Tudor and its domains: germ cell formation from a Tudor perspective

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ABSTRACT

In many metazoan species, germ cell formation requires the germ plasm, a specialized cytoplasm which often contains electron dense structures. Genes required for germ cell formation in *Drosophila* have been isolated predominantly in screens for maternal-effect mutations. One such gene is *tudor* (*tud*); without proper *tud* function germ cell formation does not occur. Unlike other genes involved in *Drosophila* germ cell specification *tud* is dispensable for other somatic functions such as abdominal patterning. It is not known how TUD contributes at a molecular level to germ cell formation but in *tud* mutants, polar granule formation is severely compromised, and mitochondrially encoded ribosomal RNAs do not localize to the polar granule. TUD is composed of 11 repeats of the protein motif called the Tudor domain. There are similar proteins to TUD in the germ line of other metazoan species including mice. Probable vertebrate orthologues of *Drosophila* genes involved in germ cell specification will be discussed.

Keywords: *Drosophila*, *tudor*, germ cells, germ plasm, Tudor domains.

INTRODUCTION

Germ cell formation is an integral part of sexual reproduction as well as certain types of asexual reproduction as germ cells are a totipotent stem cell line in which meiosis occurs. In many metazoan species, germ cell formation requires a specialized cytoplasm. In *Drosophila,* this cytoplasm, referred to as germ plasm or pole plasm, contains electron dense organelles called polar granules. Many of the proteins and mRNAs needed for germ cell formation accumulate on or in these structures. Although mammals do not appear to rely on a specialized maternally deposited cytoplasm for germ cell development, many mRNAs and proteins found in the *Drosophila* germ plasm are conserved in sequence and function in mammalian species. The following discussion focuses on one specific polar granule component, TUD and its novel protein domains (Tudor domains).

GERM PLASM

Early embryonic development in many different species depends on properly localized maternally deposited factors. Over 100 years ago, it was first observed that germ cells form from cytoplasm at the posterior of the fertilized egg [1]. This posterior cytoplasm is composed of maternal gene products, is morphologically distinct, and is essential for germ cell formation. In early experiments in beetles, physical disruption or removal of the germ plasm abolished germ cell formation [2]. Similarly, later experiments in *Drosophila* showed that UV-irradiation of the germ plasm produces adult flies that lack germ cells [3]. However, when germ plasm from non-UV-irradiated embryos was transplanted into UV-irradiated embryos, germ cell formation was rescued [4]. Interestingly, the production of germ cells can be induced if germ plasm is injected into embryos at ectopic sites [5]. Furthermore, these ectopic germ cells could complete germ cell formation when transplanted to the posterior of a host embryo [6]. Taken together the data from these studies indicate that germ plasm is not only essential but is sufficient for germ cell formation. Ultrastructural analysis of *Drosophila* germ plasm shows that it contains electron dense structures referred to as polar granules, and similar electron dense structures are found in the germ plasm or germ line of other species.

To identify genes required maternally for germ cell specification, screens for grandchildless mutations, that is, maternal-effect sterile mutations, were conducted [reviewed in 7]. Subsequent maternal-effect lethal screens

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brought about a huge leap in the understanding of germ plasm assembly and germ cell formation, as they uncovered a novel class of genes known as posterior group genes. Mutations of these genes disrupt posterior somatic patterning, as seen by the loss of one or more abdominal segments, which usually results in maternal effect lethality. Mutations in one posterior group gene, *nanos* (*nos*), do not initially disrupt germ cell formation but result in a strong posterior group phenotype [8]. When *nos* mRNA is injected into the posterior of posterior group mutant embryos, posterior segmentation is restored. It was also found that when *nos* mRNA is injected at the anterior, it can direct posterior patterning at the site of injection [9]. Not only does this show that NOS is the posterior determinant, but also that other posterior group genes are needed for the proper localization of NOS. To support this observation, mutations in other posterior group genes were found to affect NOS protein accumulation, often by abrogation of *nos* RNA localization [8, 10].

Not surprisingly, many posterior group genes encode germ plasm components. These genes have been extensively studied to get a better idea of how they interact at both the genetic and molecular levels to drive germ plasm assembly and germ cell formation. Germ plasm components are deposited into the oocyte during oogenesis, [for a review of oogenesis see 11]. *Drosophila* oogenesis begins with the asymmetrical division of a germ line stem cell. This division gives rise to a germ line stem cell and a daughter cystoblast. The cystoblast goes through 4 mitotic divisions with incomplete cytokinesis, thus forming a 16-cell interconnected germ cell cyst. 15 of these cells become nurse cells while one becomes the oocyte. Nurse cells supply the growing oocyte with the bulk of the RNAs and proteins needed for early embryonic development, including those destined for the germ plasm.

Assembly of the germ plasm during oogenesis occurs in a stepwise fashion where each molecule depends on the localization of the preceding molecules. *oskar* (*osk*) mRNA is at the top of this pathway, and mislocalization of *osk* mRNA to the anterior is capable of inducing germ cell formation at the anterior [12]. Two genes encoding downstream pole plasm components, *tud* and *vasa* (*vas*), are required in addition to *osk* for pole cell formation at any location, while other posterior-group genes are dispensable for germ cell formation at an ectopic site [12]. This suggested direct roles for *tud* and *vas* in germ cell formation (Fig. 1).

vas encodes a DEAD-box RNA helicase with similarities to eIF4A, which suggests that VAS has a role in regulating translation [14-16]. Ovaries of homozygous *vas*-null females rarely complete oogenesis and exhibit pleiotropic phenotypes, suggesting that VAS is involved

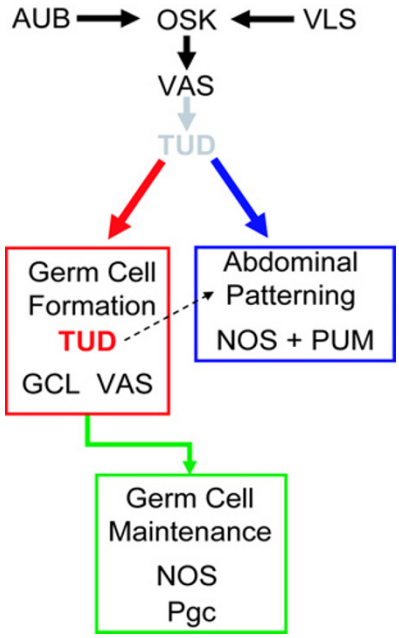

Fig. 1 Pole plasm assembly in brief. "TUD" in grey represents the position originally assigned to TUD in the pole plasm pathway, as initial mutations of *tud* affected both germ cell and abdomen formation. Recent isolation of a null allele shows that TUD is absolutely needed for germ cell formation, as indicated by the red "TUD". As implied by the dashed arrow TUD does have a role in abdominal patterning but is redundant or not absolutely required as normal abdominal patterning can occur in embryos completely lacking TUD. Thus TUD is the earliest gene absolutely needed for germ cell formation but dispensable for other somatic functions. Valois (VLS) is a co-factor needed for proper OSK accumulation in the germ plasm [13]. Some germ plasm components have roles in multiple functions of the germ plasm as shown with NOS and VAS.

in several earlier processes in oogenesis in addition to its role in the pole plasm [17]. Yeast two hybrid studies show that VAS and OSK bind directly to each other. The interaction of these two proteins plays an integral role in germ cell formation and polar granule assembly, and OSK appears to mediate accumulation of VAS in the germ plasm [18]. Although the localization of VAS does not rely on its ability to bind RNA via the DEAD box RNA helicase, germ cell formation does [19]. More recent work revealed that VAS binds the translation initiation factor eIF5B [20]. A mutated form of VAS-GFP whose eIF5B binding activity has been greatly reduced has been used to study the functional significance of the VAS-eIF5B interaction [21]. This mutated form of VAS-GFP localizes normally, and progeny of females expressing it in a *vas* mutant genetic background often have normal posterior somatic patterning and are viable. However, germ cell formation is not rescued by this form of VAS, implying

that the interaction between VAS and a general translation initiation factor is essential for germ cell formation.

It is important to identify RNAs that are essential for germ cell specification and that depend on VAS for activation of translation initiation yet none have been found to date. Two maternally-expressed genes, *gcl* and *pgc*, encode RNAs that accumulate in the pole plasm downstream of VAS and are specifically required for germ cell specification. *gcl* may be involved in establishing or maintaining transcriptional quiescence, an essential feature of primordial germ cells [21]. In the syncytial *Drosophila* embryo, the first nuclei that migrate to the posterior are destined to become germ cells and become transcriptionally silent. In *gcl*-null embryos these nuclei are transcriptionally active and do not form germ cells. GCL localizes to the nucleoplasmic surface of the nuclear membrane where it is postulated that it might interact with chromosomes directly to inhibit transcription similar to telomeric silencing in yeast.

pgc RNA localizes to polar granules. In flies that express a *pgc* antisense construct, germ cells form normally and in near-normal numbers, but many do not survive migration to the somatic gonad [22]. Germ cells lacking *pgc* exhibit precocious expression of somatic markers presumably due to the presence of active, phosphorylated RNApolII [23]. In wild-type germ cells, RNApolII is not normally phosphorylated and phosphorylation, specifically at the carboxy-terminal domain, is needed for RNApolII activity. It is unclear at this time how the reduction of *pgc* activity represses RNApolII activity at this time [24].

While much has been learned about the composition of the pole plasm, undoubtedly more germ plasm components remain to be identified. Also, many somatically required genes also have germline specific functions. While these genes can sometimes be identified through phenotype-based screens that focus on the germline or by creating germline mutant clones [25], if a mutation blocks oogenesis at an early stage or is cell-lethal then any later function in oocyte patterning will not be uncovered. Biochemical approaches and direct protein interaction screens, such as the two-hybrid screen which identified a role for eIF5B in germ cell formation, will allow identification of somatic genes required for germ cell formation [20, 26, 27].

TUDOR HAS A KEY ROLE IN GERM CELL ESTABLISHMENT

tud was one of the first posterior-group genes identified in *Drosophila melanogaster*, and some alleles give a completely penetrant grandchildless phenotype [28]. Embryos produced by *tud* mothers have substantially fewer polar granules than do wild-type embryos. Some germ cells form in the progeny of less severe *tud* mutants, and while these have more polar granule material only a small portion is taken into the newly formed germ cells, which although functional are not morphologically normal. Thus mutations in *tud* point to a potential correlation between polar granule formation and germ cell formation.

tud encodes a large mRNA of approximately 8 kb [29] that encodes a large peptide of 2515 amino acids or 285 kD. Over half of TUD is comprised of 11 copies of a novel protein motif called the Tudor domain [30]. *tud* begins to be expressed very early in oogenesis. *tud* mRNA localizes to the newly formed oocyte where it accumulates in the posterior and later dissipates. At the same time, *tud* mRNA is abundant in nurse cells. In later stages of oogenesis and early stages of embryogenesis *tud* mRNA is uniformly distributed in germ line cells. TUD protein is concentrated around the nurse cell nuclei and in the oocyte in the early stages of oogenesis. The perinuclear localization of TUD is similar to that of VAS, a protein encoded by another posterior group gene. These proteins accumulate in what is called the nuage, an electron dense structure around nurse cell nuclei. TUD protein later accumulates in the posterior of the developing oocyte where it stays until after germ cell formation [31], after which time it can no longer be detected. TUD is also found throughout the early embryo in ring-like structures in the anterior.

A null allele of *tud* was recently isolated [32]. As homozygous *tud*-null individuals allele are fully viable and do not exhibit any defects except those attributable to the pole plasm [32], *tud* does not have an essential somatic function. Unlike *osk* and *vas* [19, 36], the *tud*-null mutation did not affect oogenesis. Surprisingly, some embryos from homozygous *tud*-null mothers grew to adulthood [32]. Although all *tud*-null embryos could not form germ cells, approximately 15% had normal posterior patterning. In *tud*-null oocytes, posterior localization of OSK and VAS occurs normally. However, the localization of OSK, VAS and NOS is not maintained in embryos produced by *tud*-null mothers after the developmental stage when pole cells would normally form. This phenomenon was first observed in *Drosophila* germ plasm formation almost 2 decades ago [16, 33]. If germ cells do not form due to a mutation affecting a downstream member of the pole plasm assembly pathway, posterior localization of the upstream factors, while initially unaffected, is not maintained. It is unknown if this is an active process where the soma degrades germ plasm components, or a passive process where the germ cells provide an essential structure to store and protect the germ plasm. The *tud*-null mutant showed that TUD, unlike many other pole plasm components, is required spe-

cifically for germ cell formation. Thus isolation of a *tud*-null mutant has begun to unravel two entangled pathways, germ cell formation and abdominal patterning.

A BRIEF ANALYSIS OF TUDOR DOMAINS

The Tudor domain was first discovered during a comparison of protein sequences, which was done to find homologous regions with sequence or structural similarities [30, 34]. Several of the first Tudor domain containing proteins that were identified have well known RNA binding motifs. Based on this knowledge, it was suggested that Tudor domains may have a role in RNA metabolism [30]. However, a more complete database analysis of proteins with Tudor domains does not show an association between them and RNA binding motifs. Moreover, recent experimental evidence does not support an RNA-binding function for the Tudor domain. For example, the *Drosophila* OVARIAN TUMOR (OTU) protein contains a single Tudor domain and is associated with mRNPs, but deletion of its Tudor domain did not disrupt its ability to associate with mRNPs [35].

The mammalian SURVIVAL MOTOR NEURON (SMN) protein also contains a single Tudor domain, and mutations in the corresponding gene are responsible for autosomal recessive proximal spinal muscular atrophy (SMA) [36]. Structural analysis of the SMN Tudor domain shows that it is essentially a "barrel", closed on one end with hydrophobic residues pointing inwards, which is not consistent with an RNA binding function [37, 38] (Fig. 2). Tudor domains have highly conserved amino acids and predicted structure, which suggests that the structure of the Tudor domain in SMN is likely to be very similar to those found in other Tudor domain containing proteins. SMN binds to two SM proteins, SmD1 and SmD3, at their carboxy-terminal ends, which are arginine- and glycine-rich domains [39]. Methylation of the carboxy-terminal ends of SmD1 and SmD3 enhances the binding ability of SMN by several folds. SMN binding is also enhanced when other SMN targets are methylated, [40, 41], particularly when binding partners have symmetrical dimethylation of the arginines (sDMA). SMN cannot efficiently bind unmethylated or asymmetrically methylated carboxyl-terminal ends of SmD1 and SmD3. A single amino acid mutation of the SMN Tudor domain completely inhibits the ability of SMN to bind the sDMAs of SmD1 and SmD3, strongly suggesting that the Tudor domain of SMN is needed for binding to methylated SmD1 and SmD3 [41].

There are some experimental caveats that may make the conclusion that the Tudor domain is a methylated protein binding domain somewhat premature. First, most of the expression assays were not carried out with full length SMN constructs. Second, SMN binding was tested against only a small portion of SmD1 and SmD3. Third, SMN lacking portions of its Tudor domain can weakly bind SmD1 and SmD3. Finally, the biological effects of disrupting the SMN Tudor domain-SM protein interactions have not yet been tested. In fact, one study has suggested that deletion of the Tudor domain of SMN is not lethal to the chicken pre-B cell line DT40, while full length SMN is needed for viability of this same cell line [42]. Analysis of human type I SMA patients leads to a different conclusion, however. While 90% of SMA patients have large deletions of the *smn* gene, and are therefore not informative as to specific Tudor domain function [43], some SMA patients have missense mutations leading to amino acid changes in conserved residues in Tudor domains [43, 44]. Therefore, although in a specific cell line the Tudor domain of SMN may not be required for viability, the Tudor domain of SMN is functionally relevant in vivo. It should be noted that type I SMA is a lethal disorder, and individuals with single amino acid changes in Tudor domains still show lethality as early as those with large deletions of the *smn* gene.

Recent work with 53BP1 and its role in DNA double-stranded breaks (DSBs) has resolved the issue of Tudor domain functionality [45]. 53BP1 is a conserved checkpoint gene that has been implicated as a sensor for DNA DSBs. Recruitment of 53BP1 to DSBs is crucial for their repair. 53BP1 contains two Tudor domains and mutations specifically in residues predicted to be crucial to proper Tudor domain folding inhibit the recruitment of 53BP1 to DNA DSBs. 53BP1 was found to bind to histone H3 directly. H3 had to be methylated for this interaction, specifically at Lys 79. The removal of the methyltransferase (DOT1L) necessary for methylating Lys 79 had the same effect as disrupting the Tudor domains of 53BP1. Mutating the Tudor domains of 53BP1 or inhibiting the methylation of Lys 79 of H3 had the same effect as these cells were not able to respond to DNA DSBs. This evidence makes it clear that Tudor domains do not have a role in RNA metabolism but instead bind to methylated amino acids. It will be interesting to see if variations in Tudor domains determine what type of methylated amino acids they bind, either lysines or arginines, and if the lysines are symmetrically methylated as were the arginines in SmD1 and SmD3.

POLAR GRANULES: A MYSTERY WRAPPED IN PROKARYOTIC TRANSLATION?

The germ plasm of *Drosophila* was first observed using light microscopy over 100 years ago. It is morphologically distinct due to the presence of large electron dense structures called polar granules, which are associated with mitochondria. Polar granules are large mRNP aggregates

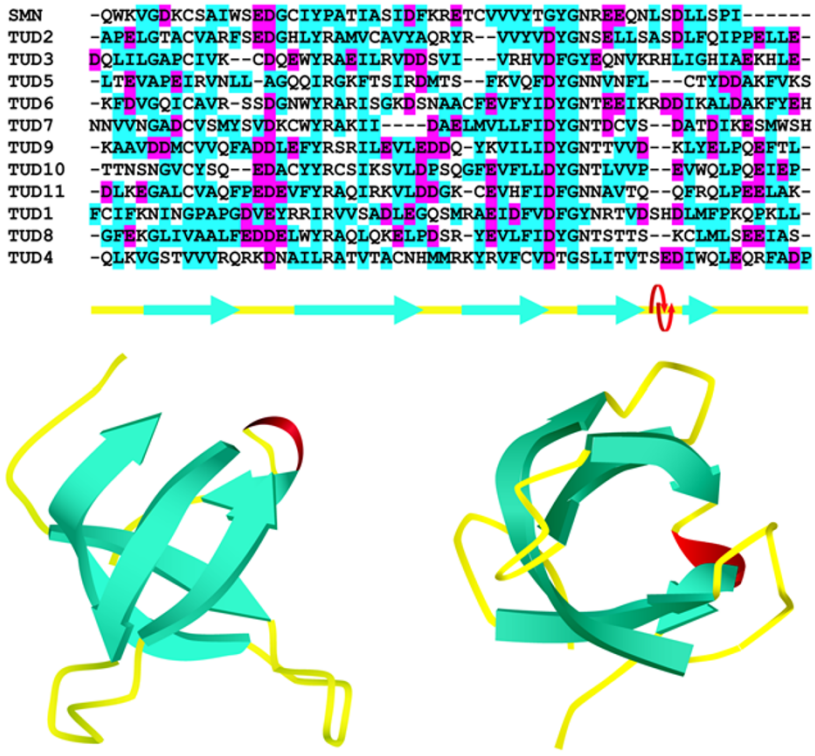

Fig. 2 The Tudor Domain. (**A**) Amino acid sequence of the Tudor domain in SMN (*Homo sapiens*) in comparison to the 11 Tudor domains of TUD. Hydrophobic residues are highlighted in blue and negatively-charged residues are highlighted in pink. Although there is a conservation of residues of Tudor domains across species, what is more conserved are the locations of hydrophobic and negative amino acids. (**B**) In spite of the many amino acid differences between the Tudor domains even in the same protein (as shown in **A**) the predicted secondary structures are very similar to that shown in **B**. α-sheets are represented in blue and red arrows show the position of the α-helix. (**C**) Front view of the Tudor fold as determined by NMR. (**D**) Bottom view of the Tudor domain showing it to be tube, the N-terminal end of the Tudor domain is predicted to fold over the top end making the Tudor domain are barrel like structures with a closed end (angle not shown). Modified from Selenko *et al* [37], see for a detailed explanation of the Tudor domain structure.

that resemble electron dense aggregates seen in the germline of other species. Polar granules are dynamic structures that change in size, shape and presumably composition throughout oogenesis and early embryogenesis [46-49]. Many components of the *Drosophila* polar granule can be found in the nuage, a perinuclear structure in ovarian nurse cells**.** These include VAS, TUD, and Aubergine (AUB), a translational regulator of *osk* mRNA [50, 51]. The fact that nuage and polar granules share some components suggests that the former might be a precursor form of the latter. However, real-time analysis of GFP-VAS and GFP-AUB localization suggests that the nuage may disassemble before localization to the posterior, and polar granules assemble *de novo* [51]. It will be interesting to see if this observation can be reconfirmed using electron microscopy.

Shortly after fertilization, polar granules lose a considerable amount of electron density due to a loss of RNA from the polar granules [52]. It is unknown if the RNAs are translated and degraded or dispersed into the newly forming germ cells. What remains of the polar granules are transported by aggregation onto the centrosomes into the newly forming germ cells [49, 53]. Many germ plasm components localize to polar granules as determined by electron microscopy (Tab. 1). The functional significance of the polar granule structure itself remains an open question, and the dynamics of the polar granules, nuage and other electron dense structures in the germline must be re-examined as the original ultrastructural analysis was done prior to the identification of several polar granule components.

MITOCHONDRIAL RRNA AND GERM CELL SPECIFICATION

As discussed above, UV-irradiation of the pole plasm of *Drosophila* disrupts germ cell formation. This effect can be rescued by injection of mitochondrial large rRNA (mtlrRNA) into the irradiated pole plasm [68]. A strong case can be made that mtrRNA is needed for germ cell formation, as ribozyme-mediated depletion of mtlrRNA results in a severe reduction of the number of pole cells formed [69]. Ultrastructural analysis of mtrRNA in the pole plasm shows that mtlrRNA localizes to the polar granules along with small mitochondrial rRNA (mtsrRNA) and mitochondrial ribosomal proteins S12 and L7/L12 [59, 60]. Electron microscopy revealed that the mtrRNAs and mitochondrial ribosomal proteins aggregate together to form mitochondrial ribosomes on the periphery of the polar granule (Fig. 3).

Are mitochondrial tRNAs and other factors involved in mitochondrial translation somehow used by polar granule ribosomes? If so, then mRNAs translated on the polar granules would be produced with mitochondrial codon usage,

Tab. 1 Known or suspected polar granule components. Relatively few polar granule components have been identified, and many that are suspected to be polar granule components have not been confirmed by electron microscopy. "EM data" indicates if a component has been shown to localize to the polar granules using electron microscopy.

Component	Description	EM DATA	Other data
Oskar	Unknown function	YES	Crucial and sufficient for germ cell formation and abdominal patterning [12, 54, 55]. Needed for polar granule formation [18].
Vasa	DEAD-BOX RNA helicase	YES	Role in translational activation needed for germ cell formation [20, 26]. Needed for polar granule formation [14-16, 56].
Tudor	Unknown, needed for germ cell formation.	YES	Localizes to polar granules and mitochondria in the germ plasm and fibers that connect the two [57]. May have a role in transporting mtrRNAs from mitochondria to polar granules [57]. Needed for polar granule formation [28, 32].
Staufen	RNA binding protein	YES	Needed for proper OSK localization [58]. Needed for polar granule formation [18].
mtlrRNA	Mitochondrially encoded large rRNA subunit	YES	Forms a mitochondrial ribosome on the surface of polar granules [59, 60].
mtsrRNA	Mitochondrially encoded small rRNA subunit	YES	Forms a mitochondrial ribosome on the surface of polar granules [60, 61].
Mitochondrial Ribosomal Protein S12	Mitochondrially encoded ribosomal protein	YES	Forms a mitochondrial ribosome on the surface of polar granules [60].
Mitochondrial Ribosomal Protein L7/L12	Mitochondrially encoded ribosomal protein	YES	Forms a mitochondrial ribosome on the surface of polar granules [60].
Polar granule component	Non-coding RNA	YES	Involved in transcriptional quiescence after germ cell formation [22, 23].
Fat Facets	Deubiquitinating enzyme (DUB)	NO	Enrichment of FAF mRNA to the posterior of early embryos, and FAF binds to VAS directly, suggesting that at least a portion is on the polar granules with VAS [27, 62].
Aubergine	Similar to eIF2C, RNAi machinery component.	NO	AUB-GFP colocalizes to the germ plasm in a punctuate pattern with other GFP labeled polar granule components [63].
***cyclin* B mRNA**	Cell cycle regulator.	NO	Punctate mRNA localization to posterior [64]. Recently shown to have a specific role in the Drosophila germline [65].
***hsp83* mRNA**	Chaperone protein	NO	Localization to posterior as determined RNA *in situ* [66].
***gcl* mRNA**	GCL is needed to maintain germ plasm transcriptional quiescence.	NO	Localization of gcl mRNA to the posterior in a granular fashion [67].
P95	UNKNOWN	N.A.	Unknown protein isolated in biochemical purification of polar granules [7].

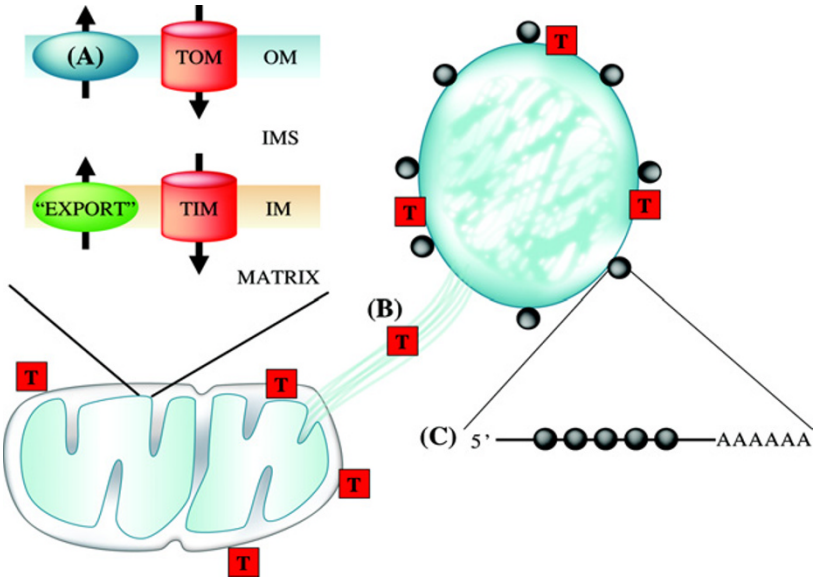

Fig. 3 Transport of mtrRNA to polar granules. (**A**) The TOM and TIM complexes are well studied complexes involved in transport into the mitochondria; the "Export" complex is a partially understood complex that moves molecules across the inner membrane. Very little is known about how molecules can be transported across the outer membrane or out of the mitochondria altogether. (**B**) It has been observed that thin fibers connect the polar granules and mitochondria. TUD localizes to these fibers, along with mtrRNAs, suggesting it is a mitochondria to polar granule transport structure. What comprises these fibers and how they function is unknown. (**C**) Mitochondrial polysomes localize to the periphery of polar granules, but which mRNAs are translated in these complexes is unknown. Red squares with a "T' represent TUD.

and one class of stop codon (UGA) would be read through. It may also be that mitochondrial translation is needed to overcome some form of translational repression in the germ plasm or to ensure that somatic genes are not translated in the germ plasm. Whatever their significance, the formation of mitochondrial ribosomes in the germ plasm is highly conserved in animal evolution. Mitochondrially-derived ribosomes also form on germinal granules, structures very similar to polar granules, that are present in *Xenopus* germ plasm [70, 71]. Recently, 16S rRNA was found outside the mitochondria in human testis, localized to the nuclei of spermatogenic cells, which suggests that mitochondrial ribosomes may have a function in the mammalian germline [72].

This leads to another interesting question: how are macromolecules such as mtrRNAs transported out of the mitochondria? The transport of macromolecules into the mitochondria via the TOM and TIM complexes is well understood [reviewed in 73]. However, the export of proteins from the inner matrix across the inner membrane is less well understood, and how macromolecules completely leave the mitochondria is still a mystery. The bidirectional mitochondrial transport of spermine, a polyamine involved in several biological processes, is one of the few examples of how molecules are transported out of the mitochondria. The transport of spermine is facilitated by high mitochondrial membrane potential and pH gradient [74]. It has been reported that the membrane potential of the mitochondria in the germ plasm is increased in the early embryo, as seen with rhodamine 123 staining [75]. However, the membrane potential of *Drosophila* germ plasm mitochondria is not affected by UV-irradiation. The injection of mtlrRNA into UV-irradiated embryos rescues germ cell formation, suggesting that mtrRNAs are not transported out of the mitochondria even with high membrane potential. This implies that high membrane potential is not a prerequisite for transport out of the mitochondria. It is possible that UV-irradiated embryos still transport mtrRNA out of the mitochondria and it is then deactivated or that UV-irradiation does affect transport of mtrRNA at a point downstream of high membrane potential. Reducing mitochondrial membrane potential in the posterior pole plasm using commercially available anti-cancer drugs could be very illuminating if it could be demonstrated that no mitochondrially encoded rRNAs or proteins are seen on the polar granules after treatment. Finding out if high membrane potential is needed for transport out of the mitochondria is a great first step but more work is clearly needed to determine how macromolecules are transported out of the mitochondria.

NOW THAT THE MTRNA IS OUT OF THE MITOCHONDRIA...WHAT NEXT?

Like other pole plasm components such as OSK and VAS, TUD localizes to the polar granules. A unique and interesting characteristic of the TUD distribution in the pole plasm is that TUD also localizes to the mitochondria, not just the polar granules [31, 57]. Closer examination shows that TUD localizes with mtrRNAs to the fibrous material that seems to connect polar granules and mitochondria. Although mutations in *vas*, *osk* and *tud* all lead to a loss of mtrRNA in the pole plasm, the observation that TUD localizes to the mitochondria and polar granules led to speculation that TUD may have a role in mtrRNA transport to the polar granules. In fact, in embryos produced by *tud* mutants mtrRNAs are not transported from the mitochondria to the polar granules. Do these embryos simply lack polar granules for mtrRNA localization or do they lose the ability to transport mtrRNAs to polar granules? To distinguish these possibilities, the localization of mtrRNAs was compared to the localization of VAS. In a *tud* mutant that still made polar granules, albeit at a greatly reduced quantity, VAS still localized to these reduced polar granules. However, no mtrRNAs localized to these polar granules, suggesting a direct role for TUD in mtlrRNA localization. However, it can still be argued that this result stems from a failure of the abnormal polar granules in *tud* mutants to receive or capture mtrRNAs, rather than a defect in mtrRNA transport.

CONSERVATION OF *DROSOPHILA* GERM CELL FORMATION GENES IN MAMMALS AND OTHER SPECIES

A wide variety of species relies on a germ plasm for germ cell formation, and germ plasm generally contains polar granule like structures [76]. During germ cell migration, in both mammals and *Drosophila*, there is a great deal of conservation of genes and gene function [reviewed in 77, 78]. The following discussion will focus on mammalian orthologues of some *Drosophila* germ plasm genes. The most notable example is *vas.* Many species appear to have one or more *vas* homologs whose products are expressed in germline cells [76, 79-84]. These *vas* homologs are essential for germ line development in many of these species [15, 16, 19, 82, 85-86]. The MVH (mouse vasa homolog) protein localizes to mammalian germ cells very early in development, and later localizes to the spermatogonia, where it is associated with the chromatoid body. The chromatoid body is a large electron dense structure located near the spermatogenic cell nucleus and is associated with mitochondria, thus in several aspects it resembles the polar granule. Mice lacking *mvh* function do not complete spermatogenesis. It is unknown at this time whether MVH associates with eIF5B.

Other mouse homologs of *Drosophila* germ plasm genes also function in germ line development. Mouse *germ cell-less* (*mgcl-1*) localizes to the germline, and like *mvh*, *mgcl-1* is needed for spermatogenesis but not for oogenesis [87]. mGCL-1 protein localizes to testicular cells and, as for GCL, a large amount of this protein localizes to the nuclear envelope. However not all mGCL-1 localizes to the nuclear envelope, a significant portion is seen in the nuclei surrounding DNA [88]. The expression of mGCL-1 in flies that are null for *Drosophila gcl* rescues germ cell formation, which suggests that not only is there sequence similarity but a conservation of function as well [89].

The *Xenopus* protein XTR (*Xenopus* Tudor Repeats) [90] and a murine protein MTR (Mouse Tudor Repeats) (MTR) [91] may be functional counterparts of TUD, as both are expressed in germ line cells. XTR was first thought to contain four Tudor domains and one short Tudor domain located at its N-terminal end, which probably cannot form a Tudor fold. However, recent protein database searches suggest that there may be as many as 7 complete Tudor repeats in XTR [92]. Although TUD, MTR and XTR have significant sequence similarity, it is premature to consider them as orthologs until more functional information is available for the vertebrate proteins. However, MTR localizes to the chromatoid bodies in the mammalian male germ line, and when expressed in cell culture, MTR is able to induce the formation of chromatoid-like bodies [91]. Based on these observations, it would not be surprising if *mtr* null mice, when generated, will be found to be compromised with regard to male fertility.

Given its position at the top of the *Drosophila* pole plasm hierarchy, it is perhaps surprising that *osk* is not widely conserved in evolution. Perhaps its function is unique to flies; for example, VAS and other downstream components may specifically require OSK to be anchored in the pole plasm in that organism. While *osk* recruits all downstream pole plasm components, it is not actually certain that it is required itself for germ cell specification, as for technical reasons it has not yet been possible to mis-localize VAS and/or TUD to an ectopic site such as the anterior pole of the embryo without also mislocalizing *osk.* It could also be that in other species, the molecular function of OSK is carried out by another gene or set of genes.

In *Drosophila*, germ cell formation is integrally connected to patterning of the posterior soma. Many other species, even those that localize maternally deposited factors to a germ plasm, do not share this characteristic. Consistent with this, the functions of some germ plasm components have diverged over evolutionary time. An example is NOS, which is highly conserved both in sequence

and function in mammals. As mentioned NOS is required for posterior somatic patterning in *Drosophila*. It is not required for initial germ cell specification, but it is critical for germ cell function as it is involved in establishing and maintaining transcriptional quiescence. In mouse, at least two *nos* homologs are expressed in the germline, *nos2* and *nos3* [93]. Mutation of *nos2* blocks spermatogenesis, and mice lacking *nos3* are blocked both for spermatogenesis and oogenesis. Similar to NOS in *Drosophila*, homologs in mice are not needed for the initial formation of germ cells but are required for the maintenance of germ cell fate. In mice, NOS1 binds the mouse PUMILIO homolog just as NOS binds PUMILIO in *Drosophila*, suggesting a conservation of molecular function [94]. However, unlike in *Drosophila* there does not seem to be a role for mouse NOS2 or NOS3 in somatic patterning.

FINAL REMARKS

Although mammals and *Drosophila* diverged over 400 million years ago and their embryonic development is very dissimilar, there is remarkable conservation of *Drosophila* genes encoding germ plasm components. However, the functions of these genes in germ line development have somewhat diverged. Most notably, while in *Drosophila* *vas*, *tud* and *gcl* are required during oogenesis for embryonic patterning and for germ cell specification, in mammals their possible counterparts appear to be essential only in spermatogenesis. This may imply that mammalian spermatogenesis employs more evolutionarily ancient mechanisms that are also used for maternal specification of germ cells in *Drosophila*, and that mammalian oogenesis is a more recently diverged developmental pathway.

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