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Convergent evolution of germ granule nucleators: A hypothesis

Arpita Kulkarni [a,*], Cassandra G. Extavour [a,b,*]

[a] Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA
[b] Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA

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ABSTRACT

Germ cells have been considered "the ultimate stem cell" because they alone, during normal development of sexually reproducing organisms, are able to give rise to all organismal cell types. Morphological descriptions of a specialized cytoplasm termed 'germ plasm' and associated electron dense ribonucleoprotein (RNP) structures called 'germ granules' within germ cells date back as early as the 1800s. Both germ plasm and germ granules are implicated in germ line specification across metazoans. However, at a molecular level, little is currently understood about the molecular mechanisms that assemble these entities in germ cells. The discovery that in some animals, the gene products of a small number of lineage-specific genes initiate the assembly (also termed nucleation) of germ granules and/or germ plasm is the first step towards facilitating a better understanding of these complex biological processes. Here, we draw on research spanning over 100 years that supports the hypothesis that these nucleator genes may have evolved convergently, allowing them to perform analogous roles across animal lineages.

1. Introduction

Germ cells form an important subset of stem cells in metazoans, and are specified during embryogenesis either by the inheritance of a specialized cytoplasm, or via zygotic induction, i.e. extra-cellular signaling from neighboring somatic cells (Extavour and Akam, 2003). Germ cells are critical in maintaining the continuity of life and hence have been called "immortal" and "the ultimate stem cell" by some authors (Cinalli et al., 2008; Gao and Arkov, 2013; Nussbaum, 1880; Wilson, 1896). In the early 1890s, August Weismann pointed out that it was not that the germ cells per se remained continuous and immortal, but rather that the passage of certain "substances" from the parent germ cell to its progeny resulted in this "immortal" continuity (Weismann, 1892). He referred to these substances collectively as "germ plasm," thus providing, to our knowledge, the earliest recorded use of this term (*Das Keimplasma*: Weismann, 1892). Although Weismann had originally used the term germ plasm to mean nuclear genetic material (discussed by Lankenau, 2008), today germ plasm refers to a specialized cytoplasm, often morphologically and spatially distinct, that is contained within and confers fate upon the germ cell lineage (Eddy, 1975; Gao and Arkov, 2013; Guraya, 1979; Ikenishi, 1998; Voronina et al., 2011; Weismann, 1892). Contemporaneous with and following Weismann's discovery and description of germ plasm came independent observations that this cytoplasm contained granular material, termed "germ granules" (Hegner, 1911; Metschnikoff, 1866; Ritter, 1890). These granules were later found to contain specific ribonucleoprotein (RNP) complexes that often included apparent molecular determinants of germ line specification (see for example Illmensee and Mahowald, 1974, 1976; Strome and Wood, 1982). Germ line RNP complexes have been referred to using various terms in the literature over the years, oftentimes as a consequence of observed morphologies at various developmental stages in different organisms (Table 1). Throughout this review we will collectively refer to germ line RNP complexes as germ granules for simplicity and consistency.

Although germ granules were discovered more than a century ago, we are only recently beginning to understand the molecular-level biology behind their formation (also referred to as nucleation) and composition across metazoans (Gao and Arkov, 2013). In this review, we limit our discussion to the evolution of proteins that appear to cause or catalyze the nucleation of germ granules in non-mammalian species. Multiple pathways (reviewed by Voronina et al., 2011) that include the (inter)action of seed proteins known as "nucleators" (e.g. Oskar, Bucky ball, Xvelo1 and PGL), mitochondria (Huang et al., 2011; Watanabe et al., 2011), Tudor-domain containing proteins (Arkov et al., 2006), low-specificity protein-RNA interactions (Brangwynne et al., 2009) and small non-coding RNAs (Sengupta and Boag, 2012) are currently implicated in this process. Using a comparative approach that focuses on recent advances in our molecular understanding of the few germ granule nucleators listed above, we look at what is known about how novel nucleators arise, and ask whether the available literature can be used to address the hypothesis that these nucleators have evolved convergently to perform analogous roles.

* Corresponding authors.
E-mail addresses: akulkarni@fas.harvard.edu (A. Kulkarni), extavour@oeb.harvard.edu (C.G. Extavour).

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Table 1
Germ granule nomenclature simplified by developmental stage and/or cell type.

Developmental stage	Referred to as	Select species examples	Select references
Immature and undifferentiated/ developing germ cells	Nuage, perinuclear granules	*Caenorhabditis elegans*	(Strome and Wood, 1982)
		Drosophila melanogaster	(Mahowald, 1968)
		Xenopus laevis	(Ikenishi et al., 1996)
		Danio rerio	(Knaut et al., 2000)
		Mus musculus	(Chuma et al., 2009)
Mature gametes such as oocytes and sperm	(Oocytes) Sponge bodies, Balbiani body, mitochondrial cloud, germ plasm	*Drosophila melanogaster*	(Cox and Spradling, 2003; Hurd et al., 2016; Snee and Macdonald, 2004)
		Xenopus laevis	(Kloc et al., 2004) (Bilinski et al., 2004)
		Danio rerio	(Bontems et al., 2009; Marlow and Mullins, 2008)
		Mus musculus	(Pepling et al., 2007; Spiegelman and Bennett, 1973)
	(Sperm) Chromatoid bodies, inter-mitochondrial cement	*Mus musculus*	(Chuma et al., 2009; Spiegelman and Bennett, 1973)
Embryos	P-granules	*Caenorhabditis elegans*	(Strome and Wood, 1982)
	Germ plasm, polar granules, Balbiani body	*Drosophila melanogaster*	(Illmensee and Mahowald, 1974, 1976)
		Xenopus laevis	(Kloc et al., 2004)
		Danio rerio	(Bontems et al., 2009; Marlow and Mullins, 2008)

2. Germ granules are characteristic of germ cells but may not always confer germ cell identity

Here we use the term "germ granules" to describe a class of cytoplasmic RNP complexes with differing morphologies and localization patterns during development (Table 1), unique to and characteristic of germ cells (Arkov and Ramos, 2010; Eddy, 1975; Gao and Arkov, 2013; Ikenishi, 1998; Schisa, 2012; Voronina et al., 2011). These complexes have been previously described as motile, electron dense, compact, highly dynamic, fibrillar or granular in appearance and lacking a membrane (Arkov and Ramos, 2010; Eddy, 1975; Gao and Arkov, 2013; Ikenishi, 1998; Schisa, 2012; Voronina et al., 2011). Although non-membrane bound, germ granules are organized in their architecture. Recently, it has been shown that some germ granules are divided into subdomains of specific protein and/or RNA composition (see for example Little et al., 2015; Schisa, 2012). Germ granules are required for germ cell function in all organisms, even though many organisms do not depend on them to specify germ cell fate (reviewed by Voronina et al., 2011). Thus, these granules can be formed *de novo* in primordial germ cells upon induction (e.g. *Mus musculus*) or inherited as part of the maternal germ plasm (e.g. *Drosophila melanogaster*, *Danio rerio* and *Xenopus laevis*) (see Table 1 for details and references). Recent advances in our understanding of animals that inherit germ granules suggest that germ plasm and germ granules are not equivalent, and may in fact represent distinct functional entities (reviewed by Marlow, 2015).

Germ cells can maintain other RNP complexes that are distinct entities from germ granules, including processing bodies (P-bodies) and stress granules. The latter two types of RNP complexes are also found in somatic cells (Balagopal and Parker, 2009; Nover et al., 1989). However, growing evidence suggests that all of these complexes share multiple components in common (Fig.1) and may therefore be related (Gallo et al., 2008; reviewed by Voronina et al., 2011). While the RNA within all of these RNP granules consists of both coding and non-coding components, three consistent protein classes are characteristic of all granules: RNA helicases (e.g. Vasa), Tudor-domain proteins (e.g. Tudor), and Piwi family proteins (e.g. Piwi) (Gao and Arkov, 2013). This may explain why the expression and function of genes such as *vasa* and *piwi*, which are often considered germ cell markers, are not restricted to the germ line, but are also integral to the maintenance and differentiation of somatic cells (see for example Alié et al., 2011; Ewen-Campen et al., 2010; Schwager et al., 2015; Yajima and Wessel, 2011).

Central to the idea of germ granule assembly was the discovery of a handful of proteins that function as germ granule inducers, assemblers or nucleators. These proteins help initiate the assembly of germ granules (and/or germ plasm) by recruiting several similar downstream components. Some of these components are highly conserved, such as Vasa and Piwi family members, and others may be more species-specific (Hanazawa et al., 2011; Hay et al., 1988a, 1988b; Lasko and Ashburner, 1988; Raz, 2000). Examples of apparent germ granule nucleators include Oskar (*osk*) from *D. melanogaster* (Lehmann and Nüsslein-Volhard, 1986), PGL proteins (*pgl-1* and *pgl-3*) from *C. elegans* (Hanazawa et al., 2011), and the vertebrate-specific Bucky ball (*buc*) from *D. rerio* (Bontems et al., 2009; Marlow and Mullins, 2008) along with its *X. laevis* homolog, Vegetally localized 1 (*xvelo1*) (Nijjar and Woodland, 2013). *osk*, *pgl* and *buc* share low sequence similarity with each other and are not orthologous, suggesting that they are novel lineage-specific genes that arose independently. *osk*, being the best understood of these genes, is discussed in a comparative context below.

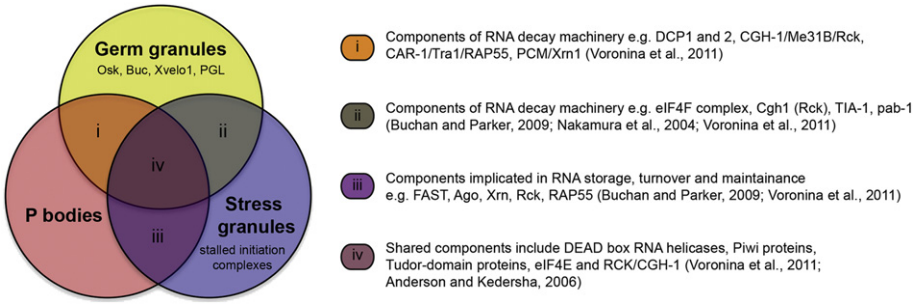

Fig. 1. Shared and distinct components of RNP granules in germ cells and somatic cells. A venn diagram (left) showing some examples of shared components (detailed at right) between germ granules, P-bodies and stress granules (Anderson and Kedersha, 2006; Buchan and Parker, 2009; Nakamura et al., 2004; Voronina et al., 2011). Components currently thought to be unique to each class of RNP complexes are also listed. It should be noted that a complete list of components for any RNP granule-type is currently lacking, limiting our understanding of shared and unique components.