oocyte, where it induces assembly of the *Drosophila* germ plasm (pole plasm) (Ephrussi and Lehmann 1992; Ephrussi et al. 1991; Kim-Ha et al. 1991; Lehmann and Nüsslein-Volhard 1986) (Figure 3). The pole plasm is essential for formation of germ cells and abdominal structures in the embryo (reviewed in Mahowald 2001). Embryos that lack Oskar fail to develop germ cells and abdominal segments, while embryos that ectopically express additional Oskar at the opposite pole form additional pole cells and a second abdomen at the anterior instead of a head (Ephrussi and Lehmann 1992; Lehmann and Nüsslein-Volhard 1986; Smith et al. 1992).

Translation of *oskar* mRNA occurs from two different in-frame start codons resulting in two Oskar protein isoforms (Markussen et al. 1995) (Figure 1). Of these, Short Oskar (aa 139–606) is the most abundant, the essential and inductive isoform, whose mis-expression or absence from flies results in the aforementioned phenotypes (Breitwieser et al. 1996; Markussen et al. 1995). Long Oskar (aa 1–606) is largely dispensable for germ cell formation and embryonic patterning (Breitwieser et al. 1996; Markussen et al. 1995), but required for tight anchoring of the germ plasm to the posterior oocyte cortex (Vanzo and Ephrussi 2002; Vanzo et al. 2007).

Despite its long known critical function during development, the molecular mechanisms that underlie Short Oskar's germ cell inductive capacity are largely unknown and its dissection just recently began. Short Oskar is an RNA-binding protein that physically associates with *nanos*, *pgc*, and *gcl* mRNAs *in vivo* (Jeske et al. 2015). These mRNAs code for proteins that are essential for germ cell formation

and/or embryonic patterning (Hanyu-Nakamura et al. 2008; Jongens et al. 1992; Leatherman et al. 2002; Lerit et al. 2017; Pae et al. 2017; Timinszky et al. 2008; Wang and Lehmann 1991), thus potentially providing a molecular link to Oskar's germ cell inductive activity.

Short Oskar carries a dimerizing eLOTUS domain, which is connected by a disordered linker to an Oskar-specific OSK domain (Figure 2). Interestingly, sequence analysis suggests that the OSK domain is of bacterial origin and that it has combined with the LOTUS domain to form Oskar through horizontal gene transfer (Blondel et al. 2020). The structure of the OSK domain resembles that of SGNH hydrolases (Jeske et al. 2015; Yang et al. 2015), a group of enzymes that catalyze the hydrolysis of ester bonds of various substrates, such as lipids, carbohydrates, and peptides (Akoh et al. 2004). The OSK domain lacks an active site and hence, is very unlikely to act as an enzyme. The surface of the OSK domain is composed of mainly hydrophobic and positively charged residues and the domain binds to RNA *in vitro* (Jeske et al. 2015; Yang et al. 2015). In our hands, RNA binding was unspecific *in vitro*, as the OSK domain bound similarly well to short ssRNA oligos with an irrelevant sequence and to several longer RNA constructs covering the full *oskar* and *nanos* mRNAs (Mandy Jeske and Anne Ephrussi, unpublished data). Furthermore, single or double mutations of two strictly conserved, positively charged surface residues of the OSK domain (R576, R593) did not interfere with RNA binding *in vitro* (Mandy Jeske and Anne Ephrussi, unpublished data), and thus to us the RNA-binding interface of the OSK domain is unclear. Finally, how Oskar recognizes *nanos*, *pgc*, or *gcl* mRNAs *in vivo* is not understood. Target recognition might be mediated by the OSK domain through a specific but unknown sequence motif. Alternatively or in addition, *in vivo* RNA binding might require the eLOTUS domain, which binds to G4 RNAs *in vitro* (Ding et al. 2020).

Oskar is required for localization and translation of *nanos*, *pgc*, and *gcl* mRNAs at the posterior pole of oocytes and/or embryos (Ephrussi et al. 1991; Hanyu-Nakamura et al. 2008; Jongens et al. 1992; Rangan et al. 2009; Wang and Lehmann 1991). While it is not known whether Oskar directly regulates *pgc* and *gcl* mRNAs, there is strong evidence that it plays an active role in translation regulation of the posterior determinant *nanos* in early embryos. There, only a small fraction of *nanos* mRNA localizes to the posterior pole and undergoes translation, while bulk *nanos* mRNA is distributed throughout the cytoplasm and remains repressed (Bergsten and Gavis 1999; Dahanukar and Wharton 1996; Dahanukar et al. 1999; Gavis and Lehmann 1994; Smibert et al. 1996; Wang and Lehmann 1991). The

newly synthesized Nanos protein diffuses towards the anterior of the embryo creating a concentration gradient that is fundamental to abdomen formation in *Drosophila* (Gavis and Lehmann 1992; Lehmann and Nusslein-Volhard 1991; Wang and Lehmann 1991). The translation of bulk *nanos* mRNA is inhibited by the protein Smaug, which is uniformly distributed in embryos but unable to silence *nanos* mRNA at the posterior pole (Dahanukar et al. 1999; Smibert et al. 1999). There, *nanos* mRNA repression is overcome by the antagonistic activity of Oskar (Dahanukar et al. 1999; Zaessinger et al. 2006). Interestingly, *nanos* mRNA repression can be faithfully recapitulated using a cell-free system derived from *Drosophila* embryos, and de-repression can be achieved by addition of recombinant Short Oskar protein to the lysates (Jeske and Wahle 2008; Jeske et al. 2006, 2011, 2014). In the presence of Short Oskar, Smaug is unable to bind to *nanos* mRNA (Jeske et al. 2011; Zaessinger et al. 2006). Furthermore, Oskar has been reported to bind to the *nanos* RNA-binding SAM-PHAT domain of Smaug, which was speculated to result in Smaug inactivation and *nanos* mRNA translation (Dahanukar et al. 1999). However, the relevance of this finding has been questioned later by the same group (Dean et al. 2002), and we were unable to detect any physical interaction between Short Oskar and the SAM-PHAT domain of Smaug using recombinant proteins (Mandy Jeske and Anne Ephrussi, unpublished data). Thus, how Oskar de-represses *nanos* mRNA at the posterior pole of embryos remains poorly understood and investigating the underlying mechanism might provide the molecular link to Oskar's capacity to induce posterior patterning.

Short Oskar was reported to physically interact with many other proteins, including the WD40 protein Valois (a.k.a. MEP50) (Anne 2010) that acts as activator of the protein arginine methyltransferase Capsuléen (a.k.a. PRTM5, DART5) (Anne and Mechler 2005; Cavey et al. 2005; Gonsalvez et al. 2006), the eIF4E-binding protein Cup (Ottone et al. 2012) that functions as translational co-repressor of *oskar*, *nanos*, and *orb* mRNAs (Igreja et al. 2014; Kinkelin et al. 2012; Nakamura et al. 2004; Nelson et al. 2004; Ote and Yamamoto 2018; Wilhelm et al. 2003; Wong and Schedl 2011), the actin-binding protein Lasp that might play a role in anchoring of Oskar to the posterior pole (Suyama et al. 2009), the dsRNA-binding protein Staufen (Breitwieser et al. 1996) that is required for the localization of *bicoid* and *oskar* mRNAs during oogenesis (Ephrussi et al. 1991; St Johnston et al. 1989), as well as the DEAD-box RNA helicase Vasa (Breitwieser et al. 1996; Lasko and Ashburner 1988; Suyama et al. 2009). When we tested Oskar interactions with all these candidates using cell-culture-based methods, split-ubiquitin yeast-two hybrid

experiments and/or interaction assays involving recombinant proteins, the only physical Short Oskar partner that we could verify was Vasa (Jeske et al. 2015, 2017; Mandy Jeske and Anne Ephrussi, unpublished data). Oskar binds to the C-terminal RecA-like helicase domain of Vasa through its eLOTUS domain and binding stimulates the ATPase activity of Vasa *in vitro* (Jeske et al. 2017). Furthermore, the specific interaction with the eLOTUS domain of Oskar is required for the recruitment of Vasa to the posterior pole of the *Drosophila* oocyte (Jeske et al. 2017). At the pole, Vasa is an essential component of germ plasm and its ATPase activity is required for germ cell formation and abdominal patterning (Dehghani and Lasko 2015; Hay et al. 1988; Lasko and Ashburner 1988, 1990). However, the molecular function of Vasa within the germ plasm is not understood, and thus the *in vivo* role of Vasa stimulation by Oskar is unclear. The *vasa* gene is essential for the posterior localization of *nanos* mRNA (Gavis and Lehmann 1992). As Short Oskar physically interacts with both *nanos* mRNA and Vasa protein *in vivo*, and Vasa has previously been implicated in translation control (Carrera et al. 2000; Johnstone and Lasko 2004; Styhler et al. 1998), Vasa might act together with Short Oskar in translation activation of *nanos* mRNA at the posterior pole of early embryos.

In contrast to Short Oskar, Long Oskar is not capable of assembling functional germ plasm. Instead it is required for proper anchoring of the germ plasm to the posterior cortex of the oocyte (Breitwieser et al. 1996; Markussen et al. 1995; Vanzo and Ephrussi 2002). In the absence of Long Oskar, the germ plasm detaches from the oocyte cortex, which can result in insufficient germ plasm material remaining at the posterior tip of the embryo to induce the formation of germ cells (Markussen et al. 1995; Vanzo and Ephrussi 2002). Thus, although germ plasm is present, a low percentage of embryos lacks precursor germ cells and displays patterning defects in the absence of Long Oskar (Markussen et al. 1995; Vanzo and Ephrussi 2002).

Consistent with the distinct cellular functions of Short and Long Oskar, the two proteins display remarkable differences in their subcellular localization as well as in their biochemical properties. Both Short and Long Oskar localize at the posterior pole of oocytes. However, while Short Oskar localizes, together with Vasa, to electron-dense particles termed polar granules, Long Oskar is excluded from these structures and instead localizes to endocytic membranes along the oocyte cortex (Breitwieser et al. 1996; Mahowald 1962; Tsai et al. 2014; Vanzo et al. 2007). Unlike Short Oskar, Long Oskar is not present in pole cells (Markussen et al. 1995). Moreover, in contrast to Short Oskar, Long Oskar seems not to bind to RNA *in vivo* and is

unable to recruit Vasa to the pole (Breitwieser et al. 1996; Jeske et al. 2015). These observations are striking, as Long Oskar comprises the entire amino acid sequence of Short Oskar, including OSK and eLOTUS domains. Although the molecular basis underlying the functional difference between Long and Short Oskar is unclear, it must be caused by the Long Oskar-specific N-terminal extension (NTE). The NTE is required for posterior anchoring of Long Oskar during the late stages of oogenesis, but it is not sufficient (Gunkel et al. 1998; Vanzo and Ephrussi 2002). This indicates that both the NTE as well as sequences common to Long and Short Oskar are needed to anchor Long Oskar and hence the germ plasm to the posterior pole of the oocyte. Our sequence analysis using PHYRE2 and HELIQUEST servers (Gautier et al. 2008; Kelley et al. 2015) has revealed three predicted α -helices within the NTE, two of which display a high propensity to form amphipathic α -helices, and thus might mediate the membrane localization of Long Oskar through peripheral insertion. Alternatively, Long Oskar might indirectly associate with membranes through formation of a complex with a specific set of yet to be identified membrane-bound proteins.

How Long Oskar anchors germ plasm to the oocyte cortex is not understood at the molecular level. Several lines of evidence indicate that Long Oskar is necessary for polarized endocytosis activity as well as actin remodeling, and that in turn, both processes are necessary for Long Oskar-dependent anchoring of germ plasm to the posterior cortex of the oocyte (Chang et al. 2011; Dollar et al. 2002; Tanaka and Nakamura 2008, 2011; Tanaka et al. 2011a; Vanzo et al. 2007). In oocytes and early embryos, Long Oskar additionally functions in tethering mitochondria to the posterior pole ensuring efficient transmission of maternal mitochondria to the newly forming pole cells (Hurd et al. 2016). The tethering of mitochondria depends on the actin cytoskeleton (Hurd et al. 2016), possibly linking mitochondria inheritance to Long Oskar's germ plasm anchoring function.

TDRD5 and TDRD7

TDRD5 and TDRD7 are highly conserved proteins with diverse functions that range, depending on the species and sex, from piRNA-mediated as well as piRNA-independent transposon silencing in the germline to organogenesis and anti-viral repression. TDRD5 and TDRD7 exhibit a similar domain topology. At the N-terminus, TDRD5 and TDRD7 harbor a single eLOTUS domain, which in most organisms is followed by one or two additional mLOTUS domains.

Within the C-terminal region, TDRD5 and TDRD7 contain one or several extended Tudor (eTudor) domains with unknown function (Figure 1).

eTudor domains are found in all germline-associated TDRD proteins and are composed of a canonical Tudor domain that is N- and C-terminally flanked by amino acid stretches, which together assemble into a single staphylococcal nuclease-like (SN-like) fold that is juxtaposed to the Tudor domain and lacks catalytic residues (Liu et al. 2010a) (Figure 2). eTudor domains mediate protein - protein interactions by recognizing symmetrical dimethylated arginine (sDMA) residues in their targets, such as PIWI proteins (reviewed in Gan et al. 2019). While an aromatic cage in the Tudor core recognizes the sDMA, both the Tudor and the SN-like fold contribute to recognition of sDMA-adjacent residues of the bound peptide (Liu et al. 2010a). The aromatic cage is composed of four aromatic and one asparagine or aspartic acid residue (Liu et al., 2010a,b; Mathioudakis et al. 2012; Zhang et al. 2018). Interestingly, none of the eTudor domains of TDRD5 and TDRD7 carries an intact aromatic cage (Figure 2). Moreover, a systematic analysis of all human TDRD proteins has revealed that the aromatic cage of more than half of the eTudor domains is incomplete, and that *in vitro* most eTudor domains with one or more substitutions in their cage, including those of TDRD7, bind only very weakly or not at all to the peptides provided, and if so then without any preference for sDMA (Zhang et al. 2017). Hence, the function of most eTudor domains that lack an intact aromatic cage is unclear.

***Drosophila* Tejas and Tapas - essential cofactors of piRNA-mediated transcript silencing**

The *Drosophila* orthologs of TDRD5 and TDRD7 have been named Tejas and Tapas, respectively (Patil and Kai 2010; Patil et al. 2014). The *tejas* and *tapas* genes are expressed in the germline and required for piRNA-mediated silencing of transcripts in both males and females (Patil and Kai 2010; Patil et al. 2014). piRNAs are a class of small non-coding RNAs that are specifically expressed in gonadal tissue of most animals and direct gene silencing at the transcriptional as well as at the post-transcriptional level (reviewed in Ozata et al. 2019; Parhad and Theurkauf 2019; Yamashiro and Siomi 2018). piRNAs function in complex with the PIWI-clade of Argonaute proteins and are mainly known to preserve genome integrity within the germline of animals by targeting active transposon genes.

In *Drosophila* ovaries, Tejas and Tapas are expressed in nurse cells, which are adjacent to the maturing oocyte within the syncytial egg chambers (Figure 3). Nurse cells provide the oocyte with proteins and mRNAs that are critical for oogenesis and early embryogenesis. Within nurse cells, Tejas and Tapas localize to a germline-specific, specialized perinuclear cytoplasm, known as nuage, which is considered as the organelle that primarily functions in piRNA-mediated degradation of transposon transcripts (Hirakata and Siomi 2019; Lim and Kai 2007; Patil and Kai 2010; Patil et al. 2014).

In ovaries, Tejas and Tapas perform related but not identical functions. In early *tejas* mutant oocytes, the nucleus fails to undergo compaction into the so-called karyosome, which is a condensed chromatin structure that normally forms as the oocyte arrests in meiotic prophase I (Roth and Lynch 2009; von Stetina and Orr-Weaver 2011) (Figure 3). In addition, *tejas* mutant ovaries display an increased expression of the germline-specific retroelements *I-element*, *TART*, *HeT-A*, and *blood*, while somatic gonadal transposons such as *gypsy* and *ZAM* are not affected (Handler et al. 2011; Patil and Kai 2010; Patil et al. 2014). Consequently, *tejas*-deficient females lay eggs that arrest in development and do not hatch. In contrast, *tapas* mutant females are viable and fertile and do not show any significant or only a slight increase in transposon transcript levels (Handler et al. 2011; Patil et al. 2014). However, when *tejas* is additionally absent, a *tapas* phenotype becomes evident. In *tejas/tapas* double mutant females the increase in germline transposon levels is stronger as compared to *tejas* single mutants, suggesting that Tejas and Tapas work together in the piRNA pathway (Patil et al. 2014). Furthermore, in *tejas/tapas* double, but not single mutants, Gurken protein fails to localize to the anterior-dorsal corner of the oocyte, indicating severe defects in the establishment of oocyte polarity (Patil et al. 2014).

The precise molecular mechanisms underlying the functions of Tejas and Tapas during oogenesis are unknown. Like Oskar, Tejas and Tapas bind to Vasa through their eLOTUS domains and stimulate its ATPase activity (Jeske et al. 2017) (Figure 2). Vasa not only functions together with Oskar at the posterior pole of oocytes, but is also an essential component of the nuage and required for piRNA-mediated transposon silencing (Malone et al. 2009) (Figure 3). The localization of Vasa to the nuage depends on its interaction with the eLOTUS domains of Tejas and/or Tapas (Jeske et al. 2017). However, as for Oskar the *in vivo* function of the stimulation of the Vasa activity by Tejas and Tapas is currently unclear. It might be that the enzymatic activity of unlocalized Vasa is too low to be productive, and that eLOTUS domain proteins recruit and stimulate Vasa

and thereby ensure that Vasa activity is spatially restricted to nuage and germ plasm (Jeske et al. 2017).

Vasa's role within the piRNA pathway has mainly been studied in cultured cells derived from ovaries of the silk moth *Bombyx mori* (BmN4), which recapitulate the so-called ping-pong cycle of the piRNA biogenesis pathway (Kawaoka et al. 2009; Nishida et al. 2015; Xiol et al. 2014) (Figure 3). There, Vasa associates with the PIWI proteins Siwi (Aubergine in *Drosophila*) and Ago3 (Nishida et al. 2015; Xiol et al. 2014), the two major players of the ping-pong loop in *Bombyx* (reviewed in Sakakibara and Siomi 2018). In this cycle, the Siwi-piRNA complex binds and cleaves transposon mRNAs with anti-sense orientation to piRNAs. The cleavage products are then loaded into Ago3 and the complex recognizes and cleaves piRNA cluster transcripts to produce mature piRNAs. Consequently, the ping-pong loop serves to specifically amplify piRNAs that target highly abundant transposon RNAs in the cytoplasm. In contrast to nonfunctional RNA fragments in the cytoplasm, transposon RNA fragments are not immediately degraded, but handed over from Siwi to Ago3, a process that requires Vasa function (Xiol et al. 2014). Additionally, *in vitro* experiments suggest that the ATPase activity of Vasa is necessary for the release of the transposon RNA from Siwi-piRNA complexes after cleavage (Nishida et al. 2015). However, these experiments leave open whether the release requires Vasa specifically or just any RNA helicase. In case of a Vasa-specific requirement and assuming a similar mechanism in the *Drosophila* germline, it is likely that the stimulation of Vasa's ATPase activity by Tejas and/or Tapas might function to enhance the rate of the ping-pong cycle of the piRNA biogenesis pathway. Nevertheless, although Tejas and Tapas share a similar domain organization and Vasa as interaction partner, Tapas is unable to rescue the *tejas* phenotype in *Drosophila* ovaries even upon overexpression (Patil et al. 2014), strongly indicating that the molecular functions of Tejas and Tapas are not identical.

In *Drosophila* males, Tejas and Tapas play a role in the piRNA pathway too. In testes, the majority of piRNAs are encoded by the Y chromosome-encoded *Suppressor of Stellate* (*Su[Ste]*) locus, which serve to inactivate the X chromosome-linked repetitive *Stellate* genes (Aravin et al. 2001, 2004; Vagin et al. 2006). *Stellate* encodes a regulatory β -subunit of the casein kinase II, which is not naturally expressed in testis, but upon ectopic overexpression forms large needle-like protein crystals within the nucleus and cytoplasm (Bozzetti et al. 2012; Egorova et al. 2009). The formation of Stellate crystals is linked to defects in meiotic chromosome condensation and segregation

eventually leading to reduced fertility (Malone et al. 2015). In *tejas* and *tapas* single and more severely in double mutant testes, the production of *Su(Ste)* piRNAs is impaired and both *Stellate* transcript and Stellate protein are upregulated leading to massive accumulation of Stellate crystals in the cells (Patil et al. 2014). Although *tejas* and *tapas* single mutant males are fertile, *tejas/tapas* double mutant males become sterile when they turn a few days old, indicating that Tejas and Tapas act synergistically also during spermatogenesis. The molecular mechanisms underlying Tejas and Tapas function in *Stellate* repression is unclear. Their binding partner Vasa is expressed in testes too and also required there for piRNA-mediated *Stellate* repression (Hay et al. 1988; Kibanov et al. 2011; Nagao et al. 2010). Thus, similar to their role during *Drosophila* oogenesis, also in males Tejas and Tapas might form a complex with Vasa that functions in piRNA-mediated transcript repression.

Mouse TDRD5 - an essential piRNA biogenesis factor during spermatogenesis

Mouse TDRD5 is expressed in the female and male germline but only essential for fertility in males (Smith et al. 2004; Yabuta et al. 2011). TDRD5 plays a fundamental role in the formation and maturation of round spermatids and is critical for the assembly of inter-mitochondrial cement (IMC) and chromatoid bodies (CB) (Yabuta et al. 2011), two characteristic granular ribonucleoprotein structures that appear at different developmental stages during spermatogenesis (Chuma et al. 2009) (Figure 3). The absence of TDRD5 leads to disorganization of IMC and CBs and to mislocalization of TDRD1, TDRD6, TDRD7, TDRD9 and of the mouse PIWI proteins MIWI, MILI, and MIWI2 (Yabuta et al. 2011). Furthermore, TDRD5-deficient spermatocytes fail to repress LINE1 transposable elements, while expression of other retrotransposons, such as IAP or SINE-B1 is unaffected (Yabuta et al. 2011).

Depending on the time of their expression during spermatogenesis, two piRNA populations are distinguished in mice (Ozata et al. 2019) (Figure 3). Fetal male germ cells express pre-pachytene piRNAs, which are enriched in transposon sequences and primarily act in transposon silencing (Aravin et al., 2007, 2008; De Fazio et al. 2011). Later during spermatogenesis, spermatocytes and round spermatids express pachytene piRNAs, the major piRNA population in mouse testes (Aravin et al. 2006; Girard et al. 2006; Grivna et al. 2006; Li et al. 2013). Pachytene piRNAs contain only a low level of transposon sequences, and

emerging evidence suggests that they regulate mRNAs and long non-coding RNAs in mouse testes (Goh et al. 2015; Gou et al. 2014; Watanabe et al. 2015).

Mouse TDRD5 is essential for the biogenesis of pachytene piRNAs (Ding et al. 2018). Crosslinking immunoprecipitation (CLIP) experiments revealed that TDRD5 is an RNA-binding protein that associates with pachytene piRNA precursors (Ding et al. 2018). The TDRD5-CLIP reads are enriched in guanines and both the eLOTUS and the first mLOTUS domain of TDRD5 have been demonstrated to specifically bind to G-rich and G4 RNA *in vitro* (Ding et al. 2020). Based on that G4 binding by TDRD5 has been speculated to drive piRNA precursor processing (Ding et al. 2020). TDRD5 associates with MIWI and MILI in co-immunoprecipitation experiments, and MIWI binding requires the eTudor domain of TDRD5 (Ding et al. 2018). As the TDRD5 eTudor domain lacks an intact aromatic cage, the MIWI-TDRD5 interaction is very likely methylation-independent.

The eLOTUS domain of human TDRD5 is capable of stimulating the ATPase activity of Vasa *in vitro* (Jeske et al. 2017). However, an *in vivo* function of the mammalian TDRD5-Vasa interaction has so far not been described. In testes, the mouse Vasa homolog (MVH) is an essential piRNA biogenesis factor required for the ping-pong cycle (Kuramochi-Miyagawa et al. 2010; Wenda et al. 2017), which mainly serves to amplify pre-pachytene piRNAs in pre-natal germ cells (Aravin et al., 2007, 2008). The ATPase activity of MVH is critical for processing of pre-piRNA intermediates into piRNAs (Wenda et al. 2017), and Vasa stimulation by TDRD5 might function to accelerate this process.

The function of TDRD5 seems not limited to piRNA biogenesis. In TDRD5-deficient spermatids the expression of the *Act* gene is greatly reduced (Yabuta et al. 2011), which encodes a testis-specific co-activator of CREM (Fimia et al. 1999), a critical transcription factor of spermiogenesis (Blendy et al. 1996; Nantel et al. 1996). Hence, TDRD5 might play additional functions in piRNA-independent post-transcriptional gene regulation.

Mammalian TDRD7 - a critical factor during spermatogenesis, organogenesis, and viral defense

Mammalian TDRD7 has first been described in rats, where it is expressed in brain and testis, and associates with the cyclin-dependent kinases (CDK) 16 and 17 (a.k.a. PCTAIRE/PCTK 1 and 2, respectively), and thus the protein was

initially named TRAP after tudor repeat associator with PCTAIRE 2 (Hirose et al. 2000). CDK16 is highly expressed in mammalian brain and testis and beyond many other functions plays an essential role during spermatogenesis (Mikolcevic et al., 2012a,b; Okuda et al. 1992; Zi et al. 2015). CDK17 is predominantly expressed in terminally differentiated neurons but poorly characterized (Besset et al. 1999; Hirose et al. 1997; Mikolcevic et al. 2012a). In mice, TDRD7 interacts with ik3-1 (a.k.a. Cables), a protein that modulates the activity of several CDKs and thus is associated with diverse functions including neurite outgrowth (Wu et al. 2001; Yamochi et al. 2001; Zukerberg et al. 2000). Both the CDK and the ik3-1 interactions are mediated by the C-terminal eTudor domain of TDRD7 (Hirose et al. 2000; Yamochi et al. 2001). However, the role and the molecular function of TDRD7 in these pathways are not known.

Further studies in mouse revealed that TDRD7 is required for male-only fertility (Lachke et al. 2011; Tanaka et al. 2011b). Similar to TDRD5 mutants, TDRD7-deficient mice arrest at round spermatid stage of spermatogenesis (Lachke et al. 2011; Tanaka et al. 2011b) (Figure 3). During spermatogenesis, TDRD7 localizes to CBs and to a lesser extent to IMC, and is essential specifically for assembly, remodeling and maintenance of CBs (Hosokawa et al. 2007; Tanaka et al. 2011b). TDRD7 associates with MVH, TDRD1, and TDRD6 in co-immunoprecipitation experiments (Hosokawa et al. 2007), and is involved in silencing of LINE1 retrotransposons during spermatogenesis (Tanaka et al. 2011b). Interestingly, although LINE1 element repression has mainly been described to occur via the piRNA pathway (De Fazio et al. 2011; DiGiacomo et al. 2013; Pezic et al. 2014; Reuter et al. 2011), TDRD7-mediated LINE1 repression does not, and instead appears to operate at the level of translation control (Tanaka et al. 2011b).

In addition to brain and testes, TDRD7 is highly enriched in the developing ocular lens of vertebrates and its deficiency in chicken, mice and human causes eye defects such as cataract and glaucoma (Chen et al. 2017; Lachke et al. 2011; Tan et al. 2019; Tanaka et al. 2011b). In the lens, TDRD7 was suggested to bind and post-transcriptionally regulate mRNAs that encode factors critical for lens development (Barnum et al. 2020; Lachke et al. 2011).

Through a genetic screen in HeLa cells with the aim to identify interferon-stimulated genes that block Sendai virus replication, human TDRD7 has been identified as antiviral effector protein, whose expression is induced by interferons, and which plays a role in the defense against paramyxo-, pneumo-, and herpes simplex viruses (Subramanian et al., 2018, 2020). TDRD7 restricts virus replication and virus-induced autophagy through

inhibition of the AMP-dependent kinase (AMPK) (Subramanian et al., 2018, 2020).

The molecular mechanisms underlying the various TDRD7 functions in mammals are not known. The eLOTUS domain of human TDRD7 binds to G4 RNAs *in vitro* (Ding et al. 2020), which might be an important function for mRNA target recognition. The eLOTUS domain is also able to stimulate the ATPase activity of Vasa *in vitro* (Jeske et al. 2017). However, as mammalian Vasa protein expression is germline-specific (Toyooka et al. 2000), a TDRD7-Vasa complex might only assemble during spermatogenesis. Consequently, outside the germline, the molecular function of TDRD7 is very likely independent of Vasa, and the eLOTUS domain might predominantly serve to recognize G4 RNA motifs. In mouse testes, MVH plays a critical role in the piRNA pathway (Kuramochi-Miyagawa et al. 2010; Wenda et al. 2017), which is in contrast to TDRD7. MVH appears to exhibit a piRNA pathway-independent role too (Wenda et al. 2017), and thus it will be interesting to investigate the function of a potential TDRD7-Vasa complex during mammalian spermatogenesis in the future.

Concluding remarks

The molecular functions of LOTUS domains, such as G-rich/G4 RNAs recognition by both eLOTUS and mLOTUS domains as well as Vasa helicase binding and stimulation by eLOTUS domains appears highly conserved among individual LOTUS-domain proteins and throughout evolution. In this light, it is remarkable that the physiological roles of the individual LOTUS-domain proteins are highly divergent. For example, the eLOTUS-Vasa module is involved in distinct biological pathways, such that in *Drosophila* oocytes, the Oskar eLOTUS-Vasa interaction is critical for germ cell specification, while the Tejas/Tapas eLOTUS-Vasa interaction plays a potential role in the piRNA biogenesis pathway (Figure 3). The physiological importance of most LOTUS-domain proteins has been known for many years, and structural biology approaches, including homology modeling, have provided molecular insight to an extent that allows visualizing their entire domain organization at atomic level (Figure 2). Still, the molecular mechanisms underlying the functions of LOTUS-domain proteins are poorly understood, and the individual contributions of their protein domains, especially of the OSK, eTudor, and RRM domains, are largely undefined. Due to the nature of animal LOTUS-domain proteins as developmental effectors, addressing the many unanswered questions of how these proteins function at the molecular level will require continuously combining

biochemical techniques with genetic approaches using animal models.

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