Shared Cell Biological Functions May Underlie Pleiotropy of Molecular Interactions in the Germ Lines and Nervous Systems of Animals

Arpita Kulkarni1*, Davys H. Lopez1† and Cassandra G. Extavour1,2*

1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States, 2 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, United States

Evolutionary developmental biology focuses on understanding the origin and evolution of extant biological variation, and the genetic basis for this variation. The genetic toolkit appears largely finite across animals, such that a combination of regulatory evolution, gene recruitment (co-option) and genetic modularity often allow morphological and developmental diversity to arise. Here we summarize a number of observations from across animals, which together suggest that many genes and gene product interaction "modules" originally characterized for their role in the germ line also have neural roles.

We explore potential explanations for this observation, noting that in the context of the germ line, these genes appear to have molecular and biochemical properties that make them well-suited to breaking symmetry within cells. The resulting asymmetry is often caused by gene products co-localizing asymmetrically to sub-cellular, non-membrane bound, electron dense compartments known as ribonucleoprotein (RNP) granules. RNP granules contain high concentrations of translationally quiescent messenger RNAs and proteins and are thought to act as hubs of localized translational control. We propose that the use of strict translational control, which may be achieved via molecular processes important for RNP granule formation and/or small RNA-related processes, is an important property of and a commonality between the germ line and nervous tissues, and helps explain, at least in part, the close relationship between these two tissue types.

Keywords: genetic toolkit, co-option, modularity, developmental function evolution, germ line, nervous system, RNP granules, pleiotropy

INTRODUCTION

Understanding the genomic basis of extant biological variation over evolutionary time scales has been the main focus of modern evolutionary developmental biology (evo-devo) research. In the pre-genomic era, it was unclear to what extent genes unique to an organism were the basis of their morphological, cellular and biological diversity (King and Wilson, 1975). Over the years, however, a large body of evo-devo work has led to the realization that much of the biological variation in extant animals has evolved based on an ancestral genetic toolkit (Peterson and Davidson, 2000).
Genes in such shared ancestral toolkits are often conserved both in sequence and developmental function across animals (e.g., Hox genes) (Hrycaj and Wellik, 2016). In other cases, conserved genes have been co-opted for additional, distinct biological roles, leading to pleiotropic gene functions (e.g., distal-less, yellow) (Panganiban et al., 1997; Gompel et al., 2005; Moczek and Rose, 2009; Khila et al., 2012). Both scenarios ultimately contribute to morphological diversity between species, within species, and between cell types within an organism, underpinned by a combination of differences in developmental gene regulation and modularity. Pleiotropy is widespread in genomes, can contribute to phenotypic variation, and may occur through a variety of molecular mechanisms (Guillaume and Otto, 2012), including alternative splicing, different substrate or binding partner affinities, localization to different cellular compartments or tissues, or the same gene product having more than one distinct biochemical property. Barring the extreme cases of “housekeeping” genes (usually ubiquitously expressed) and so-called “luxury” genes (expressed in only one tissue type) (King et al., 2013), most animal genes likely exhibit some degree of pleiotropy (Hodgkin, 1998).

Over the past two decades, multiple primary data observations and some synthetic overviews of the literature (see for example Broadus et al., 1998; Roegiers and Jan, 2000) have hinted at a potentially underappreciated example of pleiotropy that we wish to draw further attention to with this review: namely, that a number of genes sometimes dubbed “germ line genes” based on the initial primary characterization of their roles in the germ lines of animals, also have roles in the development and/or functioning of the nervous system. For example, on a genome-wide scale, tissue-specific transcriptome studies in both humans and mice have shown that the testes and nervous system are two tissues that share a larger overlap in their gene expression profiles and proteomes than they do with any other tissue types within the animal (Guo et al., 2005). Here we gather evidence for this dual tissue expression pattern across multiple metazoans. In cases where “germ line genes” are documented as playing a neural role in one species, we ask whether there is evidence that these genes play any neural role in additional species, and whether they share the same set of interactors. We provide possible molecular mechanistic explanations and suggest that these observations may be explained by co-option of pre-existing molecular interactions to new developmental contexts. Both germ cells and neurons use subcellular compartmentalization of gene products as a mechanism for proper cellular functioning. Neurons are highly compartmentalized cells, and localized translational control within and between synapses is an important mechanism regulating neuronal function (Holt et al., 2019). Likewise, germ cells often require subcellular localization of specific gene products for normal functioning of germ cells or patterning of early embryos, which can be achieved by localized translational control (Pushpa et al., 2017). In addition, both these tissues rely on small RNAs for proper functioning. Small RNAs are important for maintaining the genomic integrity of the germ line and also have key roles in memory and synaptic plasticity in the nervous system of animals (Saxe and Lin, 2011; Posner et al., 2019). Thus, we aim to summarize and synthesize data that may be relevant to understanding both the molecular and cellular basis of pleiotropy in this specific context. More generally, this approach may help shed light on the origins of cell type diversity and evolutionary novelty.

**Genes With Shared Roles in the Germ Line and Nervous System**

In the following sections, we present evidence from primary literature for genes best known for their role in the germ line, that are also expressed in the nervous system, either singly or in groups of gene products with conserved molecular interaction. Wherever possible, we present currently available data for the functions of these genes in both these tissues. For each example, we briefly summarize their roles in the germ line and in the nervous system (Table 1), providing a list of reported molecular interaction partners in both tissues (Table 2). In cases where, to our knowledge, no molecular interaction data are available, we point out evidence of their co-expression, acknowledging that co-expression may not reflect conserved molecular interactions. This gene list is not exhaustive, as it is necessarily limited to those that, to our knowledge, have been specifically examined in the context of both tissue types across animals. For each gene discussed, we note whether it predates animals or not, based on OrthoMCL-DB predictions (Chen et al., 2006). Because we aim to point out conserved molecular interactions reported in both cell types, we discuss those genes with more abundant co-IP and other interaction data in both germ line and nervous system first, and end with genes for which data are available primarily for only one tissue type. We discuss genes in groups, to indicate reported conserved molecular interactions between their gene products.

**staufen (stau) and barentsz (btz)**

*staufen (stau)* was first identified in a maternal effect genetic screen for *Drosophila melanogaster* mutants with anterior-posterior body polarity defects, while *barentsz (btz)* was identified in a female sterile chromosome screen for mutants with defects in localization of Stau protein (Schüpbach and Wieschaus, 1986; van Eeden et al., 2001). Stau belongs to a conserved family of animal proteins (Chen et al., 2006) that contain multiple double-stranded RNA binding domains, and stau orthologs are present in bilaterian outgroups, protostomes and deuterostomes (Herdal-Farlow and Kieler, 2014). *btz* genes also appear to be animal-specific (Chen et al., 2006), and Btz protein is a component of the exon junction complex (Ariz et al., 2009), which regulates spliced mRNAs (Bono et al., 2006).

**stau and btz in the germ line**

During *D. melanogaster* oogenesis, Stau and Btz localize to the cytoplasm at the posterior of the oocyte, where they both have a role in primordial germ cell (PGC) specification and establishment of the anterior-posterior axis of the embryo (St Johnston et al., 1991; van Eeden et al., 2001). Stau is required for the posterior localization and translation of the mRNAs of oskar (osk), another gene whose products likely have evolved similar molecular interactions in both germ line and nervous systems [see “oskar (osk), nanos (nos), piwi and vasa (vas)”].
TABLE 1 | Summary of germ line and nervous system roles of genes discussed in the text, listed here in alphabetical order.

| Gene name(s) | Molecular feature(s) and function(s) | Germ line functions | Nervous system functions |
|--------------|--------------------------------------|---------------------|--------------------------|
| barentsz and staufer | Stau: contains double RNA binding domains  
Btz: exon junction complex component | Germ cell specification, axonal patterning | Plasticity and learning, mRNA trafficking, mRNA localization and translation, spine morphogenesis, asymmetric cell division and differentiation of neuroblasts |
| boule and twine | Bc1: RNA-binding protein  
Tw1: putative Cad25-type tyrosine phosphatase | Gametogenesis in males | Axon and dendrite morphogenesis, neuronal development and neuronal communication |
| CPEB | RNA binding protein implicated in mRNA translation and localization via regulation of mRNA poly(A) tail length | Establishment of egg polarity and cytoskeletal network, germ cell development, meiosis entry, oskar and gurken translation and localization | Synaptic plasticity, neurogenesis, learning and memory, asymmetric cell division, RNA trafficking, translational control, regulation of mRNA poly(A) tail length |
| FMRP | RNA binding protein | Germ cell proliferation, maintenance and gamete development | Synaptic plasticity, neurogenesis, dendrite morphogenesis, RNA trafficking, translational control and regulation of mRNA poly(A) tail length |
| oskar | binds RNA (OSK domain), interacts with Vasa (LOTUS domain); predicted disordered region | Germ cell specification, nucleus involved in germ plasm assembly and posterior patterning | Neuroblast divisions in crickets; larval dendrite morphogenesis in D. melanogaster |
| nanos and pumilio | Nos: contains C2H2 Zn-finger domain; RNA-binding protein  
Pum: RNA-binding protein | Posterior embryonic patterning, translation inhibition | Long term memory, dendrite morphogenesis |
| pwi | PAZ-PWI domain family member | Germ line development, gametogenesis, transposon silencing, small RNA biogenesis, in pluripotency, pan-germ line marker | Small RNA biogenesis, transposon silencing, mRNA translational control, long term memory |
| vasa | ATP-dependent DEAD box RNA helicase | Segregation and maintenance of germ line, pan-germ line marker, pluripotency, nuage component involved in modeling RNP complexes, RNA metabolism, small RNA biogenesis, chromosome condensation | In crickets, evidence for roles in neuroblast divisions |

below] mRNA, and Stau and Btz form a complex and move together during this posterior localization event (van Eeden et al., 2001). Additionally, btz null mutants show defects in Stau protein and oskar mRNA localization to the posterior of the oocyte (van Eeden et al., 2001).

**stau and btz in the nervous system**

Evidence from multiple animals suggests that Stau and Btz function together in neuronal cells via mechanisms similar to those observed in the germ line. Stau is concentrated in ribonucleoprotein (RNP) granules within *D. melanogaster* neurites in the larval nervous system, where it co-localizes with Btz and dFMR1 (Barbee et al., 2006). Such Stau-Btz-containing neuronal granules also contain molecules that are found in yeast and mammalian somatic P-bodies (e.g., Dcp1p, Xrn1p), suggesting that neuronal and germ line Stau-containing granules may be similar to somatic P-bodies in molecular composition (Barbee et al., 2006). Stau is also present in the *D. melanogaster* neuromuscular junction (NMJ). At the NMJ, it is localized to the post-synaptic compartment, where it regulates localization and translation of *coracle (cora)* mRNA (Gardiol and St Johnston, 2014). *cora* in turn promotes synaptic bouton formation, and accordingly loss of *stau* leads to a reduction in synaptic bouton number (Macchi et al., 2003). The same Stau domain that is required in oocytes for the translation and localization of the mRNA of *oskar* [see *oskar* (osk), *nanos* (nos), *pumi* and *vasa* (vas) below] (Micklem et al., 2000), called “dsRNA binding domain 5,” is also required for local *cora* translation at the NMJ, and Cora protein fails to localize to the NMJ in *stau* mutants lacking this domain (Gardiol and St Johnston, 2014). Furthermore, Tropomyosin II, which, like Stau, localizes *oskar* to the oocyte posterior (Erdelyi et al., 1995), is also required for *cora*’s NMJ localization (Gardiol and St Johnston, 2014). Stau plays a critical role in asymmetric neuroblast divisions (Jia et al., 2015) and long-term memory formation in *D. melanogaster* (Dubbau et al., 2003), a role that appears conserved in the mollusk *Aplysia californica* (Liu et al., 2006).

In mouse and rat neurons, Stau is contained within RNP particles distributed along the somatodendrites of hippocampal neurons (Tang et al., 2001; Macchi et al., 2003). Btz also co-localizes with Stau in these granular RNPs in hippocampal neurons, and these two proteins co-immunoprecipitate from doubly transfected Baby Hamster Kidney fibroblasts (Macchi et al., 2003). Fritzsche et al. (2013) have recently reported a protein interactome for Stau- and Btz-RNPs in the rat brain, which includes some proteins also found in germ cells, such as Pum [see “nanos (nos) and pumilio (pum)” below] and FMRP [see “Fragile Mental Retardation Protein (FMRP), argonaute (AGO) piwi and staufer (stau)” below]. In mice and rats, Stau is implicated in spatial learning, novelty preference and explorative behavior (Berger et al., 2017; Popper et al., 2018), and in
### TABLE 2

Summary of selected gene products and their reported physical interactors in the germ line and in nervous system tissues, listed here in alphabetical order.

| Gene  | Interactor       | Germ Line       | Nervous System | References                                      |
|-------|------------------|-----------------|----------------|------------------------------------------------|
| Barentsz | eIF4AIII | Y2H, pull-down assay | Co-IP         | Palacios et al., 2004; Fritzsche et al., 2013 |
|         | Mago Nashi      | Co-IS           | Co-IP         | van Eeden et al., 2001; Fritzsche et al., 2013 |
|         | Staufen         | Co-IS           | Co-IP         | van Eeden et al., 2001; Fritzsche et al., 2013 |
|         | Cup             | Co-IS           | Co-IP         | Wilhelm et al., 2003                             |
|         | Oskar           | Co-IS           | Co-IP         | van Eeden et al., 2001; Fritzsche et al., 2013 |
|         | Piwi            | Co-IS           | Co-IP         | Fritzsche et al., 2013                             |
|         | FMRP            | Co-IP           | Co-IP         | Fritzsche et al., 2013                             |
|         | Pumilio         | Co-IS           | Co-IS         | Vessey et al., 2006                               |
|         | Boule           | Co-IP           |                | Xu et al., 2012                                   |
|         | Pumilio         | Co-IS, Y2H, Co-IP |                | Moore et al., 2003                                |
|         | CPEB            | Co-IS, Co-IP, pull down |                | Chang et al., 1999; Rojas-Rios et al., 2015     |
|         | Gurken          | Co-IS           |                | Davidson et al., 2016                             |
|         | Cup             | Co-IS, Co-IP    |                | Wong et al., 2011                                 |
|         | FMRP            | Co-IS, Co-IS    | Co-IS         | Costa et al., 2005                                |
|         | Pumilio         | Co-IS           |                | Eddy, 1975                                        |
|         | Boule           | Co-IS           |                | Knutson et al., 2017                              |
|         | Neuroguidin     | Co-IS           | Co-IS         | Jung et al., 2006                                 |
|         | CalMkII         | Co-IS           |                | Huang et al., 2002                                |
|         | eIF4E           | Co-IP, Y2H      | Co-IS         | Stebbins-Boaz et al., 1999                       |
|         | Cyclin B1       | Co-IS           |                | Meijer et al., 2007                               |
|         | Maskin          | Co-IP, Y2H      | Co-IS         | Stebbins-Boaz et al., 1999; Huang et al., 2003   |
|         | GLD             | Co-IP           |                | Kwak et al., 2008                                 |
|         | Staufen         | Co-IP, Co-IS    |                | Barbee et al., 2006; Fritzsche et al., 2013      |
|         | Pumilio         | Co-IS           |                | Barbee et al., 2006; Vessey et al., 2006         |
|         | Nanos           | Co-IS           |                | Barbee et al., 2006; Megosh et al., 2006         |
|         | Piwi            | Co-IS           |                | Megosh et al., 2006                               |
|         | Argonaute-1     | Co-IS           |                | Yang et al., 2007                                 |
|         | Nanos           | Cup             | Y2H, Co-IP    | Verrotti and Wharton, 2000                        |
|         | Pumilio         | Co-IS           |                | Joly et al., 2013                                 |
|         | Twine           | Co-IS           |                | Joly et al., 2013                                 |
|         | Myosin Light chain | Y2H, Pull-down assay |                | Xu et al., 2010                                   |
|         | Staufen         | Co-IS           | Co-IS         | Barbee et al., 2006                               |
|         | Homer           | Co-IS           |                | Babu et al., 2004                                 |
|         | Staufen         | Y2H             | Co-IS         | Breitwieser et al., 1996                          |
|         | Cup             | Y2H, Co-IP      |                | Ottone et al., 2012                               |
|         | Vasa            | Y2H             | Co-IS         | Breitwieser et al., 1996; Ewen-Campen et al., 2012; Jeske et al., 2015, 2017 |
|         | Lasp            | Y2H, Pull-down assay |                | Suyama et al., 2009                               |
|         | Par-1           | in vitro Kinase assay |                | Morais-de-Sa et al., 2013                         |
|         | Piwi            | Co-IS           | Co-IS         | Ewen-Campen et al., 2012                          |
|         | Vasa            | Co-IS           | Co-IS         | Megosh et al., 2006; Ewen-Campen et al., 2012     |
|         | FMRP            | Co-IS           |                | Megosh et al., 2006                               |
|         | Kumo            | Co-IS           |                | Anand and Kai, 2012                               |
|         | Veteno          | Co-IS           |                | Handler et al., 2011                              |
|         | Papi            | Y2H, Co-IP      |                | Liu et al., 2011                                  |
|         | Pumilio         | Nanos           | Co-IS         | Megosh et al., 2006                               |
|         | Twin            | Co-IS           |                | Megosh et al., 2006                               |
|         | CPEB            | Co-IS           |                | Joly et al., 2013                                 |
|         | DAZL            | Co-IS           |                | Joly et al., 2013                                 |
|         | Maskin          | Co-IS           |                | Ota et al., 2011                                  |
|         | Staufen         | Co-IS           |                | Ota et al., 2011                                  |
|         | FMRP            | Co-IS           |                | Ota et al., 2011                                  |
|         | MAPK            | Y2H, Co-IS      |                | Schuld et al., 1998                               |
|         | Barentsz        | Co-IS           | Co-IP, Co-IP   | van Eeden et al., 2001; Macchi et al., 2003      |
|         | MAPK            | Co-IP           |                | Nam et al., 2008                                  |
|         | Oskar           | Y2H, Co-IS      |                | St Johnston et al., 1991; Breitwieser et al., 1996 |
|         | Inscurteable    | Y2H             |                | Li et al., 1997                                   |

(Continued)
humans. Stau is required for normal dendritic arborization during neuroblastoma cell differentiation in vitro (Peredo et al., 2014). Interestingly, when expressed in vivo in D. melanogaster, GFP-tagged mouse Btz localizes to the oocyte posterior, suggesting that it can interact with D. melanogaster Stau (Macchi et al., 2003). However, despite this colocalization with D. melanogaster Stau, mouse Btz is unable to perform the function of D. melanogaster Btz in localizing osk mRNA to the posterior of the oocyte cytoplasm (Macchi et al., 2003), suggesting that not all Btz/Stau functional molecular interactions are conserved across species.

**Stau and Btz: additional relevant expression data**

*Stau* is expressed or required in the germ line outside of fruit flies as well. In zebrafish, morpholino-mediated knockdowns of the stau paralogs *stau1/2* abrogate the formation of Vasa-positive PGCs (Ramasamy et al., 2006). In mice, *stau* mRNA is expressed in oocytes and during meiosis in males (Saunders et al., 2000). In human oocytes, immunofluorescence studies show that STAU protein is present throughout all stages of oocyte maturation, and that its subcellular localization changes throughout oogenesis, initially dispersed throughout the cytoplasm and later localized into large discrete granules at the cortex (De Santis et al., 2015). As in *D. melanogaster* oocytes (St Johnston et al., 1991; van Eeden et al., 2001), Stau localization to a specific region of the *Xenopus laevis* oocyte cytoplasm is required to specify PGCs (Yoon and Mowry, 2004). Human Stau1/2 (Stau1/2) and Barentsz (CASC3) are both expressed in multiple tissues outside of the germ line and nervous system (Uhlen et al., 2015).

**Fragile Mental Retardation Proteins (FMRP), argonaute (AGO), piwi and stau1 (stau)**

Fragile Mental Retardation Proteins (FMRP) are conserved RNA binding proteins that may have origins predating animals, based on the prediction of a putative ortholog in the green alga *Micromonas* (Chen et al., 2006). FMRPs underlie human Fragile X syndrome, which is an X-linked dominant disorder causing mental retardation and cognitive impairment (Ashley et al., 1993; Inoue et al., 2000). This defect is caused by an expansion of a CGG trinucleotide repeat in the FMR1 gene, correlated with transcriptional silencing and loss of the gene product FMRP (Verkerk et al., 1991; Verheij et al., 1993). Mammalian FMRP is a member of a small protein family consisting of members FMRP, FXR1 and FXR2, all of which are RNA binding proteins containing two K homology (KH) domains and one RGG box (Siomi et al., 1995; Zhang et al., 1995). FMRP/FXR proteins also contain protein-protein interaction and 60S ribosomal subunit interaction domains (Ashley et al., 1993; Siomi et al., 1996; Wan et al., 2000). FMRP is predominantly detected in the cytoplasm of cells in multiple human tissues (Uhlen et al., 2015), including neurons, glial cells, and spermatogonia, but can also be detected in the nucleus (Devys et al., 1993; Verheij et al., 1993). The presence of nuclear localization (NLS) and export (NES) signals (Eberhart D. E. et al., 1996), suggest that it may function as a nucleo-cytoplasmic shuttle protein for RNA. In *in vitro* experiments suggest that FMRP binds a selective but abundant fraction of brain RNA, but little is currently known about the identity of these targets (Ashley et al., 1993; Brown et al., 1998). FMRP associates with polyribosomes (Khandjian et al., 1996; Tamanini et al., 1996; Feng et al., 1997) and negatively regulates translation (Laggerbauer et al., 2001; Li et al., 2001; Zhang et al., 2001). While all three orthologs of the FMRP/FXR family are found in multiple vertebrates, only one homolog, called *dfmr1*, has been reported in *D. melanogaster* (Wan et al., 2000).

**FMRP, AGO and piwi in the germ line**

FMRP plays roles in germ line development in *D. melanogaster* and mammals, in both cases via interactions with Piwi or Piwi-related proteins of the Argonaute family (AGO). In *D. melanogaster* Dfmr1 protein co-immunoprecipitates with Ago1 in ovaries and in adult testes (Yang et al., 2007; Bozzetti et al., 2015). Similarly, in embryos Dfmr1 forms a complex with Piwi during the formation of the specialized cytoplasm, called germ plasm, that ensures PGC specification in *D. melanogaster*.
(Megosh et al., 2006). In dfmr1 homozygous null mutants, the ovaries contain fewer germ line stem cells (GSC) than controls, suggesting that dfmr1 is required for GSC maintenance (Yang et al., 2007). dfmr1 and piwi mutants show similar phenotypes of defective pole plasm and reduced PGC number (Megosh et al., 2006). In mice, FMR1 knockout mice display macroorchidism, a disorder in which males have abnormally large testes, in this case caused by an increased postnatal proliferation of Sertoli cells (Slegtenhorst-Eegdeman et al., 1998), which are associated with and required for correct development of male gametes. In mouse testes and in human embryonal carcinoma cell lines derived from testes, FMR1 and AGO1 regulate miRNA-383, implicating FMRP in small RNA-mediated gene regulation in the mammalian germ line (Tian et al., 2013).

**FMRP, AGO, piwi and stau in the nervous system**

FMRP is implicated in multiple neuronal functions in fruit flies and mice, including synaptic plasticity (Padmashri et al., 2013; Feuge et al., 2019), dendritic morphogenesis (Feuge et al., 2019), and olfactory learning and memory (Nimchinsky et al., 2001; Bolduc et al., 2008; Sears et al., 2019). Some studies report that homozygous FMR1 knockout mice display defects in dendritic spine morphology (e.g., Nimchinsky et al., 2001; Bolduc et al., 2008) [but see Feuge et al. (2019) for a report of no abnormal dendritic spine morphology in FMRP knockout mice]. FMRP also appears important for adult mouse neurogenesis: FMR1 knockout mice show misregulation of multiple genes expressed in adult neural progenitor cells (Liu et al., 2018), increased neural progenitor cell proliferation and incorrect neuronal fate specification (Luo et al., 2010), significant reduction in hippocampal neurogenesis (Guo et al., 2011), and reduced hippocampal-dependent learning (Guo et al., 2011). Furthermore, in D. melanogaster, Dfmr1 and Ago1 are required for the regulation of synaptic plasticity (McBride et al., 2005; Bolduc et al., 2008; Sudhakaran et al., 2014). Dfmr1 loss of function mutants show ectopic axon growth (Tessler and Broadie, 2008), and trans-heterozygotes for dfmr1 and Ago1 have overgrown synapses and abnormally elaborate synaptic terminals compared to wild type flies and single heterozygotes (Jin et al., 2004). This phenotype is reminiscent of that of homozygous FMR1 knockout mice, which some researchers report have dendritic spines that are longer than controls (Comery et al., 1997; Nimchinsky et al., 2001) [but see Feuge et al. (2019)]. The molecular functions of FMRP in neurons include trafficking RNA in both fruit fly (Estes et al., 2008) and mouse (Antar et al., 2005; Dictenberg et al., 2008) neurons, regulating length of the mRNA poly(A) tail (Bienkowski et al., 2017), and local translational regulation in both dendrites and cell bodies of neurons (Darnell et al., 2011; Darnell and Klann, 2013). FMRP also co-immunoprecipitates with Stau in rat neurons (Price et al., 2006), and complexes with Stau in transfected human cells and differentiated human neuroblasts (Villacé et al., 2004). An ortholog of FMRP has been identified in the cnidarian Hydractinia echinata (HyFMR1), where it is expressed in neural precursors and nerve cells in the mature polyp (Guduric-Fuchs et al., 2004).

**nos (nos) and pumilio (pum)**

Nos (nos) and pumilio (pum) were first identified in genetic screens for D. melanogaster embryos with posterior and abdominal specification defects (Lehmann and Nüsslein-Volhard, 1987; Nüsslein-Volhard et al., 1987; Lehmann and Nüsslein-Volhard, 1991). Pum belongs to a conserved RNA-binding protein family that is found across eukaryotes (Zamore et al., 1999; Zhang et al., 1997; Gamberi et al., 2002; Chen et al., 2006). Its signature PUF domain is named after D. melanogaster Pumilio and the Caenorhabditis elegans translational regulator EBF (fem-3-binding factor) (Zhang et al., 1997). PUF proteins are implicated in post-transcriptional gene regulation (Dubnau et al., 2003), including in the germ line and nervous system, as detailed below.

**nos and pum in the germ line**

In D. melanogaster, nos and pum act together as inhibitors to repress hunchback and bicoid translation in the posterior of the embryo (Wharton and Struhl, 1991; Zamore et al., 1999). Pum is thought to directly bind hunchback and bicoid mRNAs, and to bring Nos to the repression complex (Murata and Wharton, 1995; Sonoda and Wharton, 1999). Nos and Pum are required in the germ line for continued egg chamber production during oogenesis, by regulating the germ line stem cell to cystoblast fate transition via translational repression of oocyte differentiation genes (Whang et al., 1994; Lin and Spradling, 1997; Forbes and Lehmann, 1998; Crittenden et al., 2002; Ariz et al., 2009), and learning and memory (Dubnau et al., 2003). Nos is an animal-specific gene (Chen et al., 2006) maternally required for the development and maintenance of the D. melanogaster germ line, and zygotically for embryonic patterning and PGC migration in the developing embryo (Wang and Lehmann, 1991; Wang et al., 1994; Kobayashi et al., 1996). Pum proteins often function together with Nos proteins during development (Sonoda and Wharton, 1999; Parisi and Lin, 2000; Jaruzelska et al., 2003), including in the germ line and nervous system, as detailed below.
these proteins also bind to and repress translation of cyclin B1 (Kadyrova et al., 2007). In zebrafish, Pum2 is expressed in male and female gonads, and is important for germ cell and nervous tissue development (Wang et al., 2012). Furthermore, a zebrafish homolog of nos is involved in PGC maintenance and migration into the future gonad (Koprunner et al., 2001).

nos and pum in the nervous system

nos and pum also play roles in the development and function of the nervous system of multiple taxa. For example, in D. melanogaster, Nos localizes with RNA granules in dendrites, and both nos and pum are needed for appropriate dendrite morphogenesis, suggesting that they may repress mRNA translation in the nervous system as they do in the germ line (Ye et al., 2004). In larval class IV neurons, nos mRNA requires osk for appropriate localization, as described below (Xu et al., 2013). In addition, long-term memory in D. melanogaster requires pum (Dubnau et al., 2003; Chen et al., 2008).

In mice, Pum2 is localized with RNP particles in the somatodendritic region of hippocampal neurons (Vessey et al., 2006), and Pum1 and Pum2 are required for hippocampal neurogenesis and proper functioning (Zhang et al., 2017). Furthermore, mouse Pum2 is implicated in forming stress granules under metabolic stress in neurons, in dendritic morphogenesis, and in regulating the synaptic function along dendritic shafts (Vessey et al., 2006, 2010). Interestingly, nos1 knockdown mice show no detectable neural defects in terms of behavior or fertility (Haraguchi et al., 2003). In the C. elegans genome, there are three nos-related genes and at least ten PUF-domain proteins (Lynch et al., 2011), and PUF-domain proteins have been shown to play memory-related important roles in axonal and presynaptic regions (Lee and Schedl, 2006; Arey et al., 2019). One of these Pum-like proteins, FBF-1, is needed for the change in C. elegans odor sensitivity that comes with prolonged exposure, known as odor adaptation (Kaye et al., 2009). Pum also binds to the 3′UTR of the cGMP-dependent kinase EGL-4 and promotes its translation (Kaye et al., 2009). Of the three nos-related genes, NOS-1 is required for odor adaptation (Kaye et al., 2009).

nos and pum: additional relevant expression data

Outside of bilaterians, there is also evidence for expression and function of nos and pum orthologs in the germ line. In the sexual polyp of the hydroid H. echinata, a pum ortholog and the nos ortholog nos2 are both expressed in oocytes (Kanska and Frank, 2013), as are nos orthologs in the jellyfish Podocoryne carnea (Torras et al., 2004) and Clytia hemisphaerica (Leclère et al., 2012). In H. magnipapillata, nos orthologs Cnmos1 and Cnmos2, are both expressed in the germ line (Mochizuki et al., 2000). In the anhozoan Nematostella vectensis, the nos ortholog Nvmos2 is expressed in putative germ cells during embryogenesis and in developing oocytes (Extevar et al., 2005; Torras and Gonzalez-Crespo, 2005). nos orthologs are also expressed in developing gametes in the sponges Sycon ciliatum (Leininger et al., 2014) and Oscarella lobularis (Fierro-Constatin et al., 2017). In zebrafish, Pum2 is expressed in the brain (Wang et al., 2012). In H. echinata, reduction of Nos2 causes a reduction in nematogenesis (production of stinging cells called nematocytes, considered a type of neural cell) and an increase in neurogenesis (Kanska and Frank, 2013). In sponges, while putative neural tissues remain difficult to identify based on bilaterian-centric cell type criteria (Dunn et al., 2015), expression of nos has been reported in globular cells and cross cells, two candidate sensory cell types unique to sponges (Mah and Leys, 2017).

oskar (osk), nanos (nos), piwi and vasa

The insect-specific gene oskar (osk) was first identified in the fruit fly D. melanogaster as a maternal-effect gene that is necessary and sufficient for specifying both the germ line and the posterior abdomen of the embryo (Lehmann and Nüsslein-Volhard, 1986; Ephrussi et al., 1991; Chen et al., 2006). Osk proteins have two conserved, well-folded domains on either side of a region of predicted high disorder (Jeske et al., 2015; Yang et al., 2015). The N terminal domain is a LOTUS domain (also called an OST-HTH domain) (Anantharaman et al., 2010) similar to that of TUDOR5 and TUDOR7 proteins (Ewen-Campen et al., 2012), and is predicted to dimerize (Jeske et al., 2015; Yang et al., 2015) and bind Vasa protein (Markussen et al., 1995; Breitwieser et al., 1996; Vanzo and Ephrussi, 2002; Jeske et al., 2017). The C terminal domain is known as the OSK domain and is implicated in binding nanos (see below), oskar, germ cell less and polar granule component mRNAs (Jeske et al., 2015; Yang et al., 2015).

osk, nos, piwi and vasa in the germ line

In D. melanogaster, osk is expressed from the maternal genome during oogenesis, and osk mRNA is deposited into the developing oocyte in a process dependent on Splicing oskar Location Elements (SOLE) in its 3′UTR (Ghosh et al., 2012). SOLE recruitment of Exon Junction Complex components, including barentzi, mago nashi, and tsunagi, is required for proper osk ribonucleoprotein (RNP) granule motility into the oocyte, and for posterior localization of osk within the oocyte (Ghosh et al., 2012). Posterior localization of osk also requires interactions with Staufen (St Johnston et al., 1991; see below) and Kinesin proteins (Brendza et al., 2002). Posteriorly localized osk mRNA is translated into two protein isoforms, Short Osk and Long Osk (Markussen et al., 1995). Short and Long Osk differ by an N terminal 138 amino acid (aa) addition (Markussen et al., 1995). The current model of the distinct functions of these isoforms is as follows: Long Osk localizes to endocytic membranes at the oocyte posterior (Vanzo et al., 2007, 2010; Tanaka and Nakamura, 2008), anchors both osk mRNA and Short Osk (Vanzo and Ephrussi, 2002; Tanaka et al., 2011), and stabilizes mitochondrial accumulation (Hurd et al., 2016). Short Osk localizes to electron-dense organelles called polar granules and recruits products of genes required for germ cell and posterior identity specification including vasa, nanos, and piwi (see below) (Markussen et al., 1995; Breitwieser et al., 1996; Vanzo et al., 2007). Although osk likely evolved in a last common insect ancestor (Lynch et al., 2011; Ewen-Campen et al., 2012; Blondel et al., 2020), the Long
Osk domain and isoform appear to have evolved only within the Diptera (Blondel et al., 2020).

**osk, nos, piwi and vasa in the nervous system**

Evidence for a role for osk in the nervous system comes from studies of two insects, *D. melanogaster* and the cricket *Gryllus bimaculatus*. In the cricket, *Gb-osk* mRNA and protein are enriched in neuroblasts in the embryonic nervous system (Ewen-Campen et al., 2012) and in the adult brain (Ewen-Campen and Extavour, unpublished). First identified in a grasshopper (Wheeler, 1891), neuroblasts are neural stem cells found in all pancrustaceans (insects and crustaceans) (Lear, 2001; Richter et al., 2010). Neuroblasts arise from the ventral ectoderm during embryogenesis and divide asymmetrically to produce all of the neurons of the nervous system. *Gb-osk* RNAs in cricket embryos results in broken or reduced lateral axon tracts, a phenotype that is consistent with neuronal division defects (Ewen-Campen et al., 2012). Neuroblasts of *G. bimaculatus* also express Vasa and Piwi proteins (Ewen-Campen et al., 2012), raising the possibility that Osk may interact with these proteins in neuroblasts, as it does in the germ line in other contexts (see section on Vasa below). In *D. melanogaster*, osk co-localizes with *nanos (nos)* mRNA in larval class IV neurons, and is required for correct localization of *nos* mRNA within these neurons (Xu et al., 2013).

**osk, nos, piwi, and vasa: additional relevant expression data**

Gene expression data suggest that osk also specifies germ cells in the ant *Messor pergandei*, and osk knockdown experiments in the wasp *Nasonia vitripennis* show that the germ cell and posterior identity specification roles of osk are conserved in this insect as well (Lynch et al., 2011). However, osk is not required for germ line establishment, maintenance or function in the cricket *G. bimaculatus* (Orthoptera) (Ewen-Campen et al., 2012).

**piwi, argonaute (Ago), aubergine (aub) and small RNAs**

PIWI proteins are evolutionarily conserved RNA binding proteins (e.g., Bohmert et al., 1998; Moussian et al., 1998; reviewed in Thomson and Lin, 2009; Ku and Lin, 2014) found across metazoan and plant genomes (Chen et al., 2006). The founder ortholog of this group was first identified in a *D. melanogaster* screen for genes that abolish asymmetrical divisions in germ line stem cells (GSCs) (Lin and Spradling, 1997), and named after the male sterility phenotype caused by loss of function mutations (PIWI: P-element induced wimpy testis). The PIWI clade of proteins belongs to the Argonaute/PIWI protein family (AGO/PIWI, also known as the PAZ-PIWI domain or PPD family of proteins) (Thomson and Lin, 2009; Ku and Lin, 2014).

**piwi, AGO, aub and small RNAs in the germ line**

PIWI proteins are expressed in germ cells or their progenitors in many animals, and their functions in the germ line have been extensively studied in a wide range of animals (Juliano et al., 2011). PIWI germ line functions include germ line determination, germ line stem cell (GSC maintenance), spermiogenesis, and silencing transposon expression in the germ line genome both at the epigenetic and post-transcriptional levels (Thomson and Lin, 2009; Ku and Lin, 2014). The latter role is performed via interaction with small RNAs, including but not limited to PIWI-associated small RNAs (piRNAs) (Iwasaki et al., 2015; Furrer et al., 2017; Rojas-Ríos and Simonelig, 2018). Like *vasa*, *piwi* is also expressed in multiple somatic stem cell types outside of bilaterians.

**piwi, AGO, aub and small RNAs in the nervous system**

PIWI-related proteins play critical functions in the soma as well as the germ line (Ross et al., 2014). This includes roles in the central nervous system of all major groups of animals, including deuterostomes, protostomes, and bilaterian outgroups (Juliano et al., 2011), as illustrated by the following examples: In the sea slug *A. californica*, Piwi protein interacts with a DNA methyltransferase to control the expression of CREB2, a long-term memory repressor, during long-term memory formation (Rajasekharapathy et al., 2012). The zebrafish *piwi* ortholog *ziwi* is expressed in the eye, the forebrain, and the midbrain during organogenesis (Tan et al., 2002). In the nematode *C. elegans*, the PIWI protein PRG-1 represses axonal regeneration in adult mechanosensory neurons (Kim et al., 2018). Mouse *piwi* orthologs (miwi genes) are expressed in the adult brain (Leighton et al., 2019), and *miwi* colocalizes with piRNAs to form RNP puncta in the dendrites of cultured hippocampal neurons (Lee et al., 2011). LNA-based antisense inhibition of one of these piRNAs results in a significant decrease in dendrite spine area (Lee et al., 2011). Further, knockdown of *piwi*-like genes in the mouse hippocampus affects adult behavior, as assayed in an experimental fear-conditioning paradigm (Leighton et al., 2019). In *D. melanogaster*, PIWI-related proteins Argonaute (Ago3) and Aubergine (Aub) are expressed at different levels in distinct subsets of neurons in the mushroom body (Perrat et al., 2013), the substrate for learning and memory within the insect brain (Heisenberg, 2003). Lower expression levels of Ago3 and Aub correlate with increased expression of transposable elements in the adult fly brain (Perrat et al., 2013), consistent with the proposed role of Piwi-related proteins in suppressing transposable element mobility (Thomson and Lin, 2009; Ku and Lin, 2014). This heterogeneity of Aub and Ago expression levels is speculated to contribute to behavioral variability (Perrat et al., 2013).

**piwi, AGO, aub and small RNAs: additional relevant expression data**

Expression of *piwi* orthologs during gametogenesis has been documented in multiple cnidarians (Seipel et al., 2004; Leclère et al., 2012; Plickert et al., 2012). The homoscleromorph sponge *O. lobularis* expresses a *piwi* ortholog in germ cells during spermatogenesis and oogenesis (Fierro-Constat et al., 2017).
In another sponge, the demosponge <i>Ephydatia fluviatilis</i>, a piwi homolog is expressed in choanocytes and archeocytes (Funayama, 2010; Funayama et al., 2010; Alié et al., 2015), which is relevant to the sponge germ line because gametogenous cells are thought to be derived from one or both of these cell types in these animals (Funayama, 2010). In the ctenophore <i>Pleurobrachia pileus</i>, piwi is expressed in the adult male and female germ line (Alié et al., 2010). Neural cell type expression of piwi orthologs is also present in non-bilaterians. In the cnidarian <i>Clytia hemisphaerica</i>, piwi is expressed in nematogenic and neural stem cells (Denker et al., 2008), and in the ctenophore <i>P. pileus</i>, piwi is expressed in the apical organ, which is an aboral sensory organ (Alié et al., 2010).

**vasa and piwi**

<i>vasa</i> encodes a highly conserved DEAD box-containing ATP-dependent RNA helicase (Hay et al., 1988; Lasko and Ashburner, 1988) that is expressed in the germ line of every animal studied to date (Ewen-Campen et al., 2010; Gustafson and Wessel, 2010; Yajima and Wessel, 2011b). DEAD box helicases predate animals (Chen et al., 2006) and are implicated in a broad range of biological functions including transcription, translation, splicing, ribosome biogenesis, nuclear export, and mRNA degradation (Linder, 2006; Lasko, 2013). <i>vasa</i> expression is also a hallmark of many types of stem cells, where it is proposed to interact with the products of the <i>piwi</i>, <i>broun</i>, and <i>PL10</i> genes in a conserved gene network to help maintain pluripotency (Alié et al., 2010; Juliano et al., 2010; Fierro-Constain et al., 2017).

**vasa and piwi in the germ line**

First discovered for its role in abdomen formation during embryonic development in <i>D. melanogaster</i> (Schüpbach and Wieschaus, 1986), <i>vasa</i> encodes a protein found in the cytoplasm of animal germ cells and required for one or both of germ cell specification and germ line development in multiple animals (reviewed in Yajima and Wessel, 2011b). Vasa protein is a component of germ line RNP granules, and has predicted roles in regulating mRNA translation, including that of <i>nanos</i> (Gavis et al., 1996; see below) and <i>gurken</i> (Tomancak et al., 1998), potentially by interacting with initiation factor dIF2 (Carrera et al., 2000). During the cell cycle, <i>vasa</i> may be regulated by the meiotic checkpoint pathway (Ghabrial and Schupbach, 1999), can associate with the spindle (Carré et al., 2002; Oyama and Shimizu, 2007), and is implicated in regulation of mitotic chromosome condensation (Pek and Kai, 2011; Yajima and Wessel, 2011a,b; Schwager et al., 2015). Vasa protein interacts physically with Piwi protein in the germ line of mice (Kirino et al., 2010) and <i>D. melanogaster</i> (Mgeois et al., 2006), and in cultured ovarian cells of the silkworm <i>Bombyx mori</i> (Xiol et al., 2014). Vasa, like Piwi, is involved in the small RNA biogenesis pathway in many animals (Vagin et al., 2004; Shirayama et al., 2014; Xiol et al., 2014; Dehghani and Lasko, 2016; Spracklin et al., 2017). Vasa and Osk proteins also physically interact in the germ line, where the LOTUS domain of Osk binds Vasa and facilitates its helicase activity (Jeske et al., 2015; Yang et al., 2015; Jeske et al., 2017).

**vasa and piwi in the nervous system**

To our knowledge, the only reported examples of a role for <i>vasa</i> in the nervous system come from (1) the cricket <i>G. bimaculatus</i>, where it is found co-expressed along with piwi and osk in neuroblasts (Ewen-Campen et al., 2012), and (2) cells of the apical sensory organ in the ctenophore <i>Pleurobrachia pileus</i> (Alié et al., 2010). Its function in these invertebrate nervous systems remains to be elucidated.

**vasa and piwi: additional relevant expression data**

We note that in multiple animals, <i>vasa</i> expression is also a hallmark of pluripotent and somatic stem cell lineages, which can give rise to both germ line and neural cells. These include the archaeocytes of the sponge <i>E. fluviatilis</i> (Alié et al., 2015), the interstitial cells of the cnidarians <i>H. magnipapillata</i> and <i>H. echinata</i> (Mochizuki et al., 2001; Rebscher et al., 2008), the presumptive founder cells of the larval posterior growth zone of the annelid <i>Platyneuris dumerilii</i> (Rebscher et al., 2007), the stem cells of the colonial tunicate <i>Botryllus schlosseri</i> (Sunanaga et al., 2006; Rosner et al., 2009; Kawamura and Sunanaga, 2011), and the neuroblasts of the platyhelminths <i>Macrostomum lignano</i> (Pfister et al., 2008), <i>Dugesia japonica</i> (Shibata et al., 1999), <i>Schmidtea mediterranea</i> (Wagner et al., 2012), and <i>Schistosoma mansoni</i> (Wang et al., 2013).

**boule (bol) and twine (twe)**

<i>boule</i> (bol) is a member of the Deleted in Azoosperma (DAZ) RNA-binding protein family, which contains the autosomal <i>dazl</i> and <i>bol</i> genes, and the human Y-linked <i>DAZ</i> gene (Shah et al., 2010). Although not reported in plant or fungal genomes to date, <i>bol</i> may predate animals based on identification of a putative ortholog in the slime mold <i>Dicytostelium discoideum</i> (Chen et al., 2006). In the bony fish lineage, a <i>bol</i> duplication likely gave rise to the <i>Daz-like</i> gene (<i>Dazl</i>), which then underwent a transposition to the Y chromosome in primates to give rise to <i>DAZ</i> (Shah et al., 2010). <i>DAZ</i> family members display predominant male germ line expression patterns, and <i>DAZ</i> family genes are crucial for germ cell development and meiotic progression across animals (summarized in Kim and Rhee, 2016).

**bol and twe in the germ line**

<i>bol</i> was first identified in a mutagenesis screen for <i>D. melanogaster</i> male-sterile mutants (Castrillon et al., 1993). <i>twe</i> was identified by multiple independent studies (Jimenez et al., 1990; Alphay et al., 1992; Courrot et al., 1992) that searched for orthologous or functionally analogous genes to the <i>cde25</i> phosphatase that regulates mitotic entry in <i>Schizosaccharomyces pombe</i> (Russell and Nurse, 1986). <i>bol</i> mutants fail to undergo male meiosis, but homozygous female <i>bol</i> mutants are fertile (Eberhart C. G. et al., 1996). <i>bol</i> controls the translation of <i>twe</i>,...
allowing meiotic entry in males (Courtot et al., 1992; Maines and Wasserman, 1999). The *D. melanogaster* meiotic entry defect can be rescued by the *X. laevis* bol ortholog *Xdazl* (Houston et al., 1998), and human and mouse DAZ can also partially rescue *D. melanogaster* bol loss of function (Houston et al., 1998; Xu et al., 2003). Orthologs of *bol* and *twe* also play a role in sperm maturation in haploid males in the sawfly *Athalia rosae* (Hymenoptera), which normally abort meiosis I but maintain meiosis II to produce haploid sperm (Sekine et al., 2015). As in *D. melanogaster*, *bol* knockdowns in *A. rosae* show no apparent defects in females (Sekine et al., 2015). Bol is also expressed in the testis in male mammals. In mice and humans, Bol protein is present in the cytoplasm of developing spermatoocytes and can be detected through meiosis (Xu et al., 2001). Loss of *dazl* function in mice leads to defects in gametogenesis in both sexes (Ruggiu et al., 1997). As in *D. melanogaster*, *bol* homozygous mutant male mice are infertile, but females are viable and fertile (Shah et al., 2010). Bol also co-localizes to RNPs that form under stress (called stress granules) in mouse male germ cells (Kim and Rhee, 2016). In *X. laevis*, knockdown of the maternally expressed ortholog *Xdazl* reduces the number of PGCs and perturbs PGC migration during embryogenesis (Houston and King, 2000). In contrast to the fly, mouse, human and frog *bol* genes, the *C. elegans* *bol* ortholog *daz-1* plays a role in oocyte determination rather than in spermatogenesis (Karashima et al., 2000). In wild type hermaphroditic worms, germ cells undergo two developmental decisions, the first from mitotic proliferation to meiosis in the L4 larval stage, and the second from sperm to oocyte production in young adults (Karashima et al., 2000). RNAi against *daz-1* in *C. briggsae* leads to continuous sperm production, indicating a disruption in the spermatogenesis/oogenesis switch (Karashima et al., 2000).

**bol and twe in the nervous system**

While *bol* expression is not detected in the human brain (Uhlen et al., 2015), *bol* and *twe* also function in the nervous system in adult *D. melanogaster* (Joiner and Wu, 2004), where an isoform of *bol* that is not found in the testes is expressed in the cytoplasm and extending neurites of most cells throughout the adult brain (Joiner and Wu, 2004). Bol negatively regulates developmental axon pruning in *D. melanogaster* (Hoopfer et al., 2008). Overexpression of *bol* throughout the nervous system leads to defects in neuronal communication between the retina and the lamina, abnormal locomotory behavior in wandering larvae, and lethality before the third larval stage (Joiner and Wu, 2004). The neuronal *bol* isoform interacts genetically with *twe* in the nervous system, just as *bol* does in the germ line (Joiner and Wu, 2004).

**CPEB, Maskin, and eIF4E**

Cyttoplasmic polyadenylation element binding protein (CPEB) is a member of an animal protein family implicated in binding the 3′UTRs of mRNAs at cytoplasmic polyadenylation element (CPE) sites, and in controlling their translation and cytoplasmic localization via regulation of their poly(A) tail lengths (Hake and Richter, 1994; Wells et al., 2000). Some animals have multiple paralogs of CPEB genes in their genomes: *D. melanogaster* has two CPEB genes, whereas *X. laevis*, mice, humans and *C. elegans* have four (Chen et al., 2006). The C-terminal half of the CPEB protein contains RNA binding domains (RBDs), including two RNA-recognition motifs (RRM domains) and a zinc finger domain (ZZ domain), which are used to establish CPEB gene relationships (Hake et al., 1998; Mendez and Richter, 2001; Fernandez-Miranda and Mendez, 2012). Pairwise sequence alignments of the RBDs of different CPEB genes show that CPEB genes form two subgroups (Hake et al., 1998; Mendez and Richter, 2001; Fernandez-Miranda and Mendez, 2012). One subgroup, which includes the *D. melanogaster* oo18 RNA binding protein (orb) (Christerson and McKearin, 1994; Lantz et al., 1994), mouse CPEB1 (Taylor and Richter, 2001) and *X. laevis* CPEB1 (Hake and Richter, 1994), are expressed and required in the germ line for initiation of translation of CPE-containing mRNAs. CPEB genes in the second group are more broadly expressed in several somatic tissues, including the germ system, in addition to the germ line. Their examples include *D. melanogaster* orb2 (Halier et al., 2011), mouse CPEB2-4 (Kurihara et al., 2003; Theis et al., 2003), and human CPEB3 and CPEB4 (Kikuno et al., 2004). Given that CPEB genes control mRNA expression across tissues, developmental stages and species, some have speculated that they do so via a mechanism of local translational control that is evolutionarily conserved, involving the cytoskeleton, eukaryotic initiation factor (eIF4E) and the eIF4E binding protein Maskin (Stepien et al., 2016).

**CPEB, Maskin and eIF4E in the germ line**

*D. melanogaster* orb was the first identified member of the CPEB family of translational regulators and is required to establish polarity in developing eggs and early embryos (Lantz et al., 1992, 1994; Christerson and McKearin, 1994). *orb* controls translation and polyadenylation of mRNAs including *oskar* and *gurken* (Chang et al., 1999, 2001; Tan et al., 2001; Castagnetti and Ephrussi, 2003; Norvell et al., 2015; Davidson et al., 2016), and organizes and repolarizes the microtubule cytoskeleton during *D. melanogaster* oogenesis by interacting with Actin, Dynein and Kinesin (Barr et al., 2019a,b). In *C. elegans*, CPEB homologs (called CPB-1,2,3 and FOG-1) are required for the switch from spermatogenesis to oogenesis, control germ cell fate by regulating the translation of specific mRNAs (Luitjens et al., 2000; Jin et al., 2001). CPEB interactions are also well studied in *X. laevis* oocytes, where CPEB homologs are required for normal oocyte maturation, and also regulate the cell cycle in early embryos (Stebbins-Boaz et al., 1996; Groisman et al., 2000; Groisman et al., 2002; Igea and Mendez, 2010). Co-immunoprecipitation, protein pull downs and yeast two-hybrid assays have shown that in *X. laevis*, CPEB1 directly binds both the eukaryotic translation initiation factor eIF4E, and the eIF4E binding protein Maskin (Stebbins-Boaz et al., 1999). Indeed, *D. melanogaster* Orb from ovary
extracts has also been shown to immuno precipitate with eIF4E (Wong et al., 2011).

CPEB, Maskin and eIF4E in the nervous system

*D. melanogaster* orb2 is expressed in several somatic tissues, including the nervous system at all stages of development (Hafer et al., 2011). orb2 mRNA and protein expression are detectable in the central and peripheral embryonic nervous systems (Hafer et al., 2011). In the central nervous system of embryos and larvae, Orb2 protein expression is largely limited to cell bodies, and functions in asymmetrical cell division (Hafer et al., 2011). In adult neurons, orb2 is localized at the synaptic terminals, and is required for learning and memory (Keleman et al., 2007; Kruttner et al., 2012; Majumdar et al., 2012). In the sea slug *A. californica*, CPEB forms prion-like multimers in neurons. *D. melanogaster* orb2 injected into *A. californica* neurons also forms such aggregates (Si et al., 2003, 2010), suggesting that these aggregates may be relevant to learning and memory in these animals, as they may contribute to synapse-specific differences (Fiumara et al., 2015). In sensory neurons, ApCPEB co-localizes in RNA granules that also contain eIF4E, FMRF, and Stau (Barbee et al., 2006; Chae et al., 2010). A second *A. californica* CPEB homolog, ApCPEB4, has a role in longterm facilitation, although it lacks a prion-like domain (Lee et al., 2016). In both mammalian and *A. californica* neurons, CPEB is required for mRNA shuttling, and it co-localizes with and polyadenylates multiple mRNAs (Huang et al., 2002, 2003; Chae et al., 2010). In *X. laevis* and mouse neurons, CPEB colocalizes with Maskin in a complex containing Kinesin and Dynine, suggesting that it may regulate mRNA transport and translation in dendrites (Huang et al., 2003) similar to its role in the germ line. In mice, CPEB3 interacts with the Actin cytoskeleton and has been shown to act as a functional prion as well (Stephan et al., 2015), with CPEB expression at synapses in rodent brains being required for synaptic plasticity (Wu et al., 1998), the cellular basis for memory and learning.

DISCUSSION

Here we have highlighted many genes that, following their initial characterization in the germ line, were discovered to also have neural roles. For many such genes with a neural role in one species, there is evidence for a neural role in other species as well, often with the same set of core molecular interaction partners (Table 2). We consider that the data currently available are too limited for us to propose whether the germ line roles or the neural roles of these genes represent their putative ancestral functions in a last common ancestor of animals [but see Ewen-Campen et al. (2012) for a proposal that oskar’s role in the insect germ line is derived, resulting from co-option from a putative neural role]. It is clear that relying on single gene expression patterns alone to identify homologies can be misleading (Wagner et al., 2012; Wang et al., 2013), and we are not proposing to use such data as the sole criteria for this purpose (Tautz, 1998; Nielsen and Martinez, 2003). Instead, our aim here is to suggest possible explanations for the molecular and cellular basis for this pleiotropy by looking at the properties of the molecular mechanisms of these shared genes, which may be linked to the evolution of cell-type specific functions.

Regulatory Commonalities of Germ Line and Nervous System

We begin by highlighting some independent yet interesting similarities between the germ line and the nervous system. First, germ cells, pluripotent stem cells, and undifferentiated or abnormally organized embryonic cells have been reported to differentiate towards neural cell fate under a number of circumstances. For example, in *C. elegans*, germ cells that lose P-granules can ectopically express somatic markers, including neuronal markers (Knutson et al., 2017). In induced human PGC-like cells generated from pluripotent stem cells, BLIMP1 is actively required to promote PGC fate and to repress neuronal differentiation (Sasaki et al., 2015). Dissociated *X. laevis* embryonic animal cap cells are able to upregulate the neural marker N-CAM despite the absence of normal spatial organization (Sato and Sargent, 1989). Embryonic stem (ES) cells spontaneously and readily exhibit aspects of neural identity under specific culture conditions (Trosepe et al., 2001). When plated at low densities in phosphate buffered saline, mouse ESCs can express nestin and Sox1, which is suggestive of neural stem cell differentiation (Smukler et al., 2006). It has therefore been suggested that neuronal fate is a preferred differentiation program for cells that lose their germ line identity or pluripotency (Knutson et al., 2017).

Second, the gene expression profiles of human and mouse testes and brain are highly similar to each other (Guo et al., 2003, 2005). Whether or how the two tissues communicate to regulate this similar gene expression is unclear, although Guo and colleagues (Guo et al., 2005) speculate that the hypothalamus-pituitary-gonadal axis (Plant, 2015; Kaprara and Huhtaniemi, 2018) may play a role. Finally, such observations may also help explain the link between disruption of genes with known roles in the germ line, and neural disease phenotypes. For example, the *D. melanogaster* tumor suppressor gene brain tumor (*brat*), together with *nos* and *pum*, represses translation in female germ line stem cells (Sonoda and Wharton, 2001), and *brat* loss of function mutations also cause tumors in the brain (Arama et al., 2000). Additionally, ectopic expression of at least 26 genes normally expressed in the germ line, may be linked to malignant brain tumor growth in *D. melanogaster* (Janić et al., 2010). Thus, it is possible that some shared or similar biological processes link these genes to both germ line and neural tissue types outside of mammals as well.

A Shared Molecular Basis for Pleiotropy

In this review we have summarized some of the evidence for the expression and functional requirements for a number of genes in the above mentioned two cell types. However, in most cases the molecular mechanisms linking the function of these genes to the cellular execution of neural or germ line fate remain unclear.
It is therefore difficult to determine whether this pleiotropy is a result of the same molecular function in apparently unrelated biological processes, or because some or all of these genes have multiple molecular functions per gene. In principle, it could be the case that these genes have the same immediate downstream partners in both tissue contexts, but their subsequent interactors or secondary targets are different, leading to differences in cellular responses to the activities of these genes within each tissue. Nevertheless, in the following section, we propose some possible explanations, based on shared molecular functions of these genes, for the potentially close or labile relationship between germ line and neural cell fates.

Cytoplasmic Aggregates: The Roles of RNP Granules in Germ Line and Nervous System

One way to understand the repeated conservation of expression, molecular function and interactions of these genes in neural tissues and germ lines, is by considering whether the products of these genes have functional or biochemical properties that could make them particularly suited for use by these cell types. We note that products of most of the genes discussed here share three notable properties. First, they are RNA binding proteins (e.g., Osk, Piwi, Vasa, Stau, Nos, FMRP), and play multiple roles in RNA biology including localization (e.g., Stau, Osk), translational activation (e.g., Vas, and Stau), and translational repression (e.g., Nos, Pum, Stau). Second, many of them break cellular symmetry by becoming asymmetrically localized within the cytoplasm or facilitating the asymmetric localization of other molecules (e.g., Osk, Stau/Btz, Nos). Third, the majority catalyze the formation of and/or localize to RNP granule complexes, which are in turn sometimes asymmetrically distributed within the cell (e.g., germ granules in *D. melanogaster*). RNP granules are electron dense, non-membrane bound cytoplasmic aggregates of RNAs and proteins (Eddy, 1975; Ikenishi, 1998). The assembly of proteins within RNPs is often transient or reversible, and RNPs are important for the localization, stability and translational control of their RNA (and protein) cargo (Arkov and Ramos, 2010; Voronina et al., 2011; Schisa, 2012; Gao and Arkov, 2013). Moreover, in addition to giving RNP granules their functionality in translational control, RNA Binding Proteins (RBPs) have been noted to commonly have regions of low sequence complexity and prion-like domains, both of which can mediate RNP granule assembly and disassembly (Brangwynne et al., 2009; Han et al., 2012; Kato et al., 2012; Molliex et al., 2015; Sudhakaran and Ramaswami, 2017).

RNP granules are found in both germ line and somatic cells. Depending on the tissue they are found in, RNP granules are referred to in the literature by various names, including polar or germlinal granules in germ cells, stress granules and processing bodies in somatic cells, and neuronal granules in neurons (reviewed in Voronina et al., 2011). All described classes of RNP granules share multiple components with each other (reviewed in Kulkarni and Extavour, 2017). Functional amyloid-like assemblies like RNP granules can govern cellular processes both in the germ line, including PGC specification and spermatogenesis (reviewed in Voronina et al., 2011), and in the soma, including in the consolidation of memory in the nervous system (Si et al., 2003, 2010; Si and Kandel, 2016). In the latter context, proteins with prion-like domains, which may facilitate amyloid-like assemblies, localize at neuronal synapses and form active, stable complexes with self-perpetuating properties central to memory storage (Si et al., 2003, 2010; Sudhakaran and Ramaswami, 2017). We note that Oskar and FMRP have predicted prion-like domains (McBride et al., 2012; Boke et al., 2016). Germ line and neural cells also share the commonality of regulating translation at specific sites within the cell, e.g., the oocyte posterior in the case of germ plasm formation (Lehmann, 2016), or at select neuronal synapses in the case of neurons, leading to synaptic plasticity (Kang and Schuman, 1996; Si et al., 2003).

Small RNA Biogenesis as a Regulator of Gene Expression in Germ Cells and Neurons

Piwi, its related protein Aubergine, and Vasa are among the many RNA binding proteins that are associated with and indispensable for small RNA biogenesis in the germ line (Ku and Lin, 2014). piRNAs are endogenous small non-coding RNAs that are proposed to maintain the genomic integrity of germ cells by limiting transposon mobility (Aravin et al., 2001, 2006; Girard et al., 2006; Grimson et al., 2008). piRNAs associate with the Argonaute family member Piwi (e.g., Mochizuki et al., 2002), and other members of this family (e.g., Ago3) interact with other small RNAs, including miRNAs and siRNAs (Girard et al., 2006; Vagin et al., 2006; Brennecke et al., 2007; Houwing et al., 2007; Kim et al., 2009). Small RNA-mediated gene silencing occurs at both transcriptional and post-transcriptional levels, and is an important mechanism controlling gene expression (Holoch and Moazed, 2015). piRNAs were first characterized in the germ line, but recent reports support their existence in somatic tissues as well, including neural tissues (Lee et al., 2011; Rajasethupathy et al., 2012; Ross et al., 2014). Indeed, in *A. californica*, after the germ line, the nervous system is amongst the tissue types that show relatively high selective enrichment for piRNAs (Rajasethupathy et al., 2012). There is evidence for primary piRNA biogenesis in the germ line and neurons (Rajasethupathy et al., 2012; Mani and Juliano, 2013; Kim et al., 2018) consistent, with a functional role for piRNAs in both cell types. For example, Piwi and piRNAs regulate Myosin-Va in the central nervous system of mammals (Naisbitt et al., 2000; Lee et al., 2011), control local translation in mouse neuronal dendrites (Lee et al., 2011), mouse dendritic spine development (Lee et al., 2011), neuronal migration (Viljetic et al., 2017), and may be linked to growth of malignant brain tumors (Janic et al., 2010). Finally, piRNAs regulate transposon activity both in the brain and in the germ line (reviewed in Mani and Juliano, 2013). Retrotransposons are highly active in neural tissues and contribute to proper neuronal differentiation and generation of somatic mosaicism in the brain (Muotri et al., 2005; Coufal et al., 2009). Thus, piRNAs are crucial both for...
the germ line, and for normal development and function of the nervous system, which may help explain why we observe that genes important for their biogenesis are expressed in both tissue types.

**Challenges in Determining the Evolutionary Sequence of Co-option Events**

Co-option of partial or complete gene networks in different biological contexts is common (Jacob, 1977). Novel traits may evolve either by the co-option of pre-existing gene networks that operate in functional modules, or by building a new gene network for each new developmental context (Sanetra et al., 2005; Monteiro and Podlaha, 2009). Based on the observations summarized herein, we propose that the germ line and nervous tissues of animals contain examples of gene network co-option, given that the genes involved are pleiotropic, and that we do not think it likely that the germ line and nervous system are homologous organ systems. In principle, one way of co-opting a gene network could be by recruiting an upstream regulator of an existing network into a new developmental context. This is what we previously proposed may have happened in the case of oskar in germ plasm (Ewen-Campen et al., 2012). In both cricket (Ewen-Campen et al., 2012) and fly (Xu et al., 2013) nervous systems, oskar is co-expressed with *vasa, piwi* and/or *nanos*, genes whose products function together in multiple other cellular contexts as discussed above. Given that germ plasm in insects is likely a derived mechanism of PGC specification (Extavour and Akam, 2003; Lynch et al., 2011; Ewen-Campen et al., 2013), we propose that the functional links among these genes are likely to predate the evolution of insect germ plasm, suggesting that they were co-opted to the germ line context from a preexisting somatic role (Ewen-Campen et al., 2012).

When moving beyond insects to consider all animals, because there have been fewer reported instances to date of the expression or function of these genes in the nervous system outside of bilaterians, one might wish to hypothesize that the germ line functions of these gene evolved first, and then were co-opted to the nervous system in Bilateria. However, the functions of these genes have been explored primarily in a small number of study systems, heavily biased toward the Bilateria. Moreover, the diversity of cell types, including neural cell types, outside of Bilateria are not as well studied at the molecular level as are those of bilaterians. The evidence that the earliest metazoans were highly complex animals is mounting (Halanych, 2015; Whelan et al., 2017; Paps, 2018; Laumer et al., 2019), and may well displace the traditional view that early animals were “simple” with few differentiated cell types, lacking complex reproductive or sensory systems. We therefore consider it premature to speculate on whether the ancestral function of these genes in animals, was in the germ line or in the nervous system. Rather than thinking about the patterns in their putative ancestral functions in establishing a particular cell type, we could consider the hypothesis that the cellular function of translational control in RNP granules is the relevant conserved ancestral role of this machinery in eukaryotes. This could explain why striking phenotypes are particularly or easily observed in neurons and germ cells, because these cell types rely heavily on translational regulation for their biological functions. The advent of animal-specific genes like *nos* and *osk* may have permitted the emergence of tissue-specific versions of this machinery, deployed specifically in germ lines and nervous systems to refine or augment their regulation of translation.

**CONCLUSION**

We note that an association between many of the genes discussed herein and “stemness” or cellular multipotency, has already been pointed out by several researchers: the general proposal is that these genes may have been components of an ancestral animal toolkit in stem cells, regardless of the fate of their differentiated progeny (e.g., Alié et al., 2010, 2015; Juliano et al., 2010; Fierro-Constain et al., 2017). Here we speculate that if, as in many extant animals, ancient metazoans generated gametes from germ line stem cells, and/or neurons from neuroblasts, the observed association of these genes with pluripotency may also help explain the gene expression overlap in germ line and nervous tissues. Going forward, technical advances including single-cell RNA sequencing, chromatin architecture analysis and proteomics, and improved microscopy and computational methodologies including machine learning, might make it possible to test such hypotheses experimentally (e.g., Siebert et al., 2019). The case we have discussed here, of the germ line and the nervous system, is an example of the broader, fundamental question of how the same molecular mechanisms can underlie different cell identities. Once putative ancient cell type inventories are reconstructed for important evolutionary nodes, we can perhaps begin to unravel how ancient cell types, in some cases expressing highly similar machinery, diversified into extant cell types that make up the tissues and organ systems of living animals (Kin, 2015; Arendt et al., 2016), helping answer some of the questions that we have raised here.

**AUTHOR CONTRIBUTIONS**

CE conceived of the project. DL, AK, and CE compiled the evidence from primary literature. AK and CE wrote the manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was supported by the Harvard University. DL was supported by the Harvard GSAS Research Scholar Initiative.

**ACKNOWLEDGMENTS**

We thank Samuel Church and Seth Donoughe from the Extavour lab for helpful comments, critical reading, and discussions on earlier versions of this manuscript. We also thank the reviewers for their comments and suggestions which helped improve the manuscript.
REFERENCES

Alié, A., Hayashi, T., Sugimura, I., Manuel, M., Sugano, W., Mano, A., et al. (2015). The ancestral gene repertoire of animal stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 112, E7993–E7100.

Alié, A., Leclère, L., Jager, M., Dayraud, C., Chang, P., Le Guyader, H., et al. (2010). Somatic stem cells express *Pwi* and *Vasa* genes in an adult chenopod: ancient association of “germline genes” with stemness. *Dev. Biol.* 330, 183–197. doi: 10.1016/j.ydbio.2010.10.019

Alphéy, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P., and Glover, D. M. (1992). twine, a cdc25 homolog that functions in the male and female germline of *Drosophila*. *Cell* 69, 977–988. doi: 10.1016/0092-8674(92)90616-k

Anand, A., and Kai, T. (2012). The tudor domain protein Kumo is required to assemble the nuage and to generate germline piRNAs in *Drosophila*. *EMBO J.* 31, 870–882. doi: 10.1038/emboj.2011.449

Anantharaman, V., Zhang, D., and Aravind, L. (2010). OST-HTH: a novel predicted RNA-binding domain. *Biol. Direct* 5:13. doi: 10.1186/1745-6150-5-13

Antar, L. N., Dictenberg, J. B., Plociniak, M., Afroz, R., and Bassell, G. J. (2007). The crystal structure of the exon junction complex reveals how it maintains a stable grip on mRNA. *Cell* 126, 713–725. doi: 10.1016/j.cell.2006.08.006

Balduc, F. V., Bell, K., Cox, H., Brodie, K. S., and Tully, T. (2008). Excess protein synthesis in *Drosophila* fragile X mutants impairs long-term memory. *Nat. Neurosci.* 11, 1143–1145. doi: 10.1038/nn.2175

Bozetti, M. P., Specchia, V., Catterneo, P. B., Lanève, P., Geusa, A., Sahin, H. B., et al. (2015). The *Drosophila* fragile X mental retardation protein participates in the piRNA pathway. *J. Cell. Sci.* 128, 2070–2084. doi: 10.1242/jcs.161810

Brangwynne, C. P., Eckmann, C. R., Courson, D. S., Rybarska, A., Hoege, C., Gharaikhani, J., et al. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324, 1729–1732. doi: 10.1126/science.1172046

Bretwieser, W., Markussen, F.-H., Horstmann, H., and Ephrussi, A. (1996). Oskar protein interaction with Vasa represents an essential step in polar granule assembly. *Genes Dev.* 10, 2179–2188. doi: 10.1101/gad.10.17.2179

Brendza, R., Serbus, L., Saxton, W., and Duffy, J. (2002). Posterior localization of dynein and dorsal-ventral axis formation depend on kinesin in *Drosophila* oocytes. *Curr. Biol.* 12, 1541–1545. doi: 10.1016/s0960-9822(02)00104-8

Brennecke, J., Aravin, A. A., Stark, A., Dus, M., Kellis, M., Sachidanandam, R., et al. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell* 128, 1089–1103. doi: 10.1016/j.cell.2007.01.043

Broadus, J., Fuerstenberg, S., and Doe, C. Q. (1998). Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate. *Nature* 391, 792–795. doi: 10.1038/358661

Brown, V., Small, K., Lakki, L., Feng, Y., Gunter, C., Wilkinson, K. D., et al. (1998). Purified recombinant Fmrp exhibits selective RNA binding as an intrinsic property of the fragile X mental retardation protein. *J. Biol. Chem.* 273, 15521–15527. doi: 10.1074/jbc.273.25.15521

Cao, Q., and Richter, J. D. (2002). Dissolution of the maskin-eIF4E complex by controlled dissolution/condensation. *Science* 291, 975–979. doi: 10.1126/science.1064543

Carr, J., Djejdat, C., and Sardet, C. (2002). Formation of a large vasa-positive granule and its inheritance by germ cells in the enigmatic chaetognaths. *Development* 129, 661–670.

Carrera, P., Johnstone, O., Nakamura, A., Casanova, J., Jackle, H., and Lasko, P. (2000). VASA mediates translation through interaction with a Drosophila yif2 protein. *Curr. Biol.* 10, 2179–2188. doi: 10.1016/s0960-9822(02)00104-8

Castagnetti, S., and Ephrussi, A. (2003). Orb and a long poly(A) tail are required for efficient oskar translation at the posterior pole of the *Drosophila* oocyte. *Development* 130, 835–843. doi: 10.1242/dev.00309

Castrillon, D. H., Gönczi, P., Alexander, S., Rawson, R., Eberhat, C. G., et al. (2006). Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Nature* 422, 997–1002. doi: 10.1038/nature01491

Chae, Y. S., Lee, S. H., Cheang, Y. H., Lee, N., Rim, Y. S., Jang, D. J., et al. (1993). Toward a molecular genetics analysis of oocyte. *Exp. Mol. Med.* 15:222.

Chen, F., Mackey, A. J., Stoeckert, C. J. Jr., and Roos, D. S. (2006). OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. *Nucleic Acids Res.* 34, D363–D368.

Chen, G., Li, W., Zhang, Q. S., Regulski, M., Sinha, N., Barditch, J., et al. (2008). Identification of synaptic targets of Drosophila pumilio. *PLoS Computat. Biol.* 4:e1000026. doi: 10.1371/journal.pcbi.1000026

Böhmer, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., and Benning, C. (1998). AGO1 defines a novel locus of Arabidopsis controlling leaf development. *EMBO J.* 17, 170–180. doi: 10.1093/emboj/17.1.170

Boke, E., Ruer, M., Wuhr, M., Coughlin, M., Lemaître, R., Gygi, S. P., et al. (2016). Amyloid-like self-assembly of a cellular compartment. *Cell* 166, 637–650. doi: 10.1016/j.cell.2016.06.051
Christelson, L. B., and McKearin, D. M. (1994). orb is required for anteroposterior and dorsoventral patterning during Drosophila oogenesis. Genes Dev. 8, 614–628. doi: 10.1101/gad.8.3.614

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., et al. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc. Natl. Acad. Sci. U.S.A. 94, 5401–5404. doi: 10.1073/pnas.94.10.5401

Costa, A., Wang, Y., Dockendorff, T. C., Erdjument-Bromage, H., Tempst, P., Schedl, P., et al. (2005). The Drosophila fragile X protein functions as a negative regulator in the orb autoregulatory pathway. Dev. Cell 8, 331–342. doi: 10.1016/j.devcel.2005.01.011

Coufal, N. G., Garcia-Perez, J. L., Peng, G. E., Yeow, G. W., Mu, Y., Lovci, M. T., et al. (2009). L1 retrotransposition in human neural progenitor cells. Nature 460, 1127–1131. doi: 10.1038/nature08248

Courtot, C., Fankhauser, C., Simanis, V., and Lehner, C. F. (1992). The Drosophila cd25 homolog twine is required for meiosis. Development 116, 405–416.

Crittenden, S. L., Bernstein, D. S., Bachorik, J. L., Thompson, B. E., Gallegos, M., Petcherski, A. G., et al. (2002). A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. Nature 417, 660–663. doi: 10.1038/nature754

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., Maines, J. Z., and Wasserman, S. A. (1996). Meiotic cell cycle machinery of germ line specification. Cell 85, 1083–1091. doi: 10.1016/0092-8674(96)80070-4

Ephrussi, A., Dickinson, L. K., and Lehmann, R. (1991). Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell 66, 37–50. doi: 10.1016/0092-8674(91)90137-n

Erdelyi, M., Michon, A. M., Guichet, A., Glotzer, J. B., and Ephrussi, A. (1995). Requirement for Drosophila cytoplasmic tropomyosin in oskar mRNA localization. Nature 377, 524–527. doi: 10.1038/377524a0

Estes, P. S., O’Shea, M., Clasen, S., and Zarnescu, D. C. (2008). Fragile X protein controls the efficacy of mRNA transport in Drosophila neurons. Mol. Cell. Neurosci. 39, 170–179. doi: 10.1016/j.mcn.2008.06.012

Ewen-Campen, B., Donoughue, S., Clarke, D. N., and Estavouge, C. G. (2013). Germ cell specification requires zygotic mechanisms rather than germ plasm in a basally branching insect. Curr. Biol. 23, 835–842. doi: 10.1016/j.cub.2013.06.063

Ewen-Campen, B., Schwager, E. E., and Estavouge, C. G. (2010). The molecular machinery of germ line specification. Mol. Reprod. Dev. 77, 3–18. doi: 10.1002/mrd.21091

Ewen-Campen, B., Srouji, J. R., Schwager, E. E., and Estavouge, C. G. (2012). oskar Predates the evolution of germ plasm in insects. Curr. Biol. 22, 2278–2283. doi: 10.1016/j.cub.2012.10.019

Estavouge, C. G., and Akam, M. E. (2003). Mechanisms of germ cell specification across the metazoa: epigenesis and preformation. Development 130, 5869–5884. doi: 10.1242/dev.00804

Estavouge, C. G., Pang, K., Matus, D. Q., and Martindale, M. Q. (2005). Vasa and nanos expression patterns in a sea anemone and the evolution of bilaterian germ cell specification mechanisms. Evol. Dev. 7, 201–215. doi: 10.1111/j.1525-142X.2005.0023.x

Feng, Y., Absher, D., Eberhart, D. E., Brown, V., Malter, H. E., and Warren, S. T. (1997). FMRP associates with polyribosomes as an MRNP and the 130kDa mutation of severe fragile X syndrome abolishes this association. Mol. Cell 1, 109–118. doi: 10.1016/s1097-2765(00)80012-x

Fernandez-Miranda, G., and Mendez, R. (2012). The CPEB-family of proteins, translational control in senescence and cancer. Ageing Res. Rev. 11, 460–472. doi: 10.1016/j.arr.2012.03.004

Feuge, J., Scharkowski, F., Michaela-Preusse, K., and Korte, M. (2019). FMRP modulates activity-dependent spine plasticity by binding Cofilin1 mRNA and regulating localization and local translation. Cereb. Cortex 29, 5204–5216. doi: 10.1093/cercor/bhz059

Fierz-Constain, L., Schenkelaars, Q., Gazave, E., Hagueuener, A., Rocher, C., Ereskovsky, A., et al. (2017). The conservation of the germline multipotency program, from sponges to vertebrates: a stepping stone to understanding the somatic and germline origins. Genome Biol. Evol. 9, 474–488.

Fiumara, F., Rajasepathathy, P., Antonov, I., Kosmidis, S., Sossin, W. S., and Kandel, E. R. (2015). MicroRNA-22 gates long-term heterosynaptic plasticity in alpysia through presynaptic regulation of CPEB and downstream targets. Cell Rep. 11, 1866–1875. doi: 10.1016/j.celrep.2015.05.034

Forbes, A., and Lehmann, R. (1998). Nanos and pumilio have critical roles in the development and function of Drosophila germline stem cells. Development 125, 679–690.

Furuta, R., Karra, D., Bennett, K. L., Ang, F. Y., Heraud-Farlow, J. E., Tolino, M., et al. (2013). Interactome of two diverse RNA granules links mRNA localization to translational repression in neurons. Cell Rep. 5, 1749–1762. doi: 10.1016/j.celrep.2013.11.023

Funayama, N. (2010). The stem cell system in demospontes: insights into the origin of somatic stem cells. Dev. Growth Differ. 52, 1–14. doi: 10.1111/j.1440-169X.2009.01162.x

Funayama, N., Nakatsuka, M., Mohri, K., Masuda, Y., and Agata, K. (2010). Piwi expression in archeocytes and choanoocytes in demospontes: insights into the stem cell system in demospontes. Evol. Dev. 12, 275–287. doi: 10.1111/j.1525-142X.2010.00413.x

Furrer, D. I., Swart, E. C., Kraft, M. F., Sandoval, P. Y., and Nowacki, M. (2017). Two sets of piwi proteins are involved in distinct sRNA pathways leading to elimination of germline-specific DNA. Cell Rep. 20, 505–520. doi: 10.1016/j.celrep.2017.06.050

Gambelli, C., Peterson, D. S., He, L., and Gottlieb, E. (2002). An anterior function for the posterior determinant pumilio. Development 129, 2699–2710.

Gao, M., and Arkov, A. L. (2013). Next generation organelles: structure and role of germ granules in the germline. Mol. Reprod. Dev. 80, 610–623. doi: 10.1002/mrd.22115

Gardiol, A., and St Johnston, D. (2014). Staufen targets coracle mRNA to Drosophila neuromuscular junctions and regulates GlurRNA synaptic accumulation and bouton number. Dev. Biol. 392, 153–167. doi: 10.1016/j.ydbio.2014.06.007
Gavis, E. R., Lunsford, L., Bergsten, S. E., and Lehmann, R. (1996). A conserved 90 nucleotide element mediates translational repression of nanos RNA. Development 122, 2791–2800.

Ghribal, A., and Schupbach, T. (1999). Activation of a meiotic checkpoint regulates translation of Gurken during Drosophila oogenesis. Nature Cell Biol. 1, 354–357. doi:10.1038/14018

Ghosh, S., Marchand, V., Gaspar, I., and Ephrussi, A. (2012). Control of RNP motility and localization by a splicing-dependent structure in oskar mRNA. Nat. Struct. Mol. Biol. 19, 441–449. doi:10.1038/nsmb.2257

Girard, A., Sachidanandam, R., Hannon, G. J., and Carmell, M. A. (2006). A xenopus DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of Drosophila boule. Development 127, 447–456.

Hake, L. E., and Richter, J. D. (1994). CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation. Cell 79, 617–627. doi:10.1016/0092-8674(94)90547-9

Han, T. W., Kato, M., Xie, S., Wu, L. C., Mirzaei, H., Pei, J., et al. (2012). Cell-free translation of RNA germline inducer oskar identifies two domains with distinct vasa helicase- and RNA-binding activities. Cell Rep. 12, 587–598. doi:10.1016/j.celrep.2015.06.055

Jaruzelska, J., Kotecki, M., Kusz, K., Firpo, M., and Reijo, R. A. (2003). Conservation of a pumilio-nanos complex from Drosophila to Xenopus. Dev. Genes Evol. 213, 120–126. doi:10.1007/s00427-003-1053003

Jeske, M., Muller, S. M., Rybin, V., Muller, C. W., et al. (2015). The LOTUS domain is a conserved DEAD-box RNA helicase regulator essential for the recruitment of ATP-dependent helicases. Cell 55, 577–587. doi:10.1016/0092-8674(88)90216-4

Jeske, M., Bordi, M., Slott, M., Muller, S., Ryrbin, V., Muller, C. W., et al. (2015). The crystal structure of the Drosophila RNA helicase inducer oskar identifies two domains with distinct vasa helicase- and RNA-binding activities. Cell Rep. 12, 587–598. doi:10.1016/j.celrep.2015.06.055

Janic, A., Mendizabal, L., Llamazares, S., Rossell, D., and Gonzalez, C. (2010). Ectopic expression of germline genes drives malignant brain tumor growth in Drosophila. Science 330, 1824–1827. doi:10.1126/science.1195481

Jarzembowska, L., Kotecki, M., Kusz, K., Firpo, M., and Reijo, R. A. (2003). Conservation of a pumilio-nanos complex from Drosophila to Xenopus. Dev. Genes Evol. 213, 120–126. doi:10.1007/s00427-003-1053003

Hryciw, S. M., and Wellik, D. M. (2016). Hox genes and evolution. F1000Res 5:859.

Huang, Y. S., Carson, J. H., Barbar ese, E., and Richter, J. D. (2003). Facilitation of dendritic mRNA transport by CPEB. Genes Dev. 17, 638–653. doi:10.1101/gad.103594.100

Huang, Y. S., Jung, M. Y., Sarkissian, M., and Richter, J. D. (2002). N-methyl-t-asparte receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. EMBO J. 21, 2139–2148. doi:10.1093/emboj/d2139

Ikegami, A., and Mendizabal, L. (2010). Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. EMBO J. 29, 2182–2193. doi:10.1038/emboj.2010.111

Ikenishi, K. (1998). Germ plasm in Caenorhabditis elegans. Drosophila and Xenopus. Dev. Growth Differ. 40, 1–10. doi:10.1046/j.1440-1699.1998.001400001.x

Iwasaki, Y. W., Siomi, M. C., and Siomi, H. (2015). PIWI-Interacting RNA: its biogenesis and functions. Annu. Rev. Biochem. 84, 405–433. doi:10.1146/annurev-biochem-0677-08.2008

Ikegami, A., and Mendizabal, L. (2010). Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. EMBO J. 29, 2182–2193. doi:10.1038/emboj.2010.111

Hooper, E. D., Penton, A., Watts, R. J., and Luo, L. (2008). Genomic analysis of Drosophila neuronal remodeling: a role for the RNA-binding protein Boule as a negative regulator of axon pruning. J. Neurosci. 28, 6092–6103. doi:10.1523/jneurosci.0677-08.2008

Houston, D. W., and King, M. L. (2000). A critical role for Xda1, a germ plasm-localized RNA, in the differentiation of primordial germ cells in Xenopus. Development 127, 171–180.

Houston, S., Kamminga, L. M., Berezikov, E., Crombeldonk, D., Girard, A., van den Elst, H., et al. (2007). A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. Cell 129, 69–82. doi:10.1016/j.cell.2007.03.026

Hwang, Y. S., Carbon, J. H., Barbar ese, E., and Richter, J. D. (2003). Facilitation of dendritic mRNA transport by CPEB. Genes Dev. 17, 638–653. doi:10.1101/gad.103594.100

Huang, Y. S., Jung, M. Y., Sarkissian, M., and Richter, J. D. (2002). N-methyl-t-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. EMBO J. 21, 2139–2148. doi:10.1093/emboj/d2139

Hurd, T. R., Herrmann, B., Sauerwald, J., Sanny, J., Grosch, M., and Lehmann, R. (2016). Long oskar controls mitochondrial inheritance in Drosophila melanogaster. Dev. Cell 39, 560–571. doi:10.1016/j.devcel.2016.11.004

Ikegami, A., and Mendizabal, L. (2010). Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. EMBO J. 29, 2182–2193. doi:10.1038/emboj.2010.111

Ikonomov, S. B., Siomi, M. C., and Siomi, H. (2015). PIWI-Interacting RNA: its biogenesis and functions. Annu. Rev. Biochem. 84, 405–433. doi:10.1146/annurev-biochem-0677-08.2008

Ikenishi, K. (1998). Germ plasm in Caenorhabditis elegans. Drosophila and Xenopus. Dev. Growth Differ. 40, 1–10. doi:10.1046/j.1440-1699.1998.001400001.x

Iwasaki, Y. W., Siomi, M. C., and Siomi, H. (2015). PIWI-Interacting RNA: its biogenesis and functions. Annu. Rev. Biochem. 84, 405–433. doi:10.1146/annurev-biochem-060614-034258

Jacob, F. (1977). Evolution and tinkering. Science 196, 1161–1166. doi:10.1126/science.860134

Janic, A., Mendizabal, L., Llamazares, S., Rossell, D., and Gonzalez, C. (2010). Ectopic expression of germline genes drives malignant brain tumor growth in Drosophila. Science 330, 1824–1827. doi:10.1126/science.1195481

Jarzembowska, L., Kotecki, M., Kusz, K., Firpo, M., and Reijo, R. A. (2003). Conservation of a pumilio-nanos complex from Drosophila to Xenopus. Dev. Genes Evol. 213, 120–126. doi:10.1007/s00427-003-0303-2

Jeske, M., Bordi, M., Slott, M., Muller, S., Rybin, V., Muller, C. W., et al. (2015). The crystal structure of the Drosophila RNA helicase inducer oskar identifies two domains with distinct vasa helicase- and RNA-binding activities. Cell Rep. 12, 587–598. doi:10.1016/j.celrep.2015.06.055

Jeske, M., Muller, C. W., and Ephrussi, A. (2017). The LOTUS domain is a conserved DEAD-box RNA helicase regulator essential for the recruitment of
Vasa to the germ plasm and nuage. Genes Dev. 31, 939–952. doi: 10.1101/gad.297051.117

Jia, M., Shan, Z., Yang, Y., Liu, C., Li, J., Luo, Z. G., et al. (2015). The structural basis of miranda-mediated staufen localization during Drosophila neuroblast asymmetric division. Nat. Commun. 6, 6831.

Jimenez, I., Alphs, L., Nurse, P., and Glover, D. M. (1990). Coomittal fission yeast cdc2ts and cdc25s mutants identifies two cell cycle genes from Drosophila: a cdc2 homologue and string. EMBO J. 9, 3565–3571. doi: 10.1002/j.1460-2075.1990.tb07567.x

Jin, P., Zarnescu, D. C., Ceman, S., Nakamoto, M., Mowrey, J., Jongens, T. A., et al. (2004). Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat. Neurosci. 7, 113–117. doi: 10.1038/nn1174

Jin, S. W., Arno, N., Cohen, A., Shah, A., Xu, Q., Chen, N., et al. (2001). Evidence that fragile X mental retardation protein is a negative regulator of mRNA translation. Cell 104, 1587–1593. doi: 10.1016/0092-8674(01)00247-7

Joiner, M. L., and Wu, C. F. (2004). Nervous system function for the testis RNA-decay enzyme Dicer. Proc. Natl. Acad. Sci. U.S.A. 101, 11307–11312. doi: 10.1073/pnas.0402212101

Kawamura, K., and Sunanaga, T. (2011). Role of Vasa, Piwi, and Myc-expressing coelomic cells in gonad regeneration of the colonial tunicate, Ciona intestinalis. J. Cell Sci. 124, 4277–4287. doi: 10.1242/jcs.127233

Kerkhoff, M., van den Bergh, M., Belousov, E., Weidinger, A., and De Deken, X. (2015). The fragile X mental retardation protein is recruited to stress granules in the mouse male germ cells. PLoS ONE 11:e0163015. doi: 10.1371/journal.pone.0163015

Kim, K. W., Tang, N. H., Andrusiak, M. G., Wu, Z., Chisholm, A. D., and Jin, Y. (2018). A neuronal piRNA pathway inhibits axon regeneration in C. elegans. Neuron 97, 86.

Kim, V. N., Han, J., and Somi, M. C. (2009). Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell. Biol. 10, 126–139. doi: 10.1038/nrm2632

Kin, K. (2015). Inhibiting cell type innovations by phylogenetic methods—concepts, methods, and limitations. J. Exp. Zool. B Mol. Dev. Evol. 324, 653–661. doi: 10.1002/ezmb.22657

King, M. C., and Wilson, A. C. (1975). Evolution at two levels in humans and chimpanzees. Science 188, 107–116. doi: 10.1126/science.1090005

King, R. C., Mulligan, P. K., and Stansfield, W. D. (2013). A Dictionary of Genetics. New York, NY: Oxford University Press.

Kirk, T., Latt, V., Kaiser, D., Estrada, S. E., and Ohm, J. H. (2006). Complementation of the Caenorhabditis elegans, piRNA-binding domain of the cytoplasmic polyadenylation element binding protein POG-1 are needed to regulate germ cell fates. Genetics 169, 1517–1630.

Joiner, M. L., and Wu, C. F. (2004). Nervous system function for the testis RNA-decay enzyme Dicer. Proc. Natl. Acad. Sci. U.S.A. 101, 11307–11312. doi: 10.1073/pnas.0402212101

Kulkarni, A., and Extavour, C. G. (2017). Convergent evolution of germ granule nucleators: a hypothesis. Stem Cell Res. 24, 188–194. doi: 10.1016/j.scr.2017.07.018

Kurilova, Y., Tokuriki, M., Myojin, R., Hori, T., Kuroiwa, A., Matsuda, Y., et al. (2003). CPEB2, a novel putative translational regulator in mouse haploid germ cells. Biol. Reprod. 69, 261–268. doi: 10.1095/biolreprod.103.015677

Kuwata, T., and Sato, K. (2008). The neuropeptide Y-Y2 receptor is a transmembrane receptor associated with germ cells in gonadal tissue. J. Neurosci. 28, 12033–12043. doi: 10.1523/JNEUROSCI.3220-08.2008

Kulshrestha, P., and Cohen, A. (2009). RNA metabolism in the germ line and oogenesis. Biochim. Biophys. Acta 1788, 612–622. doi: 10.1016/j.bbamrew.2009.04.001

Kulkarni et al. Genes With Germline and Neural Roles
Lear, B. (2001). Roles of Intrinsic Factors During Cell Fate Decisions in the Insect Central Nervous System, Department of Molecular Genetics and Cell Biology. Chicago: University of Chicago, 150.

Leclère, L., Jager, M., Barreau, C., Chang, P., Le Guyader, H., Manuel, M., et al. (2012). Maternally localized germ plasm mRNAs and germ cell/stem cell formation in the cnidarian hydra. Dev. Biol. 364, 236–248. doi: 10.1016/j.ydbio.2012.01.018

Lee, E. J., Banerjee, S., Zhou, H., Jammaladamaka, A., Arcila, M., Manjunath, B. S., et al. (2011). Identification of piRNAs in the central nervous system. RNA 17, 1090–1099. doi: 10.1261/rna.2565011

Lee, M.-H., and Schedl, T. (2006). RNA-binding Proteins. WormBook: The Online Review of C. elegans Biology. Pasadena, CA: WormBook, 1–13.

Lee, S. H., Shim, J., Cheong, Y. H., Choi, S. L., Jun, Y. W., Lee, S. H., et al. (2016). ApCPEB4, a non-prion domain containing homolog of ApCPEB, is involved in the initiation of long-term facilitation. Mol. Brain 9:91.

Lehmann, R. (2016). Germ plasm biogenesis—an oskar-centric perspective. Curr. Top. Dev. Biol. 116, 679–707. doi: 10.1016/bs.ctdb.2015.11.012

Lehmann, R., and Nüsslein-Volhard, C. (1996). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of oskar, a maternal gene in Drosophila. Cell 47, 144–152.

Lehmann, R., and Nüsslein-Volhard, C. (1998). Involvement of the pumilio gene in the transport of an abdominal signal in the Drosophila embryo. Nature 329, 167–170. doi: 10.1038/329167a0

Lehmann, R., and Nüsslein-Volhard, C. (1991). The maternal gene nanos has a central role in posterior pattern formation of the Drosophila embryo. Development 112, 679–691.

Leighton, I. L., Wei, W., Marshall, P. R., Ratnu, V. S., Li, X., Zajaczkowski, E. L., et al. (2019). Disrupting the hippocampal Piwi pathway enhances contextual fear memory in mice. Neurobiol. Learn. Mem. 161, 202–209. doi: 10.1016/j.nlm.2019.04.002

Leininger, S., Adamski, M., Bergum, B., Guider, C., Liu, J., Laplante, M., et al. (2014). Crystal structure of Pumilio, a protein that regulates stress granules and translation. Nat. Struct. Mol. Biol. 21, 842–848. doi: 10.1038/nsmb.2630

Li, Z., Zhang, Y., Ku, L., Wilkinson, K. D., Warren, S. T., and Feng, Y. (2001). The fragile X mental retardation protein inhibits translation via interacting with mRNAs. Nucleic Acids Res. 29, 2276–2283. doi: 10.1093/nar/29.11.2276

Lin, H., and Spradling, A. C. (1997). A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. Development 124, 2463–2476.

Linder, P. (2006). Dead-box proteins: a family affair—active and passive players in RNA metabolism. Nucleic Acids Res. 34, 4168–4188. doi: 10.1093/nar/gkl048

Liu, B., Li, Y., Stackpole, E. E., Novak, A., Gao, Y., Zhao, Y., et al. (2018). Regulatory discrimination of mRNAs by FMRP controls mouse adult neural stem cell differentiation. Proc. Natl. Acad. Sci. U.S.A. 115, E11397–E11405.

Liu, J., Hu, J. Y., Wu, F., Schwartz, J. H., and Schacher, S. (2006). Two mRNA-binding proteins regulate the distribution of syntaxin mRNA in Aplysia sensory neurons. J. Neurosci. 26, 5204–5214. doi: 10.1523/jneurosci.4917-05.2006

Liu, Q., Qi, H., Wang, J., and Lin, H. (2011). PAPI, a novel TUDOR-domain protein, complexes with AGO3, ME31B and TRAL in the nuage to silence transposition. Development 138, 1863–1873. doi: 10.1242/dev.059287

Lutjens, C., Gallegos, M., Kraemer, B., Kimble, J., and Wickens, M. (2000). CPEB4, a non-prion domain containing homolog of CPEB, is involved in the initiation of long-term facilitation. Mol. Brain 9:91.

McBride, S. M., Bell, A. J., and Jongens, T. A. (2012). Behavior in a Drosophila model of fragile X. Results Probl. Cell Differ. 54, 83–117. doi: 10.1007/978-3-642-21697-6_7

McBride, S. M., Choi, C. H., Wang, Y., Liebelt, D., Braunstein, E., Ferreiro, D., et al. (2005). Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron 45, 753–764. doi: 10.1016/j.neuron.2005.01.038

Meggioro, H. B., Cox, D. N., Campbell, C., and Lin, H. (2006). Campbell, and Lin, H., the role of PIWI and the mRNA machinery in Drosophila germline determination. Curr. Biol. 16, 1884–1894. doi: 10.1016/j.cub.2006.08.051

Meijer, H. A., Radford, H. E., Wilson, L. S., Lissendend, S. de C., and Moor, R. (2007). Translational control of maskin mRNA by its 3′ untranslated region. Biol. Cell 99, 235–249. doi: 10.1042/bc20060112

Mendez, R., and Richter, J. D. (2001). Translational control by CPEB: a means to complex traits evolve? Proc. Natl. Acad. Sci. U.S.A. 100, 538–543. doi: 10.1073/pnas.023441799

Micklem, D. R., Adams, J., Grunert, S., and St Johnston, D. (2000). Distinct roles of two conserved Staufen domains in oskar mRNA localization and translation. EMBO J. 19, 1366–1377. doi: 10.1093/emboj/19.6.1366

Mochizuki, K., Fine, N. A., Fujisawa, T., and Gorovsky, M. A. (2002). Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena. Cell 110, 689–699. doi: 10.1016/s0092-8674(02)00909-1

Mochizuki, K., Nishimaya-Fujisawa, C., and Fujisawa, T. (2001). Universal occurrence of the vasa-related genes among metazoans and their germ line expression in Hydra. Dev. Genes Evol. 211, 299–308. doi: 10.1007/s00710-002-0156-8

Mochizuki, K., Sano, H., Kobayashi, S., Nishimiya-Fujisawa, C., and Fujisawa, T. (2000). Expression and evolutionary conservation of nanos-related genes in Hydra. Dev. Genes Evol. 210, 591–602. doi: 10.1007/s00710-000-0105

Mocek, A. P., and Rose, D. J. (2009). Differential recruitment of limb patterning genes during development and diversification of beetle horns. Proc. Natl. Acad. Sci. U.S.A. 106, 8992–8997. doi: 10.1073/pnas.0806681106

Molliex, A., Temirov, J., Lee, J., Couglin, M., Kanagaraj, A. P., Kim, H. J., et al. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Curr. Biol. 163, 123–133. doi: 10.1016/j.cub.2015.09.015

Monteiro, A., and Podlaha, O. (2009). Wings, horns, and butterfly eyespots: how do complex traits evolve? PLoS Biol. 7:e1000037. doi: 10.1371/journal.pbio.1000037

Moore, F. L., Jaruzelska, J., Fox, M. S., Urano, J., Firpo, M. T., Turek, P. J., et al. (2003). Human pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. Proc. Natl. Acad. Sci. U.S.A. 100, 538–543. doi: 10.1073/pnas.0224471100

Morais-de-Sa, E., Vega-Rioja, A., Trovisco, V., and St Johnstone, D. (2013). Oskar is targeted for degradation by the sequential action of Par-1, GSK-3, and the SCF(-)Slm ubiquitin ligase. Dev. Cell 26, 303–314. doi: 10.1016/j.devcel.2013.06.011
Moussian, B., Schoof, H., Haecker, A., Jurgens, G., and Lauk, T. (1998). Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J. 17, 1799–1809. doi: 10.1093/emboj/17.6.1799

Moustri, A. R., Chu, V. T., Marchetto, M. C., Deng, W., Moran, J. V., and Gage, F. H. (2005). Somatic mosaicism in neuronal precursor cells mediated by L1 retrotansposion. Nature 435, 903–910. doi: 10.1038/nature03663

Murata, Y., and Wharton, R. P. (1995). Binding of pumilio to maternal hunchback mRNA is required for posterior patterning in Drosophila embryos. Cell 80, 747–756. doi: 10.1016/0092-8674(95)90353-4

Naïsbit, S., Valtschanoff, J., Allison, D. W., Sala, C., Kim, E., Craig, A. M., et al. (2008). Flatworm stem cells and the germ line: developmental and evolutionary implications of macuva expression in Macrostomum lignano. Dev. Biol. 319, 146–159. doi: 10.1016/j.ydbio.2008.02.045

Plant, T. M. (2015). The hypothyalamo-pituitary-gonadal axis. J. Endocrinol. 226, T41–T54.

Plickert, G., Frank, U., and Muller, W. A. (2012). Hydractinia, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. Int. J. Dev. Biol. 56, 519–534. doi: 10.1534/ijdb.123302g

Popper, B., Demeliter, A., Bolivar, V. J., Kusek, G., Snyder-Keller, A., Schieweck, R., et al. (2018). Staufen2 deficiency leads to impaired response to novelty in mice. Neurobiol. Learn. Mem. 150, 107–115. doi: 10.1016/j.nlm.2018.02.027

Posner, R., Toker, I. A., Antonova, O., Star, E., Anava, S., Azmon, E., et al. (2019). Neuronal small RNAs control behavior transgenerationally. Cell 177, e15.

Price, T. J., Flores, C. M., Cervero, F., and Hargreaves, K. M. (2006). The RNA binding and transport proteins staufen and fragile X mental retardation protein are expressed by rat primary afferent neurons and localize to peripheral and central axons. Neuroscience 141, 2107–2116. doi: 10.1016/j.neuroscience.2006.05.047

Pushpa, K., Kumar, G. A., and Subramaniam, K. (2017). Transcriptional control of germ cell decisions. Results Probl. Cell Differ. 59, 175–200. doi: 10.1007/978-3-319-44820-6_6

Rajaseethupathy, P., Antonov, I., Sheridan, R., Frey, S., Sander, C., Tuschl, T., et al. (2012). A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. Cell 149, 693–707. doi: 10.1016/j.cell.2012.02.057

Ramasamy, S., Wang, H., Quach, H. N., and Sampath, K. (2006). Zebrafish Staufen1 and Staufen2 are required for the survival and migration of primordial germ cells. Dev. Biol. 292, 393–406. doi: 10.1016/j.ydbio.2006.01.014

Rebscher, N., Volk, C., Teo, R., and Plickert, G. (2008). The germ plasm component Vasa allows tracing of the interstitial stem cells in the cnidarian Hydractinia echinata. Dev. Dyn. 237, 1736–1745. doi: 10.1002/dvdy.21562

Rebscher, N., Zelada-Gonzalez, F., Banisch, T. U., Raible, F., and Arendt, D. (2007). Vasa unveiled a common origin of germ cells and of somatic stem cells from the posterior growth zone in the polychaete Platynereis dumerilii. Dev. Biol. 306, 599–611. doi: 10.1016/j.ydbio.2007.03.021

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., et al. (2010). Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. Front. Zool. 7:29. doi: 10.1186/1749-8105-7-29

Roegiers, F., and Jan, Y. N. (2000). Staufen: a common component of mRNA transport in oocytes and neurons! Trends Cell Biol. 10, 220–224. doi: 10.1016/S0962-8924(00)01767-0

Rojas-Rios, P., Chartier, A., Pierson, S., Severac, D., Dantec, C., Busseau, I., et al. (2015). Translational control of autophagy by orb in the Drosophila germline. Dev. Cell 35, 622–631. doi: 10.1016/j.devcel.2015.11.003

Rojas-Rios, P., and Simonel, M. (2018). piRNAs and PIWI proteins: regulators of gene expression in development and stem cells. Development 145:dev161786. doi: 10.1242/dev.161786

Rosner, A., Moisseeva, E., Rinkevich, Y., Lapidot, Z., and Rinkevich, B. (2009). Vasa and the germ line lineage in a colonial urochordate. Dev. Biol. 326, 519–534. doi: 10.1016/j.ydbio.2008.09.025

Ross, R. J., Weiner, M. M., and Lin, H. (2014). PIWI proteins and PIWI-interacting RNAs in the soma. Nature 503, 353–359. doi: 10.1038/nature12897

Ruggiu, M., Speed, R., Taggart, M., McKay, S. J., Kilanowski, F., Saunders, P., et al. (1997). The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature 389, 77–77. doi: 10.1038/37987

Russell, P., and Nurse, P. (1986). cdc25+ functions as an inducer in the mitotic control of fission yeast. Cell 45, 145–153. doi: 10.1016/0092-8674(86)90546-5

Sanetra, M., Begemann, G., Becker, M. B., and Meyer, A. (2005). Conservation and co-option in developmental programmes: the importance of homology implications of macuva expression in Macrostomum lignano. Dev. Biol. 319, 146–159. doi: 10.1016/j.ydbio.2008.02.045

Sano, H., Mukai, M., and Kobayashi, S. (2001). Maternal nanos and pumilio regulate vasa expression autonomously in the germ-line progenitors of Drosophila melanogaster embryos. Dev. Growth Diff. 43, 545–552. doi: 10.1046/j.1440-169x.2001.00593.x
Sasaki, K., Yokobayashi, S., Nakamura, T., Okamoto, I., Yabuta, Y., Kurimoto, K., et al. (2015). Robust in vitro induction of human germ cell fate from pluripotent stem cells. Cell Stem Cell 17, 178–194. doi: 10.1016/j.stem.2015.06.014

Sato, K., Hayashi, Y., Ninomiya, Y., Shigenobu, S., Arita, K., Mukai, M., et al. (2007). Maternal Nanos represses hid/okl-dependent apoptosis to maintain the germ line in Drosophila embryos. Proc. Natl. Acad. Sci. U.S.A. 104, 7455–7460. doi: 10.1073/pnas.0610052104

Sato, S. M., and Sargent, T. D. (1989). Development of neural induction capacity in dissociated Xenopus embryos. Dev. Biol. 134, 236–266. doi: 10.1016/0012-1606(89)90096-1

Saunders, P. T., Pathirana, S., Maguire, S. M., Doyle, M., Wood, T., and Bownes, M. (2000). Mouse stau1 genes are expressed in germ cells during oogenesis and spermatogenesis. Mol. Hum. Reprod. 6, 983–991. doi: 10.1093/molhr/6.11.983

Siebert, S., Farrell, S., Abeykoon, Y. L., Primack, A. S., Schnitzer, C. E., et al. (2019). Stem cell differentiation trajectories in Hydra resolved at single-cell resolution. Science 365:eaaq9314. doi: 10.1126/science.aaq9314

Sonoda, J., and Wharton, R. P. (1999). Recruitment of nanos to hunchback mRNA by Pumilio. Genes Dev. 13, 2704–2712. doi: 10.1101/gad.13.20.2704

Sprenger, M., and Wieschaus, E. (1986). Maternal-effect mutations altering the localization in the developing nervous system. Genes Dev. 2, 1847–1857. doi: 10.1101/gad.2.12.1847

Stern, C. H., Lee, T., Weeraratne, S. D., Kozh, V., Lim, T.-M., and Gong, Z. (2002). Zwi, the zebrafish homologue of the Drosophila piwi gene, co-localization with vasa at the embryonic genital ridge and gonad-specific expression in the adults. Gene Exp. Patterns 2, 257–260. doi: 10.1016/s1567-333x(02)00052-2

Tamanini, F., Meijer, N., Verheij, C., Hülsken, J., and Schilling, T. F. (2007). prx1 and prx2 encode transcriptional repressors with a C2H2 zinc finger motif. Gene 392, 174–184. doi: 10.1016/j.gene.2006.08.073

Taniwaki, T., Kato, Y., Matsuda, K., Hanyu-Nakamura, K., and Nakamura, A. (2011). Drosophila Mom2 couples Oskar-induced endocytosis with actin remodeling for cortical anchorage of the germ plasm. Development 138, 2523–2532. doi: 10.1242/dev.062208
Tanaka, T., and Nakamura, A. (2008). The endocytic pathway acts downstream of Oskar in Drosophila germ plasm assembly. Development 135, 1107–1117. doi: 10.1242/dev.017293

Tang, S. J., Meulemans, D., Vazquez, L., Colaco, N., and Schuman, E. (2001). A role for a rat homolog of staufen in the transport of RNA to neuronal dendrites. Neuron 32, 463–475. doi: 10.1016/S0896-6273(01)00493-7

Tautz, D. (1998). Debateable homologies. Nature 395, 17–19. doi: 10.1038/25604

Tay, J., and Richter, J. D. (2001). Germ cell differentiation and synaptomorph complex formation are disrupted in CPEB knockout mice. Dev. Cell 1, 201–213. doi: 10.1016/S1534-5807(01)00025-9

Tessler, C. R., and Broadie, K. (2008). Drosophila fragile X mental retardation protein developmentally regulates activity-dependent axon pruning. Development 135, 1547–1557. doi: 10.1242/dev.015867

Theis, M., Si, K., and Kandel, E. R. (2003). Two previously undescribed members of the mammalian neural stem cell stage acquired through a default mechanism. Cell Stem Cell 10, 299–311. doi: 10.1016/j.stem.2012.01.016

Wang, L., Dockendorff, T. C., Jongens, T. A., and Dreyfuss, G. (2000). Characterization of dFMR1, a Drosophila melanogaster homolog of the fragile X mental retardation protein. Mol. Cell. Biol. 20, 8536–8547. doi: 10.1128/mcb.20.22.8536-8547.2000

Wang, B., Collins, J. J. III, and Newmark, P. A. (2013). Functional genomic characterization of neoblast-like stem cells in larval Schistosoma mansoni. eLife 2:e00774

Wang, C., Dickinson, L. K., and Lehmann, R. (1994). Genetics of nanos localization in Drosophila. Dev. Dyn. 199, 103–115.

Wang, C., and Lehmann, R. (1991). Nanos is the localized posterior determinant in Drosophila. Cell 66, 637–647. doi: 10.1016/0092-6273(91)90101-k

Whelan, N. V., Kocot, K. M., Moroz, T. P., Mukherjee, K., Williams, P., Paulay, G., et al. (2017). Ctenophore relationships and their placement as the sister group to all other animals. Nat. Ecol. Evol. 1, 1737–1746. doi: 10.1038/s41559-017-0313-3

Wilhelm, J. E., Hilton, M., Amos, Q., and Henzel, W. J. (2003). Cup is an eIF4E binding protein required for both the translational repression of oskar and the recruitment of Barentsz. J. Cell Biol. 163, 1197–1204. doi: 10.1083/jcb.200309088

Wong, L. C., Costa, A., McLeod, I., Sarkeshik, A., Yates, J. III, Kyin, S., et al. (2011). The functioning of the Drosophila CPEB protein Orb is regulated by phosphorylation and requires casein kinase 2 activity. PLoS ONE 6:e24355. doi: 10.1371/journal.pone.0024355

Wu, L., Wells, D., Tay, D., Mendis, D., Abbott, M. A., Barnitt, A., et al. (1998). CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of alpha-CaMKII mRNA at synapses. Neuron 21, 1129–1139. doi: 10.1016/S0896-6273(00)00630-3

Yevike, B., Diao, L., Liu, J., Krsnik, Z., Vijverman, M. A., and Ephrussi, R., et al. (2017). Multiple roles of PIWI1 in mouse neocorticogenesis. bioRxiv [Preprint] doi: 10.1101/06070

Villacé, P., Marín, M. R., and Ortín, J. (2004). The composition of staufen-containing RNA granules from human cells indicates their role in the transport and translation of messenger RNAs. Nucleic Acids Res. 32, 2411–2420. doi: 10.1093/nar/gkh552

Voronina, E., Seydoux, G., Sassone-Corsi, P., and Nagamori, I. (2011). RNA granules in germ cells. Cold Spring Harb. Perspect. Biol. 3:a002774. doi: 10.1101/cshperspect.a002774
Xu, E. Y., Moore, F. L., and Pera, R. A. (2001). A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. Proc. Natl. Acad. Sci. U.S.A. 98, 7414–7419. doi: 10.1073/pnas.131090498

Xu, S., Hafer, N., Agunwamba, B., and Schedl, P. (2012). The CPEB protein Orb2 has multiple functions during spermatogenesis in Drosophila melanogaster. PLoS Genet. 8:e1003079. doi: 10.1371/journal.pgen.1003079

Xu, X., Brechbiel, J. L., and Gavis, E. R. (2013). Dynein-dependent transport of nanos RNA in Drosophila sensory neurons requires rumpelstiltskin and the germ plasm organizer oskar. J. Neurosci. 33, 14791–14800. doi: 10.1523/jneurosci.5864-12.2013

Xu, Y., Wang, H., Zhou, J., Lei, Y., Zhou, Y., Yang, Q., et al. (2010). Zebrafish nanos interacts with and regulates the phosphorylation of Mylz2. Biochimie 92, 1812–1817. doi: 10.1016/j.biochi.2010.07.010

Yajima, M., and Wessel, G. M. (2011a). The DEAD-box RNA helicase Vasa functions in embryonic mitotic progression in the sea urchin. Development 138, 2217–2222. doi: 10.1242/dev.065052

Yajima, M., and Wessel, G. M. (2011b). The multiple hats of Vasa: its functions in the germline and in cell cycle progression. Mol. Reprod. Dev. 78, 861–867. doi: 10.1002/mrd.21363

Yang, L., Duan, R., Chen, D., Wang, J., Chen, D., and Jin, P. (2007). Fragile X mental retardation protein modulates the fate of germline stem cells in Drosophila. Hum. Mol. Genet. 16, 1814–1820. doi: 10.1093/hmg/ddm129

Yang, N., Yu, Z., Hu, M., Wang, M., Lehmann, R., and Xu, R. M. (2015). Structure of Drosophila Oskar reveals a novel RNA binding protein. Proc. Natl. Acad. Sci. U.S.A. 112, 11541–11546. doi: 10.1073/pnas.1515568112

Ye, B., Petritsch, C., Clark, I. E., Gavis, E. R., Jan, L. Y., and Jan, Y. N. (2004). nanos and pumilio are essential for dendrite morphogenesis in Drosophila peripheral neurons. Curr. Biol. 14, 314–321. doi: 10.1016/j.cub.2004.01.052

Yoon, Y. J., and Mowry, K. L. (2004). Xenopus staufen is a component of a ribonucleoprotein complex containing Vg1 RNA and kinesin. Development 131, 3035–3045. doi: 10.1242/dev.01170

Zamore, P. D., Bartel, D. P., Lehmann, R., and Williamson, J. R. (1999). The PUMILIO-RNA interaction: a single RNA-binding domain monomer recognizes a bipartite target sequence. Biochemistry 38, 596–604. doi: 10.1021/bi982264s

Zamore, P. D., Williamson, J. R., and Lehmann, R. (1997). The pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. RNA 3, 1421–1433.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., et al. (1997). A conserved RNA-binding protein that regulates sexual fates in the C. elegans hermaphrodite germ line. Nature 390, 477–484. doi: 10.1038/37297

Zhang, M., Chen, D., Xia, J., Han, W., Cui, X., Neuenkirchen, N., et al. (2017). Post-transcriptional regulation of mouse neurogenesis by Pumilio proteins. Genes Dev. 31, 1354–1369. doi: 10.1101/gad.298752.117

Zhang, Y., O’Connor, J. P., Siomi, M. C., Srinivasan, S., Dutra, A., Nussbaum, R. L., et al. (1995). The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. EMBO J. 14, 5358–5366. doi: 10.1002/j.1460-2075.1995.tb00220.x

Zhang, Y. Q., Bailey, A. M., Matthies, H. J., Renden, R. B., Smith, M. A., Speese, S. D., et al. (2001). Drosophila fragile X-related gene regulates the MAP1B homolog futsch to control synaptic structure and function. Cell 107, 591–603. doi: 10.1016/s0092-8674(01)00589-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Kulkarni, Lopez and Extavour. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.