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## Phase Separation in Germ Cells and Development

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### Abstract

The animal germline is an immortal cell lineage that gives rise to eggs and/or sperm each generation. Fusion of an egg and sperm, or fertilization, sets off a cascade of developmental events capable of producing an array of different cell types and body plans. How germ cells develop, function, and eventually give rise to entirely new organisms is an important question in biology. A growing body of evidence suggests that phase separation events likely play a significant and multifaceted role in germ cells and development. Here, we discuss the organization, dynamics, and potential functions of phase-separated compartments in germ cells and examine the various ways in which phase separation might contribute to the development of multicellular organisms.

### Introduction

Eukaryotic cells produce a multitude of subcellular compartments that concentrate specific molecules in space and time to help organize cellular processes. Some of these compartments, such as mitochondria, are organized and delimited by lipid membranes. However, many other compartments lack a membrane barrier, yet remain distinct from the surrounding nucleoplasm or cytoplasm. These membraneless compartments contribute to a wide range of cellular processes. More prominent examples of membraneless compartments include nucleoli, Cajal bodies, stress granules, processing bodies (P-bodies), and germ granules. However, the list of known membraneless compartments extends far beyond these examples and will likely continue to grow.

The question of how membraneless compartments form is under intense investigation. Mounting evidence suggests that many membraneless compartments such as nucleoli and germ granules form via liquid-liquid phase separation (LLPS), similar to how oil separates from vinegar in a vinaigrette (Banani *et al.*, 2017). LLPS occurs when the contents of a homogenous liquid demix into two distinct liquid phases. In cells, LLPS can occur when a given set of molecules demixes from the surrounding cytoplasm (or nucleoplasm) to form liquid-like droplets, or condensates (Hyman, Weber and Jülicher, 2014). Current models posit that LLPS is driven in part by weak, multivalent interactions between proteins and nucleic acids, and tends to involve proteins containing intrinsically disordered domains,

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which lack well-defined three-dimensional structures (Shin and Brangwynne, 2017). The resulting liquid-like condensates are structurally dynamic: they exhibit rapid internal molecular rearrangements and rapid exchange of molecules with the surrounding cytoplasm (or nucleoplasm). Due to their dynamic and labile nature, liquid-like condensates are highly sensitive to shifts in temperature, pH, and other environmental factors. LLPS can also be regulated by post-translational modifications and changes in the concentration of constituent molecules. In some cases, phase separation within cells can result in condensates exhibiting gel-like or even solid-like properties (Alberti, 2017). Recently, the field of phase separation has adopted the term “biomolecular condensates” to refer to phase-separated subcellular compartments.

Recent studies show that several proteins involved in key developmental processes such as gene regulation and signal transduction undergo phase separation (Hnisz *et al.*, 2017; Case, Ditlev and Rosen, 2019). It is intriguing, therefore, to speculate that cells use phase separation to coordinate the timing, location, and/or efficacy of developmental processes. Support for this idea is beginning to strengthen, especially as advancements in technology improve our ability to explore the dynamics of biomolecular condensates in living cells and tissues. Here, we review the ways that phase separation might facilitate development. We focus on germ cells, which are known to form a wide array of biomolecular condensates that contribute to germ cell identity and function. Because germ cell condensates are well-studied, diverse, and cell-type-specific, they serve as excellent model systems for studying phase separation in the context of development. We examine how germ cell condensates assemble, disassemble, and change properties in response to developmental cues. We also discuss how these condensates are spatially organized, both internally and in relation to other condensates, and speculate on how this organization could help coordinate different germ cell processes. Finally, we reflect on how different cell types may exploit the unique properties of LLPS and biomolecular condensates to carry out key developmental processes. For in-depth discussions on the role of phase separation in signaling and transcription, we refer the reader to other reviews in this special issue.

## Biomolecular Condensates in Germline Development

Metazoans develop via a cascade of highly choreographed events that regulate the timing of cell divisions, cell migrations, cell-to-cell communication, and pattern formation. Remarkably, this cascade of developmental events begins with a single cell - the fertilized egg, or zygote. As the zygote begins to divide, it gives rise to a diverse array of cell types with specialized functions. These cell types can be grouped into two general categories: 1) somatic cells, which make up most of the body and include skin cells, neurons, and blood cells; and 2) germ cells, which produce haploid gametes (eggs and sperm) for reproduction. Whereas somatic cells die out with the organism, gametes can fuse to produce a new zygote and begin the cascade of development anew.

Because germ cells give rise to new life generation after generation, the germline is considered to be “immortal”. Each generation, the germline cycles through the following phases: specification of the primordial germ cells (the precursors of gametes) during embryogenesis; proliferation, meiosis, and differentiation into gametes during later stages of

development; and, finally, fusion between an egg and sperm to produce a new, totipotent zygote (Lesch and Page, 2012). In addition to being immortal, germ cells are also unique in that they contain a class of membraneless compartments known as germ granules. Examples of germ granules include P granules in *C. elegans*, polar granules in *Drosophila*, and chromatoid bodies in mammals (Table 1) (Mahowald, 1962; Fawcett, Eddy and Phillips, 1970; Strome and Wood, 1982). Germ granules are present in the germ cells of most, if not all, animals and contain many proteins important for the function and development of germ cells (Voronina *et al.*, 2011; Gao and Arkov, 2013).

Germ granules contain RNA, as well as many proteins involved in RNA regulation and quality control; thus, germ granules are thought to play important roles in various RNA-related processes, including transposon silencing, mRNA surveillance by small regulatory RNAs, mRNA localization, and mRNA storage (Table 1) (Voronina *et al.*, 2011; Gao and Arkov, 2013). Germ granules likely coordinate such processes by concentrating specific proteins and RNAs together in space and time. Germ granules often localize to the outer nuclear periphery, where they associate with nuclear pores (Voronina *et al.*, 2011). One function of perinuclear germ granules may be to surveil and process nascent mRNAs as they exit the nuclear pore (see Significance of Phase Separation in Development). In some animals such as *C. elegans*, *Drosophila*, *Xenopus*, and zebrafish, germ granules are maternally inherited and segregate asymmetrically with germline blastomeres during early embryonic cell divisions. Germ granules are cytoplasmic during this stage of development and contain multiple maternal mRNAs, including some required for germ cell specification. Early embryonic germ granules may help promote germ cell fate by concentrating maternally inherited germ cell factors in germline blastomeres (Voronina *et al.*, 2011).

A growing body of evidence suggests that germ granules assemble through phase separation (Table 1). In fact, *C. elegans* P granules were the first membraneless compartment reported to form via LLPS, and P granules have since emerged as a leading model for the study of biomolecular condensates (Brangwynne *et al.*, 2009). P granules exhibit multiple liquid-like behaviors: they are spherical except when perinuclear, they readily fuse together, they drip off the nucleus in response to shear stress, and fluorescence recovery after photobleaching (FRAP) experiments suggest that they are highly dynamic (Brangwynne *et al.*, 2009; Sheth *et al.*, 2010; Putnam *et al.*, 2019). Germ granules in other animals also show hints of liquid-like behavior, as germ granules in zebrafish can undergo liquid-like fusion events (Strasser *et al.*, 2008), and multiple types of germ granules in *Drosophila* (polar granules, nuclear germ granules, perinuclear nuage, and piRNA nuage giant bodies, or piNG-bodies) rapidly exchange molecules with the surrounding cytoplasm (Snee and Macdonald, 2004; Nosov, Kibanov and Olenina, 2014; Kistler *et al.*, 2018). Further work characterizing the biophysical properties of different types of germ granules should help determine whether phase separation is a widespread mechanism of germ granule assembly.

In addition to driving germ granule formation, phase separation-related processes likely coordinate other aspects of germline development, as well. For instance, developing vertebrate oocytes compartmentalize protein, RNA, and various membrane-bound organelles into a large, membraneless compartment known as the Balbiani body (Table 1) (Kloc, Bilinski and Etkin, 2004; Jamieson-Lucy and Mullins, 2019). Proposed functions of the

Balbiani body include germ cell specification and oocyte quality control (Lei and Spradling, 2016; Jamieson-Lucy and Mullins, 2019). In *Xenopus*, the Balbiani body is rigid, seemingly non-dynamic, resistant to harsh conditions *ex vivo*, and stains with an amyloid-specific dye (Chang *et al.*, 2004; Boke *et al.*, 2016). Unlike typical amyloids, however, the Balbiani body is spherical and its formation is reversible, as the Balbiani body disassembles in later stages of oocyte development. To account for all these properties of the Balbiani body, (Woodruff, Hyman and Boke, 2018) propose that Balbiani bodies initially assemble via LLPS, but then rapidly harden into a solid-like state by forming an amyloid-like matrix (Woodruff, Hyman and Boke, 2018). Understanding the mechanisms by which LLPS-generated condensates harden into solid-like states will likely lead to a greater understanding of how such organelles contribute to development. Interestingly, the Balbiani body forms specifically in early oocytes, which have the remarkable ability to remain dormant for months or even years. It is therefore intriguing to speculate that one function of Balbiani body formation and hardening is to preserve the contents of the oocyte during prolonged periods of meiotic arrest (Boke *et al.*, 2016). Other types of condensates may serve similar functions in oocytes of animals that do not form Balbiani bodies. For instance, in *C. elegans*, oocytes subjected to prolonged arrest form large, P-body-like compartments (often termed grP-bodies) that are thought to store and protect maternal mRNAs in the absence of sperm (Table 1) (Schisa, Pitt and Priess, 2001; Boag *et al.*, 2008; Jud *et al.*, 2008; Noble *et al.*, 2008; Hubstenberger *et al.*, 2013). Thus, phase separation may play a common and important role in preserving the quality of arrested oocytes in animals.

## Drivers and Regulators of Phase Separation in Germ Cells

Proteins containing RNA-binding domains, intrinsically disordered regions, and/or low sequence complexity play key roles in the assembly of germ cell condensates. For example, intrinsically disordered proteins rich in serine (MEG-3, MEG-4, and DEPS-1), proteins containing RG-rich RNA-binding domains (PGL-1, PGL-3, and LAF-1), and multiple FG-repeat proteins including the RNA helicase GLH-1 are all known to contribute to P granule formation in *C. elegans* (Spike *et al.*, 2008; Marnik and Updike, 2019). Some or all of these proteins may help assemble P granules by promoting LLPS. In support of this idea, recombinant MEG-3, PGL-3, and LAF-1 can all phase separate into liquid-like condensates *in vitro* (Elbaum-Garfinkle *et al.*, 2015; Saha *et al.*, 2016; Smith *et al.*, 2016). Furthermore, when MEG-3, PGL-3, and RNA are all combined in the same buffer, MEG-3 and PGL-3 co-assemble into condensates with substructures similar to those of P granules (see Internal Organization of Germ Granules) (Putnam *et al.*, 2019). GLH-1 and other FG-repeat proteins may undergo phase separation to promote the physical association of P granules with nuclear pores, as tethering of P granules to nuclei requires the presence of both FG-rich proteins in P granules and FG-rich nucleoporins in the nuclear pore complex, and nucleoporin FG domains readily phase separate *in vitro* (Frey, Richter and Görlich, 2006; Updike and Strome, 2009; Voronina and Seydoux, 2010; Schmidt and Görlich, 2015; Marnik *et al.*, 2019; Chen *et al.*, 2020). GLH-1 is an ortholog of *Drosophila* Vasa and other Vasa-like helicases, which localize to germ granules in many animals (Voronina *et al.*, 2011). In *Drosophila*, Vasa interacts with the intrinsically disordered RNA-binding protein Osk to help assemble germ granules (Breitwieser *et al.*, 1996; Jeske *et al.*, 2015; Lehmann, 2016;

Kistler *et al.*, 2018). Notably, Ddx4, the human ortholog of Vasa, forms phase-separated condensates *in vitro* and when expressed ectopically in HeLa cells (Nott *et al.*, 2015). Specific sequence features of Ddx4 and *C. elegans* LAF-1 govern the phase behavior of these proteins *in vitro* and in heterologous cells, and it will be interesting to test whether these features impact germ granule formation and/or function (Nott *et al.*, 2015; Schuster *et al.*, 2020).

Post-translational modifications are emerging as important regulators of LLPS (Hofweber and Dormann, 2019; Owen and Shewmaker, 2019). For instance, the *C. elegans* P granule assembly protein MEG-3 is a substrate for the kinase MBK-2 and the phosphatase PP2A, and phosphorylation and dephosphorylation of MEG-3 is thought to promote P granule dissolution and condensation, respectively (Wang *et al.*, 2014). Additionally, arginine methylation of Ddx4 dramatically impairs Ddx4 condensation *in vitro*, perhaps by disrupting cation- $\pi$  interactions between FG and RG repeats within Ddx4 (Nott *et al.*, 2015). In addition to regulating condensate formation, post-translational modifications may also affect the material properties of condensates. In zebrafish, Balbiani body assembly requires the intrinsically disordered protein Bucky ball (Buc) (Marlow and Mullins, 2008; Bontems *et al.*, 2009). Recently, the multi-Tudor domain-containing protein Tdrd6a was shown to promote the mobility of Buc within Balbiani bodies (Roovers *et al.*, 2018). Tdrd6a interacts directly with methylated arginines in Buc that reside within a tri-RG motif, suggesting that Balbiani body dynamics may be subject to regulation by post-translational modifications (Roovers *et al.*, 2018). Thus, post-translational modifications of intrinsically disordered proteins likely trigger a variety of effects on phase separation in germ cells.

RNA is also likely to play an important role in the phase separation of germ cell condensates. In the case of *C. elegans* P granules, phase separation is thought to be driven in part by interactions between mRNAs and RNA-binding proteins. Support for this idea originated from studies showing that RNA stimulates the condensation of both MEG-3 and PGL-3 *in vitro* (Saha *et al.*, 2016; Smith *et al.*, 2016). PGL-3 binds mRNA *in vitro* with low sequence specificity via the C-terminal RGG domain (Saha *et al.*, 2016). Although MEG-3 lacks a recognizable RNA-binding domain, MEG-3 also binds RNA *in vitro*, as well as *in vivo* (Smith *et al.*, 2016; Lee *et al.*, 2020). *In vivo*, MEG-3 preferentially binds ribosome-poor mRNAs independent of mRNA sequence (Lee *et al.*, 2020). Interestingly, translational repression is sufficient to direct mRNAs to P granules, and increasing cellular pools of ribosome-poor mRNAs (via heat shock or treatment with certain translation inhibitors) causes P granules to enlarge (Lee *et al.*, 2020; Parker *et al.*, 2020). Altogether, these observations suggest that non-specific RNA-protein interactions - particularly between ribosome-poor mRNAs and MEG-3 - help drive P granule condensation in *C. elegans* germ cells. RNA could potentially regulate the viscosity of P granules, as well: adding short RNAs (15–50 nt) to LAF-1 condensates *in vitro* increases condensate fluidity, whereas long RNAs (3,000 nt) have the opposite effect (Elbaum-Garfinkle *et al.*, 2015; Wei *et al.*, 2017). A challenge for the future will be to determine the extent to which cells use RNA to tune and regulate phase separation in germ cells and beyond.

## Internal Organization of Germ Granules

The composition of germ granules and other germ cell condensates is often non-uniform, with specific proteins and RNAs localizing to distinct subcompartments within a given condensate. Perhaps due to their relatively large size (up to 10  $\mu\text{m}$ ), *C. elegans* grP-bodies exhibit especially striking subdomains, including clusters marked by the poly(A)-binding protein PAB-1, the mRNA decay factor PATR-1, and the RNA-binding protein PGL-1 (Figure 1A) (Jud *et al.*, 2008; Hubstenberger *et al.*, 2013). The mechanistic underpinnings by which condensates like grP-bodies form and maintain internal subcompartments is an area of current investigation. In some cases, condensate subcompartments may be maintained in part by differences in subcompartment surface tensions (Feric *et al.*, 2016; Shin and Brangwynne, 2017). Future studies are likely to reveal the mechanisms by which condensate subcompartments form and may reveal additional mechanisms by which these structures are maintained.

In *C. elegans* embryos, P granules contain at least two subcompartments: an inner core, and an outer layer marked by the intrinsically disordered protein MEG-3 (Wang *et al.*, 2014; Putnam *et al.*, 2019). MEG-3 and its paralog MEG-4 function specifically during the early stages of embryogenesis to assemble P granules in germ cell precursors (Wang *et al.*, 2014). As a part of this process, MEG-3 condenses into structures that coat the P granule periphery (Figure 1B) (Wang *et al.*, 2014; Putnam *et al.*, 2019). In comparison to the P granule core, which exhibits many liquid-like behaviors, the outer MEG-3 layer is less dynamic and less sensitive to perturbations such as an up-shift in temperature (Putnam *et al.*, 2019). The distinct, gel-like properties of the MEG-3 phase are thought to help nucleate and stabilize the more labile P granule core (Putnam *et al.*, 2019). Embryonic P granules house maternal mRNAs, including some that function in germ cell specification, and the MEG-3 phase helps recruit and retain such mRNAs within P granules (Seydoux and Fire, 1994; Subramaniam and Seydoux, 1999; Schisa, Pitt and Priess, 2001; Smith *et al.*, 2016; Lee *et al.*, 2020; Parker *et al.*, 2020). By preferentially condensing in the germline-destined portion of the cytoplasm during early embryonic divisions, P granules - and the MEG-3 phase, in particular - may help enrich germline-defining mRNAs in the germline founder cell (Lee *et al.*, 2020).

In addition to *C. elegans* P granules, other germ cell condensates also possess distinct core and surface layers. For instance, polar granules in *Drosophila* primordial germ cells, piNG-bodies (piRNA nuage giant bodies) in *Drosophila* male germ cells, and piP-bodies in mouse prospermatogonia all contain proteins that localize to either the condensate core or the condensate periphery (Figure 1C) (Aravin *et al.*, 2009; Kibanov *et al.*, 2011; Vo *et al.*, 2019). Distinctions between the core and surface are also apparent in other types of condensates, including stress granules, nucleoli, and paraspeckles (Shin and Brangwynne, 2017). Therefore, core and surface subdivisions are a common feature of biomolecular condensates. Proteins that localize to the surface of condensates could potentially serve a variety of roles in condensate biology, as they could act as “gatekeepers” that regulate which molecules enter or exit the condensate, as stabilizers of condensate cores, as adaptors that facilitate interactions with other subcellular structures, or as biological surfactants that tune condensate stability.



Additionally, specific RNAs can also localize to distinct subregions within condensates. For instance, RNA-defined subcompartments are a notable feature of *Drosophila* polar granules. During late oogenesis, several mRNAs important for germ cell development, including *nos*, *gcl*, and *CycB*, accumulate in the oocyte posterior, where they incorporate into polar granules (Lehmann, 2016; Trcek and Lehmann, 2019). Upon entering polar granules, mRNAs organize into discrete homotypic clusters (Figure 1D) (Little *et al.*, 2015; Trcek *et al.*, 2015, 2020; Niepielko, Eagle and Gavis, 2018). Interestingly, homotypic clusters can localize to different positions within polar granules: clusters of *cycB* mRNA typically reside in the center of *Vasa*-marked granules, clusters of *nos* mRNA tend to occupy a middle zone, and clusters of *gcl* mRNA coat the granule edge (Trcek *et al.*, 2015, 2020). RNA cluster positions appear to depend on the concentration of the mRNA within the granule (Trcek *et al.*, 2020). Homotypic mRNA clusters have also been observed in the Balbiani body/germ plasm of zebrafish and in *C. elegans* P granules, suggesting that this phenomenon may be common among germ cell condensates (Roovers *et al.*, 2018; Eno, Hansen and Pelegri, 2019; Parker *et al.*, 2020). Future studies exploring the organization of RNA within condensates will likely reveal if and how such organization impacts condensate function.

## Germ Granule Remodeling during Development

During development, germ granules undergo regulated changes in composition, morphology, and localization. P granules, for instance, are present during all stages of the *C. elegans* life cycle, but show distinct characteristics at different stages of germline development. Unlike P granules in early embryos, P granules in later stages of development do not harbor a MEG-3-marked surface phase and do not require MEG-3 and MEG-4 for assembly/maintenance (Wang *et al.*, 2014). Such post-embryonic P granules also lack other proteins found in embryonic P granules, including PIE-1, POS-1, MEG-1, and MEG-2 (Mello *et al.*, 1996; Tabara *et al.*, 1999; Leacock and Reinke, 2008). In addition to losing and gaining various proteins during development, P granules also show changes in subcellular localization. For most of the *C. elegans* life cycle, P granules associate with clusters of nuclear pores at the outer nuclear periphery of germ cell nuclei (Pitt, Schisa and Priess, 2000; Sheth *et al.*, 2010). In oocytes, however, P granules detach from the nucleus and remain cytoplasmic post-fertilization for the first few cell divisions of embryogenesis before reattaching to the nucleus (Strome and Wood, 1982). Changes in P granule localization and composition likely underlie changes in P granule function: Whereas cytoplasmic P granules in early embryos may assist in germ cell specification, as noted above (Lee *et al.*, 2020), perinuclear P granules in later stages of development are thought to help maintain germ cell identity by surveilling and processing nascent mRNAs as they exit the nucleus (Updike *et al.*, 2014; Knutson *et al.*, 2017).

At least two proteins that localize to P granules in early germline blastomeres (the RNA helicase ZNFX-1 and the Argonaute protein WAGO-4) separate from P granules at a precise time during early embryogenesis (during the birth of primordial germ cells) to form an independent condensate termed the Z granule (Figure 2) (Wan *et al.*, 2018). Z granules are liquid-like in nature and localize adjacent to P granules at the nuclear periphery (Wan *et al.*, 2018). Interestingly, Z granules appear to initially form by demixing from other components in the P granule (Figure 2A) (Wan *et al.*, 2018). How and why Z granules emerge from P

granules at a defined point in development is not yet known. P granules contain multiple small RNA pathway proteins including the Argonautes WAGO-1, CSR-1, and PRG-1, and P granules are thought to coordinate small RNA pathways (Batista *et al.*, 2008; Claycomb *et al.*, 2009; Gu *et al.*, 2009; Dodson and Kennedy, 2019; Lev *et al.*, 2019; Ouyang *et al.*, 2019). ZNFX-1 and WAGO-4 also contribute to small RNA-based gene regulation, as they help propagate a subset of small RNA-based gene silencing signals across generations (Ishidate *et al.*, 2018; Wan *et al.*, 2018). Thus, one role of embryonic P granules may be to sort out mRNAs destined for transgenerational silencing, while demixing of ZNFX-1/WAGO-4 to form Z granules may facilitate downstream silencing of these targets.

Notably, Z granule formation roughly coincides with the onset of germline transcription, as well as the attachment of P granules to nuclei, and these concurrent events could potentially trigger Z granule demixing via a number of mechanisms (Figure 2B) (Strome and Wood, 1982; Seydoux *et al.*, 1996; Wan *et al.*, 2018). It is possible, for instance, that the docking of P granules to nuclei is sufficient to promote Z granule demixing, as membrane surfaces are known to alter phase separation dynamics (Snead and Gladfelter, 2019). Alternatively, Z granule formation may be driven by post-translational modifications and/or by one or more proteins produced as a consequence of renewed germline transcription. Another intriguing possibility is that newly transcribed mRNAs play a direct role in Z granule formation. Time-lapse imaging of mRNAs transcribed from various inducible transgenes suggests that, as nascent mRNAs exit the nuclear pores of germ cell nuclei, they accumulate temporarily in P granules before exiting into the cytoplasm (Sheth *et al.*, 2010). Since RNA can influence the assembly and organization of condensates (Berry *et al.*, 2015; Rhine, Vidaurre and Myong, 2020), (Wan *et al.*, 2018) speculate that, as nascent germline mRNAs enter P granules, they interact with RNA-binding proteins such as ZNFX-1 and WAGO-4 to drive Z granule formation. Other types of perinuclear germ granules (piNG-bodies in *Drosophila* and chromatoid bodies in mice) also form during waves of transcription, hinting that transcriptional activation may be a common driver of phase separation at or near the outer nuclear membrane (Kibanov *et al.*, 2011; Meikar *et al.*, 2011). In support of this idea, injecting the RNA Polymerase II transcriptional inhibitor  $\alpha$ -amanitin into *C. elegans* gonads causes at least some P granule components to detach from the nucleus, whereas injecting translation inhibitors does not have the same effect (Sheth *et al.*, 2010).

Phase demixing may underlie other instances of germ granule remodeling, as well. For instance, *Drosophila* polar granules likely undergo internal demixing events during early embryogenesis: Initially, polar granule proteins including Oskar, Aubergine, and Tudor are distributed somewhat evenly within polar granules; however, later in development, these proteins relocate to distinct core and surface subcompartments (Harris and Macdonald, 2001; Jones and Macdonald, 2007; Trcek *et al.*, 2015; Vo *et al.*, 2019). Thus, phase demixing may be a common form of germ granule remodeling. Conversely, germ granule remodeling may also occur via the mixing of two or more different phases. In support of this idea, two different types of germ granules - piP-bodies and early chromatoid bodies - appear to merge together during mouse spermatogenesis to form mature chromatoid bodies (Figure 3A) (Tanaka *et al.*, 2011). The mixing of these two condensates depends on *Tdrd7*, which encodes a Tudor domain-containing protein that localizes to chromatoid bodies (Hosokawa *et al.*, 2007; Tanaka *et al.*, 2011). In *Tdrd7*<sup>-/-</sup> mutants, chromatoid bodies and piP-bodies fail



to merge completely, and instead form striking multi-condensate assemblages that, at least in some cases, comprise one piP-body “sandwiched” by two chromatoid bodies (Figure 3B) (Tanaka *et al.*, 2011). Given that Tdrd7 is required for spermatid differentiation, the merging of chromatoid bodies and piP-bodies may be an important step in male germ cell development in mammals (Tanaka *et al.*, 2011). Altogether, these observations suggest that regulated mixing and demixing of phase-separated structures may be a common mechanism by which cells coordinate developmental processes.

## Interactions between Germ Cell Condensates

Prior to merging with chromatoid bodies during mouse spermatogenesis, piP-bodies contact another type of condensate termed the pi-body (also referred to as intermitochondrial cement) (Figure 3A) (Aravin *et al.*, 2009; Shoji *et al.*, 2009). In prospermatogonia, piP-bodies often localize adjacent to or partially overlap with pi-bodies (Aravin *et al.*, 2009; Shoji *et al.*, 2009). Interestingly, pi-bodies and piP-bodies are thought to coordinate sequential steps in the processing of pre-pachytene PIWI-interacting RNAs (piRNAs), a class of small RNAs that mediates transposon silencing during early stages of mammalian spermatogenesis (Aravin *et al.*, 2009; Chuma and Pillai, 2009; Shoji *et al.*, 2009; Wang *et al.*, 2020). Thus, pi-bodies and piP-bodies are linked in terms of both location and function. Current models posit that pi-bodies and piP-bodies cooperate to produce and amplify pre-pachytene piRNAs that then silence transposable elements (Figure 3A) (Lehtiniemi and Kotaja, 2018). A second population of piRNAs, pachytene piRNAs, is expressed later in spermatocytes and spermatids and may target mRNAs for silencing within the chromatoid body, as the chromatoid body houses multiple piRNA pathway proteins, including the piRNA-binding PIWI proteins MILI and MIWI and the Tudor domain-containing proteins TDRD1, TDRD6, and TDRD7 (Figure 3A) (Lehtiniemi and Kotaja, 2018). Pachytene piRNAs may silence transposons and also regulate meiotic gene expression (Ernst, Odom and Kutter, 2017). In addition to mice, *Drosophila*, *C. elegans*, and likely other animals also appear to compartmentalize the piRNA silencing pathway into distinct, yet interconnected condensates (Dennis *et al.*, 2013; Murota *et al.*, 2014; Dennis, Brasset and Vaury, 2019; Hirakata and Siomi, 2019; Manage *et al.*, 2020). Such organization may help partition different steps of the piRNA pathway in space and time, while still allowing pathway intermediates to flow in an organized and highly choreographed fashion from one step to the next.

Regulated interactions between different condensates are especially evident in the germline of *C. elegans*. As noted above, ZNFX-1 and WAGO-4 demix from P granules during early embryogenesis to form Z granules, which remain directly adjacent to, and may be in direct contact with, P granules for most of development (Wan *et al.*, 2018). Interestingly, P granules and Z granules reside in multi-condensate assemblages that also include compartments known as *Mutator* foci and SIMR foci (Figure 4) (Phillips *et al.*, 2012; Wan *et al.*, 2018; Manage *et al.*, 2020). These assemblages are spatially ordered, as each typically contains a single Z granule sandwiched by a P granule on one end and a *Mutator* focus on the other (Wan *et al.*, 2018). Like P granules and Z granules, *Mutator* foci are thought to form via phase separation (Uebel *et al.*, 2018). The dynamics of SIMR foci and the exact position of SIMR foci with respect to other compartments remain undetermined. For

simplicity, we hereinafter refer to these multi-condensate assemblages as perinuclear nuage. The mechanisms underlying the formation and spatial ordering of *C. elegans* perinuclear nuage remain unclear. One possibility is that each type of condensate contains a distinct network of intermolecular interactions that partially overlaps with the networks of neighboring condensates (Sanders *et al.*, 2020).

Each subcompartment of *C. elegans* perinuclear nuage contains a distinct set of proteins involved in small RNA-based gene regulation. *Mutator* foci, for example, contain proteins needed to silence transposable elements (Phillips *et al.*, 2012). Many of these proteins are involved in amplifying small RNA populations by directing the synthesis of secondary small interfering RNAs (siRNAs) via RNA-dependent RNA polymerases (RdRPs) (Billi, Fischer and Kim, 2014). For instance, *Mutator* foci contain the nucleotidyl transferase RDE-3, which adds tails of alternating uridine and guanosine ribonucleotides (termed pUG tails) to the ends of mRNA fragments (Phillips *et al.*, 2012; Shukla *et al.*, 2020). pUG tails are thought to recruit RdRPs, which then synthesize secondary siRNAs using the pUGylated mRNA fragments as templates (Shukla *et al.*, 2020). RDE-3 and other *Mutator* foci components, including the RdRP RRF-1 and the RNA helicase MUT-14, are recruited to *Mutator* foci by the intrinsically disordered protein MUT-16, which nucleates *Mutator* foci assembly (Phillips *et al.*, 2012; Uebel *et al.*, 2018). *Mutator* foci are thought to serve as hubs for siRNA amplification because loss of MUT-16 (and therefore *Mutator* foci) substantially reduces the levels of a major class of secondary siRNAs (Zhang *et al.*, 2011). Further supporting this idea, RNA FISH coupled with immunofluorescence suggests that pUG-tailed RNAs enrich in *Mutator* foci (Shukla *et al.*, 2020). siRNA amplification in *C. elegans* germ cells is also mediated by the RdRP EGO-1, which may localize to P granules (Claycomb *et al.*, 2009). EGO-1 produces a specific class of secondary siRNAs that associate with the Argonaute CSR-1 (another P granule component), but also shows some redundancy with RRF-1 (the RdRP in *Mutator* foci) in the production of other types of secondary siRNAs (Claycomb *et al.*, 2009; Gu *et al.*, 2009). As noted earlier, P granules contain multiple Argonaute proteins, and Z granules contain ZNFX-1 and WAGO-4, which promote the inheritance of siRNA-based gene silencing across generations (Ishidate *et al.*, 2018; Wan *et al.*, 2018). SIMR foci are known to contain the Tudor domain-containing protein SIMR-1 and the small RNA factor RSD-2 (Manage *et al.*, 2020). Together, these observations show that several small RNA-related proteins localize to different subcompartments within *C. elegans* perinuclear nuage. Given that Z granules and SIMR foci were only recently identified, future studies are likely to provide additional insights into the components and functions of these structures, as well as potentially identify additional types of nuage subcompartments.

*Mutator* components amplify multiple types of small RNA-based gene-silencing signals, including those triggered by piRNAs (Das *et al.*, 2008; Bagijn *et al.*, 2012; Luteijn *et al.*, 2012; Shirayama *et al.*, 2012). Interestingly, piRNA targeting is thought to occur in P granules, which contain the piRNA-binding protein PRG-1 (Batista *et al.*, 2008; Wang and Reinke, 2008). It is therefore intriguing to speculate that different subcompartments of *C. elegans* perinuclear nuage orchestrate distinct steps in piRNA-based silencing: target recognition in P granules, and signal amplification in *Mutator* foci (Figure 4). SIMR foci may also function in piRNA-based silencing, downstream of P granules and upstream of

*Mutator* foci (Manage *et al.*, 2020). Because the different subcompartments of perinuclear nuage may function in related processes, these compartments are likely to communicate with one another, perhaps by relaying messages in the form of RNA. Why compartmentalize piRNA targeting and downstream silencing steps into different condensates? *C. elegans* piRNAs interact with essentially all germline mRNAs, yet only a subset of these mRNAs are silenced by the piRNA pathway (Shen *et al.*, 2018; Zhang *et al.*, 2018; Reed *et al.*, 2019). Therefore, one function of P granules may be to restrict piRNA activity, perhaps by ensuring that only unwanted mRNAs are routed to other subcompartments for downstream silencing. In support of this idea, the P granule assembly proteins MEG-3 and MEG-4 are required to protect certain germline-expressed mRNAs (e.g. *sid-1* and *rde-11*) from piRNA-based silencing (Dodson and Kennedy, 2019; Ouyang *et al.*, 2019). *sid-1* and *rde-11* mRNAs normally localize to P granules, suggesting that P granules play a direct role in protecting these mRNAs from silencing (Ouyang *et al.*, 2019). Because piRNAs can trigger robust transgenerational silencing in *C. elegans* (Ashe *et al.*, 2012; Lee *et al.*, 2012; Luteijn *et al.*, 2012; Shirayama *et al.*, 2012), nuage-based organization of the piRNA pathway may be particularly important for preventing runaway heritable silencing of the wrong germline mRNAs (Dodson and Kennedy, 2019; Lev *et al.*, 2019).

## Significance of Phase Separation in Development

Biomolecular condensates exhibit a variety of unique properties that cells and organisms could exploit to regulate and drive developmental processes. For instance, condensates are highly sensitive to multiple factors that guide and/or influence development, including post-translational modifications, temperature, pH, and local concentrations of key molecules: small changes in any of these factors can trigger rapid, switch-like effects in terms of phase separation. Switch-like events are common in development and often decide the developmental fate of cells; therefore, one role of phase separation in development may be to drive cell fate decisions by converting subtle developmental cues into robust, all-or-nothing outputs. In support of this idea, phase-separated condensates known as keratohyalin granules (KGs) are thought to promote the terminal differentiation of epidermal keratinocytes in mammals by rapidly dissolving in response to a pH shift (Figure 5A) (Quiroz *et al.*, 2020). Keratinocytes differentiate as they travel from the innermost basal layer of the epidermis to the outermost layer - the skin surface (Fuchs and Raghavan, 2002). During the late stages of differentiation, the intrinsically disordered protein profilaggrin helps assemble KGs, which grow and stiffen over time and eventually occupy a significant portion of the cytoplasm (Quiroz *et al.*, 2020). Once keratinocytes reach the acidic surface of the skin, however, KGs rapidly dissolve, and nuclei and other organelles degrade, leaving flat “ghost” cells that form the skin barrier (Figure 5A) (Quiroz *et al.*, 2020). Current models posit that the change in pH triggers the dissolution of KGs, which in turn promotes enucleation, perhaps by releasing degradation factors residing within KGs into the cytoplasm (Quiroz *et al.*, 2020).

The switch-like nature of phase separation may also be important for spatial patterning, as phase separation is known to occur at precise concentrations and can thereby amplify the effects of weak concentration gradients (Lee *et al.*, 2013). Imagine, for instance, that a condensate assembles only when the concentration of a key component rises above a certain

threshold. If that component were distributed in a concentration gradient across a cell, or groups of cells, then phase separation-related processes could turn subtle differences in concentration gradients into a sharp, spatial asymmetry in condensate formation with a boundary dictated by the threshold concentration. A variation on this scenario drives spatial patterning in the *C. elegans* zygote, which forms multiple protein concentration gradients, including an anterior-rich gradient of the RNA-binding protein MEX-5 (Griffin, 2015). In turn, the MEX-5 gradient gives rise to a pronounced asymmetry in P granule formation: P granules condense only in the posterior half of the zygote, and, therefore, segregate with the posterior blastomere as the zygote divides (Figure 5B) (Schubert *et al.*, 2000; Brangwynne *et al.*, 2009; Gallo *et al.*, 2010). MEX-5 may antagonize P granule formation in the zygote anterior by limiting the availability of RNAs, as MEX-5 binds RNA with high affinity, and RNA is thought to stimulate P granule condensation (Pagano *et al.*, 2007; Lee *et al.*, 2013; Saha *et al.*, 2016; Smith *et al.*, 2016). Notably, P granule asymmetry in zygotes also requires the kinase MBK-2, which phosphorylates the P-granule assembly protein MEG-3 to destabilize P granules (Pellettieri *et al.*, 2003; Quintin *et al.*, 2003; Wang *et al.*, 2014). Thus, *C. elegans* germ cells exploit the sensitivity of phase-separated P granules to both molecular concentrations and post-translational modifications to drive asymmetric cell division.

In addition to driving developmental decisions, phase separation may also regulate the fidelity of such decisions. During transmembrane signaling, for example, phase separation is thought to help ensure that only genuine signals (and not stochastic interactions) are able to activate downstream signaling components (Figure 5C) (Huang *et al.*, 2016, 2019; Case *et al.*, 2019). In two different actin regulatory signaling pathways (nephrin signaling in podocyte cells and T cell receptor signaling), receptor activation causes multiple components in the pathway to undergo phase separation at the membrane surface (Banjade and Rosen, 2014; Su *et al.*, 2016). Phase-separating components include the membrane receptor Nephrin and its cytoplasmic targets Nck and N-WASP in podocytes, and the membrane adaptor LAT and its cytoplasmic binding partners Grb2 and Sos1 in activated T cells. *In vitro* reconstitution assays suggest that multivalent interactions within the condensates help retain cytoplasmic signaling molecules at the membrane and thereby prolong communication between membrane signaling components and their cytoplasmic binding partners (Huang *et al.*, 2016, 2019; Case *et al.*, 2019). Signal transmission from the membrane to the cytoplasm is a kinetically slow, multi-step process; thus, phase separation provides a means for cells to both complete this transmission and to spatially restrict this process to genuine sites of activation.

Proper spatiotemporal regulation of gene expression contributes to many developmental processes, and certain biomolecular condensates are thought to serve as sites of gene regulation. *C. elegans* P granules, for example, contain several factors involved in post-transcriptional gene regulation (e.g. piRNA- and siRNA-related proteins, the mRNA decapping enzymes DCAP-1 and DCAP-2, and CCF-1, a subunit of the CCR4/NOT deadenylase complex) and are required for maintaining proper germline gene expression programs: Upon the depletion of P granules, germ cells lose their totipotency and begin to accumulate soma-specific transcripts (Updike and Strome, 2010; Updike *et al.*, 2014; Knutson *et al.*, 2017). As noted earlier, P granules associate with nuclear pores for much of development, and nascent mRNAs traffic through perinuclear P granules before entering the

cytoplasm (Strome and Wood, 1982; Pitt, Schisa and Priess, 2000; Sheth *et al.*, 2010). Therefore, current models posit that P granules help maintain germ cell totipotency by intercepting nascent soma-specific transcripts as they exit the nuclear pore, thereby preventing their translation (Knutson *et al.*, 2017). Given that many types of germ granules are perinuclear, phase separation may play a widespread role in curating the nascent transcriptome of germ cells.

Phase-separated condensates can adopt a wide range of material properties, which could be exploited by animals to drive and regulate developmental processes. P granules, for instance, exhibit a glycerol-like consistency, whereas the viscosity of grP-bodies is more comparable to peanut butter, and Balbiani bodies are virtually solid (Brangwynne *et al.*, 2009; Hubstenberger *et al.*, 2013; Boke *et al.*, 2016). Notably, different viscosities may be conducive to different functions: liquid-like properties could facilitate dynamic processes such as RNA processing, whereas more solid-like properties could suppress molecular dynamics during periods of dormancy, for instance. *In vitro*, liquid-like condensates can transition over time into more solid-like states, suggesting that cells may normally regulate condensate viscosity to prevent such transitions from occurring inappropriately (Lin *et al.*, 2015; Mollieux *et al.*, 2015; Patel *et al.*, 2015). In support of this idea, loss of the RNA helicase CGH-1 causes the grP-body component CAR-1 to condense into solid, sheet-like structures in the *C. elegans* germline (Audhya *et al.*, 2005; Boag, Nakamura and Blackwell, 2005; Hubstenberger *et al.*, 2013). It is possible that condensates also undergo phase transitions as a normal part of development, and that such transitions assist in developmental processes. For instance, as keratohyalin granules stiffen during epidermal differentiation, they begin to physically deform the nucleus (Figure 5A) (Quiroz *et al.*, 2020). Stiffening of keratohyalin granules (and subsequent deformation of the nucleus) could be a controlled process that promotes enucleation (Quiroz *et al.*, 2020). Understanding if and how cells might regulate condensate viscosity will likely lead to a better understanding of many biological processes including development.

## Open Questions

The topics discussed in this review raise a number of questions worthy of further exploration. How, for example, might cells regulate and exploit the physical properties of condensates to regulate key processes in germ cells and development? Does phase separation underlie the formation and function of other cell-type-specific organelles in addition to germ granules and keratohyalin granules? For development, in particular, it will be interesting to determine how and to what extent biomolecular condensates act as sensors to help organisms process external stimuli. Although *in vitro* experiments have provided significant insight into phase separation, *in vivo* experiments will be critical for understanding how biomolecular condensates function and change over the course of development. Genetic manipulations, imaging of living cells and tissues, and the use of phase-separation “sensors” should be particularly informative in terms of tools and approaches (Quiroz *et al.*, 2020). In addition, proximity labeling techniques, which have recently been used to probe the transcriptome and proteome of stress granules, could be useful for extracting a comprehensive list of all the proteins and RNAs that localize to different condensates (Markmiller *et al.*, 2018; Padrón, Iwasaki and Ingolia, 2019).



Future studies of germ cell condensates are likely to provide key insights into the functional significance of phase separation, as germ cells form a diverse array of condensates known to contain many proteins important for germ cell function. This review focuses mainly on the idea that condensates spatially organize cellular processes, serve as storage sites for protein and RNA, and/or facilitate switch-like decisions. However, additional/alternative functions are also possible. For instance, some condensates may function as noise buffers that stabilize protein levels/activity in the cytoplasm (or nucleoplasm) (Klosin *et al.*, 2020). Another possibility is that condensates limit or inactivate certain reactions in the cytoplasm by sequestering a key component. Pinning down the exact function(s) of a given condensate remains difficult, as it would require disrupting the assembly or integrity of that condensate without disrupting any of its individual components. Therefore, additional studies exploring the functional impacts of phase separation are needed and will likely require innovative approaches. Tools enabling the control of phase separation *in vivo* (e.g. light-activatable “Corelets”) may help advance this endeavor (Bracha *et al.*, 2018; Bracha, Walls and Brangwynne, 2019).

Lastly, we note that germ cell condensates form a variety of subcompartments and multi-condensate assemblages and that these structures are particularly ripe for exploration. Advancements in super-resolution imaging may be especially useful in determining how such structures are organized. In the case of multi-condensate structures, understanding how different condensates might interact and communicate should provide insight into the organization of complex pathways in germ cells and beyond.

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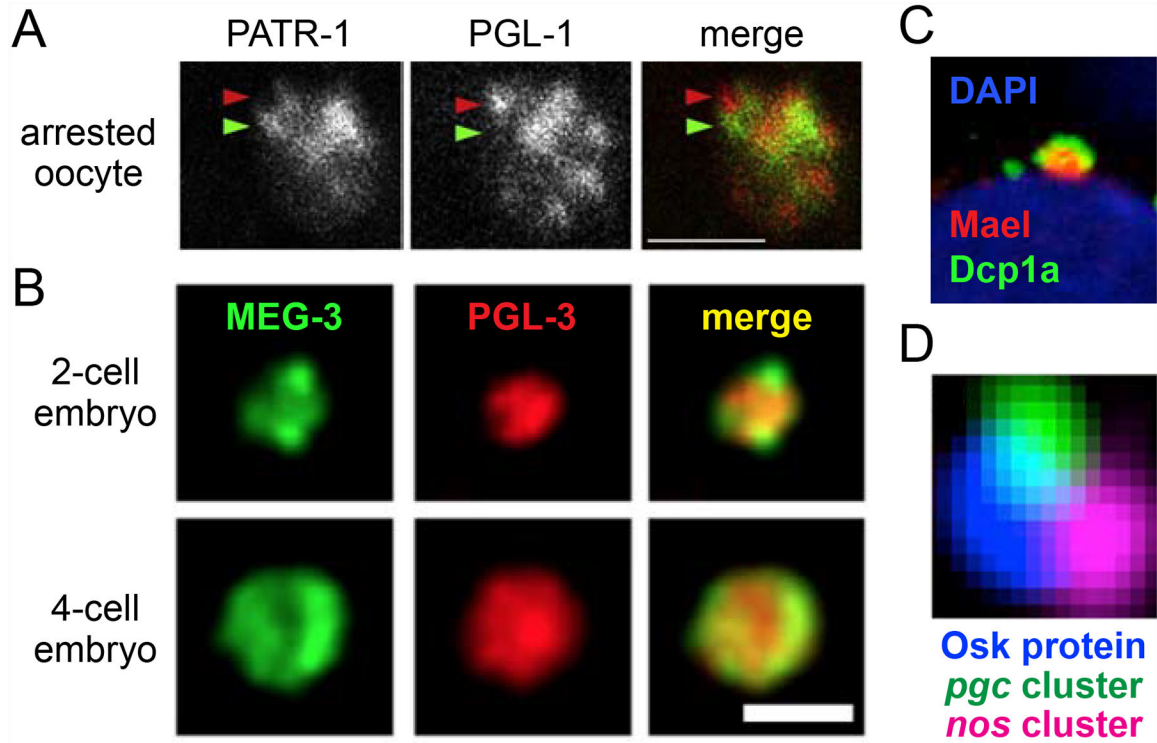
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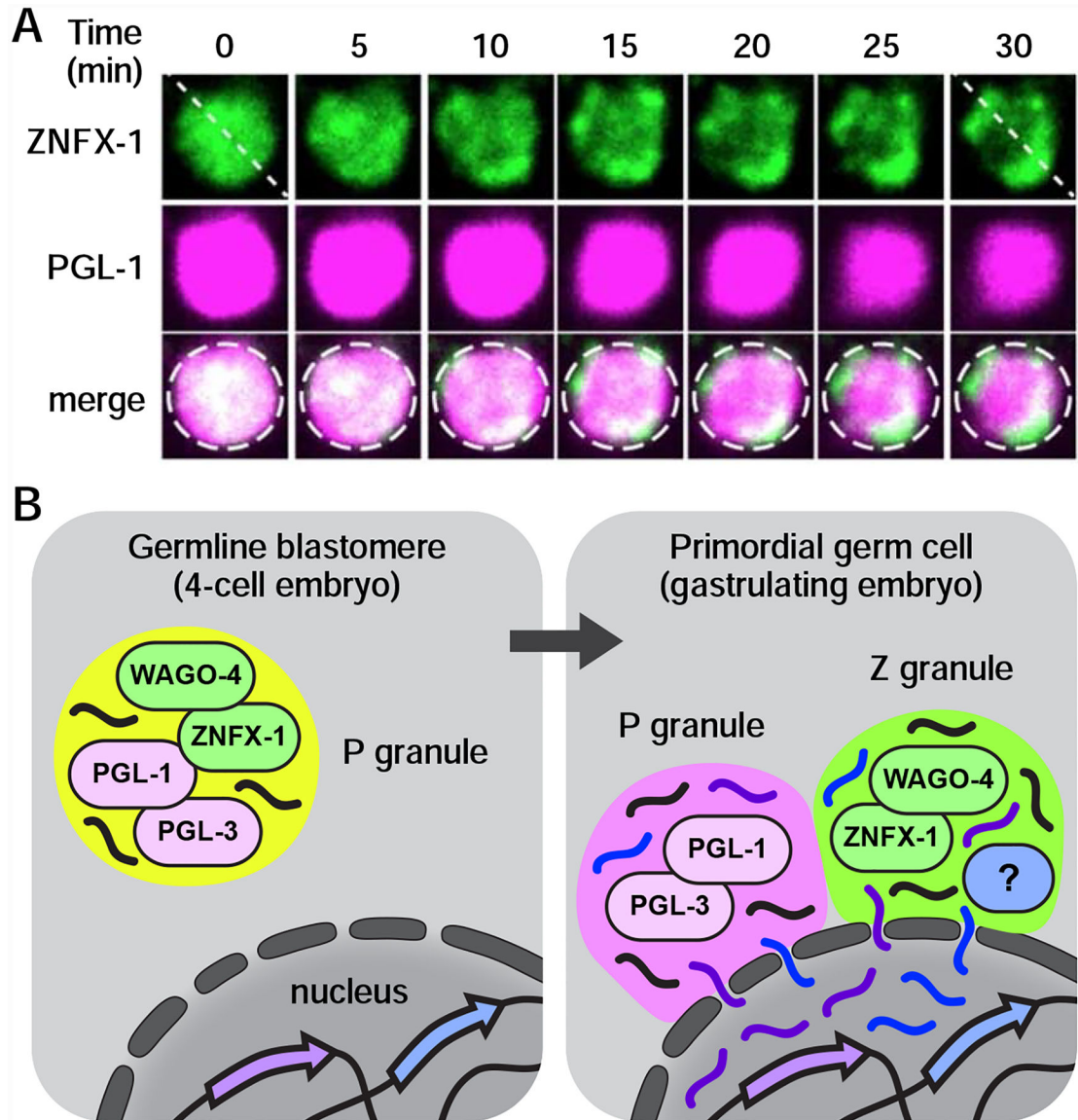


Phase separation is a versatile and widespread biophysical phenomenon that contributes to subcellular organization, gene regulation, environmental sensing, and a variety of other cellular processes. Dodson and Kennedy review how animals regulate and exploit the properties of phase separation in the germline and during development.



**Figure 1. Spatial organization of germ cell condensates.**

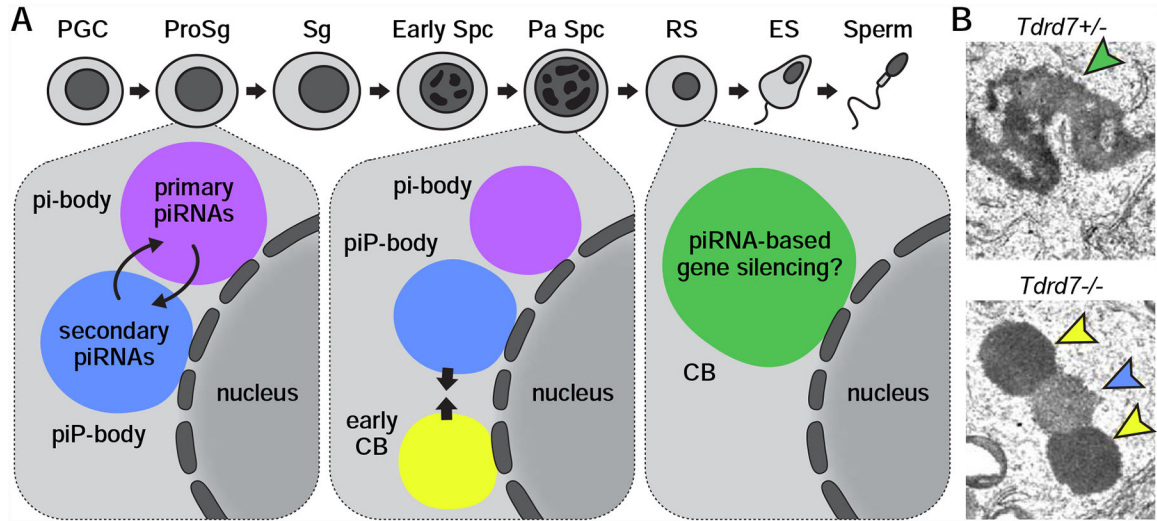
(A) PATR-1 and PGL-1 localize to distinct subregions (arrowheads) within germline ribonucleoprotein granules related to P-bodies (grP-bodies) in arrested *C. elegans* oocytes. Scale bar, 2  $\mu$ m. Image originally from (Hubstenberger *et al.*, 2013). (B) P granules in the germline blastomeres of early *C. elegans* embryos. MEG-3 unevenly coats a PGL-3-marked core. Scale bar, 1  $\mu$ m. Reprinted by permission from Springer Nature: (Putnam *et al.*, 2019). (C) Representative piP-body in mouse prospermatogonia. Dcp1a surrounds a Mael-marked core. Image originally from (Aravin *et al.*, 2009). (D) Homotypic mRNA clustering in *Drosophila* polar granules. Osk-marked polar granule containing distinct clusters of *pgc* and *nos* mRNA. Image originally from (Niepielko, Eagle and Gavis, 2018).



**Figure 2. Z granule formation in *C. elegans* embryos.**

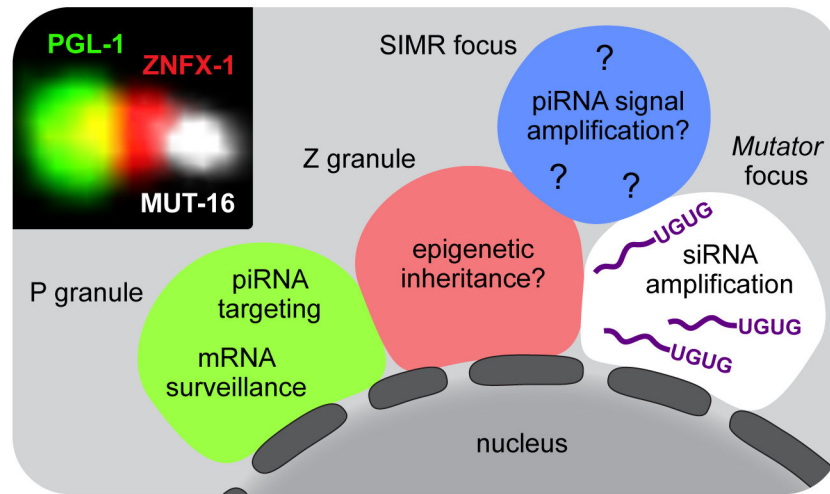
(A) Time-lapse micrographs showing ZNFX-1 demixing from PGL-1 in a primordial germ cell at approximately the 300-cell stage. Reprinted by permission from Springer Nature: (Wan *et al.*, 2018).

(B) Z granule formation coincides roughly with nuclear attachment and the onset of germline transcription. In early germline blastomeres (left), ZNFX-1 and WAGO-4 co-localize with P granule proteins. Later in embryogenesis (right), ZNFX-1 and WAGO-4 demix from P granules to form Z granules. Z granule demixing may be driven by newly synthesized mRNAs (purple and blue lines), one or more newly synthesized proteins (blue question mark), post-translational modifications, and/or attachment to the outer nuclear envelope. Note, P granules (and likely Z granules, as well) contain additional proteins not shown here.



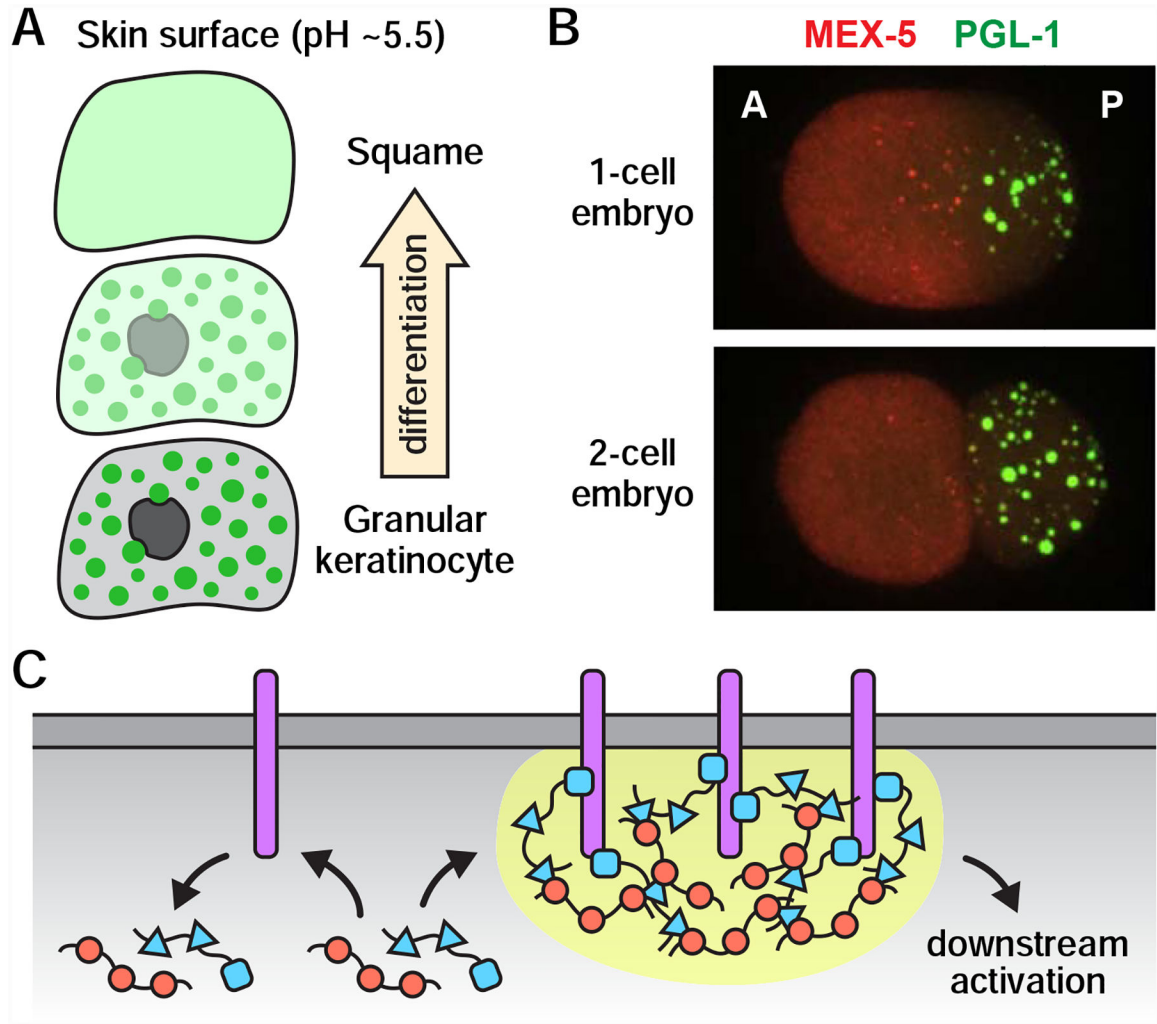
**Figure 3. Germ granule interactions during mouse spermatogenesis.**

(A) Model of germ granule-based organization of the piRNA pathway. In prospermatogonia (ProSg), pi-bodies and piP-bodies facilitate the processing of primary and secondary piRNAs, respectively. piP-bodies are often in close proximity to pi-bodies, and cross-talk between the two compartments (arrows) may promote piRNA amplification. Early chromatoid bodies (CBs) first appear in pachytene spermatocytes (Pa Spc) and are distinct from pi-bodies and piP-bodies. By the round spermatid (RS) stage, chromatoid bodies and piP-bodies have merged to form a mature chromatoid body, which likely functions in piRNA-based gene silencing and other types of RNA processing. PGC, primordial germ cell; Sg, spermatogonium; early Spc, early spermatocyte; ES, elongating spermatid. (B) Electron micrographs of spermatids in *Tdrd7*<sup>+/-</sup> and *Tdrd7*<sup>-/-</sup> mice. Green arrowhead, mature chromatin body; yellow arrowheads, early chromatin bodies; blue arrowhead, piP-body. Image originally from (Tanaka *et al.*, 2011).



**Figure 4. Subcompartments of *C. elegans* perinuclear nuage.**

Model: Nuage subcompartments are spatially ordered and specialize in distinct yet related aspects of RNA processing. pUG RNAs (purple) are enriched in *Mutator* foci. The exact location of SIMR foci relative to other subcompartments is not yet known (question marks). Inset: Representative micrograph of PGL-1 (marker of P granules), ZNF-X-1 (marker of Z granules), and MUT-16 (marker of *Mutator* foci) in a pachytene germ cell. Reprinted by permission from Springer Nature: (Wan *et al.*, 2018).



**Figure 5. Phase separation and developmental decisions.**  
**(A)** Dissolution of keratohyalin granules (green dots) in response to a pH shift during epidermal differentiation. As keratinocytes approach the acidic skin surface, keratohyalin granules dissolve, nuclei degrade, and the resulting “ghost” cells, or squames, build the skin barrier. **(B)** Spatial patterning in the *C. elegans* zygote. An anterior-rich MEX-5 concentration gradient generates a sharp asymmetry in the formation of P granules (marked by PGL-1). As the zygote divides, P granules segregate with the posterior blastomere, which eventually gives rise to the germline. A, anterior; P, posterior. Images originally from (Gallo *et al.*, 2010). Reprinted with permission from AAAS. **(C)** Model: Phase separation enhances signaling amplification and specificity. Activation of transmembrane proteins (purple) recruits cytoplasmic adaptor proteins (red and blue) to the membrane surface. Multivalent interactions between membrane proteins and cytoplasmic adaptors drive phase separation (yellow), which prolongs the length of time that cytoplasmic signaling molecules reside at the membrane and thereby increases the likelihood of downstream activation. Stochastic interactions between unclustered membrane proteins and cytoplasmic adaptors (left) do not



retain cytoplasmic adaptors at the membrane surface and are therefore unlikely to result in downstream activation.

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**Table 1.**

Prominent membraneless compartments specific to germ cells in animals.

Compartment	Potential function(s)	Physical properties	Components that condense <i>in vitro</i> and/or in heterologous cells	<i>In vivo</i> FRAP			References for physical properties, condensation, and FRAP
				Component	$t_{1/2}$ (seconds) <sup>a</sup>	Mobile fraction (%)	
P granules ( <i>C. elegans</i> )	Maintenance of germ cell totipotency, RNA processing and storage	Spherical. Undergo fusion, Deform under shear stress	LAF-1, MEG-3, PGL-3	CAR-1	2.6	16	Brangwynne et al., 2009; Elbaum-Garfinkle et al., 2015; Saha et al., 2016; Smith et al., 2016 Putnam et al., 2019
				GLH-1	18.4–30.1	100	
				LAF-1	1.5		
				MEG-3	128.3–384.3		
				PGL-1	4.7–20.73	68	
				PGL-3	21.9		
Z granules ( <i>C. elegans</i> )	Transgenerational epigenetic inheritance, RNA processing	Spherical, Resistant to shear stress		PIE-1	5.4	51	Wan et al., 2018
				ZNFX-1	8		
<i>Mutator</i> foci ( <i>C. elegans</i> )	siRNA amplification, Transposon silencing		MUT-16		7.2	35	Uebel et al., 2018
SIMR foci ( <i>C. elegans</i> )	piRNA-based gene silencing						
grP-bodies ( <i>C. elegans</i> )	RNA storage	Spherical, Highly viscous			~940		Hubstenberger et al., 2013
Polar granules ( <i>Drosophila</i> )	Germ cell specification and development	Spherical, Fusion not observed	Osk	Osk	6.9	43.6	Jeske et al., 2015; Kistler et al., 2018
				Vasa	23.9	46	
				Aub	35		
Nuage ( <i>Drosophila</i> )	piRNA processing				52.1–59	72	Snee and Macdonald, 2004; Nosov et al., 2014
Sponge bodies ( <i>Drosophila</i> )	RNA processing and/or storage						
piNG-body ( <i>Drosophila</i> )	piRNA-based gene silencing				5.3	71	Nosov et al., 2014
Perinuclear germ granules (zebrafish)	Germ cell development	Spherical, Undergo fusion					Strasser et al., 2008
Balbiani body, or mitochondrial cloud (vertebrates and other animals)	Germ cell specification ( <i>Xenopus</i> , zebrafish), Oocyte quality control	Spherical, Resistant to shear stress	Buc (zebrafish)	Buc (zebrafish)	Slow and partial recovery		Boke et al., 2016; Roovers et al., 2018
				Xvelo ( <i>Xenopus</i> )		20	

Compartment	Potential function(s)	Physical properties	Components that condense <i>in vitro</i> and/or in heterologous cells	<i>In vivo</i> FRAP			References for physical properties, condensation, and FRAP
				Component	$t_{1/2}$ (seconds) <sup>a</sup>	Mobile fraction (%)	
pi-bodies, or intermitochondrial cement (mammals)	Primary piRNA processing						
piP-bodies (mammals)	Secondary piRNA processing						
Chromatoid body (mammals)	piRNA-based gene silencing, RNA processing and storage		Ddx4 (human)				Nott et al., 2015

<sup>a</sup>FRAP recovery half-times from Brangwynne et al., 2009 were derived from the time constant  $\tau$  using the following equation:  $t_{1/2} = \tau \ln 2$ .