A quiet space during rush hour: Quiescence in primordial germ cells

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Abstract

Quiescence is a common character in stem cells. Low cellular activity in these cells may function to minimize the potential damaging effects of oxidative stress, reduce the number of cells needed for tissue replenishment, and as a consequence, perhaps occupy unique niches. Quiescent stem cells are found in many adult human tissues, the hematopoietic stem cells are paradigmatic, and more recently it appears that stem cell of the germ line in many animals display quiescence characters. Here we explore the diversity of quiescence phenotypes in primordial germ cells, leveraging the diverse mechanisms of germ cell formation to extract evolutionary significance to common processes.

The light turns green and they are off. Through the intersection they zip – one, ten, and a flood of followers. The rush of activity is overwhelming, signals are flashing, tempers are flaring, and it is not clear if they are being drawn forward, or pushed from behind. They do know though that they have to get there – “where” is not clear yet – but they do have to get there, and fast! Off to one side, a few leave the rush, into a quiet place. There they gather and plan and change their strategy as they avoid the commotion next to them.

So it is after fertilization and during early embryonic development. The rush of cell divisions in some embryos is rapid, often chaotic, as if speed is the goal. And often it is! Some become entangled or straddled between lanes of cell fate. Others are stuck in the middle and accept their eventual fate further down the road. But in many embryos, the cells that enter the germ line, the primordial germ cells (PGCs), avoid the crunch and find a quiet spot. If only for a little while, and sometimes only parking one frenetic activity. There they plot their fate with care and know their time and important job will come.

The quiet space for PGCs is distinct from the somatic rush, but varied for diverse animals. PGCs of some animals stop cell cycling, transcription, some even change metabolism. The mechanism of these changes, and what purpose might they hold though, is yet unclear.

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Perhaps by looking at diverse animals we might see an underlying theme that could help in this explanation.

The PGCs of Drosophila, for example, are derived from the pole cells, formed first during cellularization at the posterior pole from the syncytial blastoderm stage. The incipient PGCs seem to exit the rush even when within the syncytium of nuclei; they begin to divide asymmetrically before cellularization (cycles 9 and 10; (Su et al., 1998)) and are distinct from their somatic nuclei counterparts with whom they share a cytoplasm. Once cellularized the pole cells continue to divide, but not with the synchrony or rapidity seen in the adjacent somatic cells. By cell cycle 14 (stage 4–5), the pole cells cease dividing while the adjacent somatic cells rush on. Subsequently, the PGCs are displaced passively from their origin at the posterior pole to a dorsal region of the embryo by the process of germ band extension. This major morphogenetic transition is an elongation of a stripe of tissue along the midline by convergent extension of cells into the midline. The dorsally displaced PGCs then migrate across an epithelium to access the internal mesoderm, from which the somatic gonad forms. Re-entry into the cell cycle for these PGCs does not occur significantly until interaction with the somatic gonadal cells.

Coincident with cell cycle cessation in Drosophila, the germ cell nuclei become transcriptionally quiescent. The polar granule component (Pgc; unfortunately the same acronym as Primordial Germ Cells, PGCs. Here we will distinguish the terms by the plurality of PGCs and the lower case of Pgc.) is a 71 amino acid peptide that interacts with the transcription elongation factor b (P-TEFb) and with RNA Polymerase II to inhibit phosphorylation of the carboxy-terminal domain (CTD) Ser 2 phosphorylation that is essential for transcriptional elongation. Although the Pgc is sufficient to inactivate transcription, even in somatic cells under experimental conditions, its normal accumulation specifically in the PGCs causes cell-type specific transcriptional repression (Hanyu-Nakamura et al., 2008).

This same strategy of germ cell transcriptional quiescence functions in the C. elegans germline, suggestive of a common theme in PGC behavior (Batchelder et al., 1999; Mello et al., 1996; Zhang et al., 2003). PIE-1 is present in the 2-cell stage of the embryo, and after each cell division, the cell destined to become the P4 cell (the blastomere of the germ line) retains PIE-1, but all other cells degrade this factor. PIE-1 prevents CTD Ser 2 phosphorylation through interaction with CycT, the regulatory subunit of the cdk9, kinase domain of P-TEFb. Thus, P-TEFb seems to be a common regulatory target for the repression of mRNA transcription during germ cell specification both in Drosophila and C. elegans, yet Pgc and PIE-1, the mechanisms of repression, are unrelated. They therefore must have arisen independently in evolution, to target the same regulator by different mechanisms. Furthermore, loss of Pgc function leads to continuous transcriptional activity in the PGCs and their death soon after formation, whereas loss of PIE-1 function results in acquisition of somatic fates by the lineage that would otherwise become the germ line. Therefore, fertility is lost when this mechanism is disrupted, serving as high-level selection. Distinct mechanisms in germ cell regulation seen here, especially in diverse organisms, is consistent with the hypothesis that germ cell specification by the maternally inherited germ plasm evolved independently among diverse animal groups (Extavour and Akam, 2003). This is

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particularly significant given that PIE-1 and Pgc appear to be of distinct origins, and are each present only within one taxon.

We recently learned of a most extreme form of quiescence in primordial germ cells. Echinoderms are sister to the Chordates, and in most sea urchins, the primordial germ cells originate at the 5th cell division (32 cell stage) by two sequential asymmetric divisions as the small micromeres (Figure 1). The cell cycle of this new lineage is immediately slowed so that instead of dividing every 30–60 minutes as their siblings do, they divide only once by the end of gastrulation when the embryo has approximately 2000 cells. Thus, the PGC lineage expands 2-fold while other lineages expand ~50-fold. Quiescence in this cell is, however, more than just in the cell cycle. We learned that the PGCs of the sea urchin also change significantly relative to their sibling somatic cells by reducing their overall translational activity to 6% +/- 2.7%, decreasing their transcriptional activity to 14.9% +/- 8.4, reducing their mitochondrial activity to 1.7% +/- 2.3, and likely decreasing the pH of their cytoplasm reflective of a shift in metabolism from oxidative phosphorylation to glycolysis. Importantly, each of these quiescence phenotypes are transient – these cells return to elevated activities following gastrulation, demonstrating a rapid, predictable, and transient quiescent activity (Figure 2). These changes in the PGCs occur in the midst of their sibling cells rapidly dividing, with dynamic transcriptional changes (McClay, 2011).

What is the mechanism of this broad and deep quiescence? Nanos was first identified in Drosophila as a translational repressor (Cho et al., 2006; Irish et al., 1989). It functions through its interaction with Pumilio, which binds RNAs containing a conserved motif usually found in the 3’ UTR of mRNAs; this motif is referred to as the Pumilio Response Element (PRE) (Sonoda and Wharton, 1999; Wharton and Struhl, 1991). Only a few mRNAs have been identified as nanos/pumilio targets: cyclin B (Asaoka-Taguchi et al., 1999; Dalby and Glover, 1993; Kadyrova et al., 2007; Lai et al., 2011), hid (Hayashi et al., 2004; Sato et al., 2007), hunchback (Murata and Wharton, 1995; Wreden et al., 1997), fem 3 (Ahringer and Kimble, 1991; Zhang et al., 1997), VegT (Lai et al., 2012) and CNOT6 (Swartz et al., 2014). In the sea urchin Strongylocentrotus purpuratus (the purple sea urchin), three nanos orthologs are present in the genome, but Sp nanos 2 (mRNA and protein) is the only nanos that accumulates specifically in the PGCs at the blastula stage when the quiescence phenotype is detected (Juliano et al., 2010). Nanos 2, by targeting several mRNAs for degradation specifically in the PGCs, is responsible in part for the quiescence seen in these early born PGCs.

Targets of nanos in this animal that have functionally defined PREs, and whose removal leads to quiescence in the PGCs include: 1) cyclin B (causing cell cycle cessation); 2) EF1 (an essential translation factor that when absent contributes to lack of protein synthesis); 3) CNOT6 (a deadenylase that degrades mRNAs, and in whose absence means the PGCs retain many of the maternal mRNAs); 4) ADP/ATP translocase(a mitochondrial transmembrane protein that shuttles ADP into the mitochondrial matrix for high-energy phosphate recharging, and then transporting the newly energized ATP out of the mitochondria. Its absence contributes to low/no mitochondrial activity in the PGCs). Over 150 mRNAs are preferentially depleted (FDR<0.05) in the PGCs relative to the somatic cells, and many of these are also candidates for nanos-mediated mRNA degradation selectively in the PGCs.
Clearly nanos is capably of transitioning an early embryonic cell with broad maternal mRNA populations into a phenotypically distinct cell with broad quiescence.

Quiescence in this animal appears to be a multi-step process. The first detectible step that we currently know of is the accumulation of Nanos2 specifically in the incipient PGCs by about the 128 cell stage. Both Nanos mRNA and protein appear to accumulate selectively in the PGCs, yet this is accomplished largely post-transcriptionally and even post-translationally, respectively (e.g. (Oulhen and Wessel, 2016a; Oulhen et al., 2013; Swartz et al., 2014). The gene encoding Nanos 2 appears to be broadly transcribed throughout the embryo in most, if not all, cell types. The mRNA is only retained, however, in the PGCs. At least in the sea urchin *S. purpuratus*, this retention mechanism results from an element in the 3′ UTR of the Nanos mRNA. This element, GNARLE (Global Nanos Associated RNA Lability Element), directs degradation in all cells of the embryo except the PGCs, and functions independently of the coding region or sequences adjacent to it (Oulhen et al., 2013). The mechanism of what directs recognition and degradation so selectively outside of the germ line is yet unknown, but it has sufficient fidelity so that when Nanos2 mRNA is introduced broadly into an embryo, it is rapidly and effectively cleared from all cells of the embryo except the germ line.

So too, the Nanos 2 protein accumulates largely post-translationally (Oulhen and Wessel, 2016b). When an mRNA encoding Nanos2 is forced to broadly translate throughout the embryo (lacking GNARLE), the Nanos 2 protein is detected only within the germ line. It is translated everywhere, but it only accumulates in the PGCs. The mechanism of this post-translational process is also not understood, but appears to be independent of ubiquitylation, and sumoylation. Indeed, a 45 amino acid sequence of Nanos 2 was found to be necessary and sufficient to direct degradation of any chimeric protein linked to it. Thus, what appears to be an essential step for success of germ line formation is independent of specific transcriptional and translational events that we might normally associate with making a new cell type. Instead, the embryo broadly makes the products, the mRNAs and the protein, and then selectively degrades both to pare down functionality into the future germ line.

Nanos may not, however, be the singular step in initiating quiescence. The PGCs appear to shift their cytoplasm into becoming acidic and acquiring Hif-1 (Ben-Tabou de-Leon et al., 2013), the hypoxia inducible factor. This is surprising considering that the PGCs of this organism are exposed to the media (Figure 1) which is favorably oxygenated and the normal accumulation of Hif-1 is post-translational under low-oxygen conditions. In cells within which Hif is studied intensively, such as tumors and endothelial cells, Hif-1 is translated but tagged by prolyl-hydroxylase for ubiquitylation and proteolysis. It is the prolyl-hydroxylase activity domain, coordinated with an iron element, which senses the oxygen levels and decreases its activity – and thereby Hif-1 turnover.

This potential post-translational regulation of Hif-1 in the germ cells of this animal is a common theme among diverse gene products. In addition to Nanos regulation post-translationally (and post-transcriptionally) documented above, the conserved RNA helicase, Vasa, is regulated exceptionally by post-translational activity. Vasa – like Nanos 2 – is translated throughout the early embryo. In early cleavage divisions it accumulates on the
spindles of the large, rapidly dividing blastomeres and is essential for general protein translation on the spindles (Yajima and Wessel, 2011). The E3-ubiquitin-ligase, Gustavus, appears to degrade Vasa in all cells, except in the PGCs, and thereby making Vasa germ lineage selective by the blastula stage in this animal (Gustafson et al., 2011).

How might Hif-1 be an important node in PGC quiescence? Hif-1 is a transcription factor of the bHLH family and binds specific sequences for transcriptional activation of many genes. These Hif-1 targets are often involved in metabolic regulation that shifts energy generation from oxidative phosphorylation to glycolysis and include e.g. VEGF, erythropoietin, glucose transporters, and lactate dehydrogenase (Imanirad et al., 2014; Lopez-Iglesias et al., 2015; Majmundar et al., 2015). In the sea urchin embryo, the PGCs have a low mitochondrial activity and an apparent acidic cytoplasm, both indicative of a dependence of glycolysis with a lactate-based acidification. The low mitochondrial activity in the PGCs is of particular interest and is likely caused perhaps simply from decreased levels of essential proteins from targeted mRNA degradation e.g. ADP/ATP translocase, that make the mitochondrial less functional.

The overall presumed shift in metabolism in the PGCs of the sea urchin though may be different than seen in other cases of embryonic cells where Hif-1 is expressed in normoxic environments. Many mammalian early embryos actually display increased glycolytic activity – especially well documented in the pig (Redel et al., 2012). This activity appears to fall under the strategy of the Warburg effect ((Warburg, 1956a, b; Warburg et al., 1927) in which glycolytic activity is increased substantially, perhaps for the generation of increased levels of pyruvate for the TCA cycle, in addition to many precursor elements for amino acid, lipid, and nucleic acid biogenesis. Different from the Warburg effect though, in the sea urchin PGCs, the mitochondria are not active, and likely lead to a buildup of lactate, and lactic acidosis, whereas in the Warburg phenomenon, the mitochondrial oxidative phosphorylation remains active, and a recipient of pyruvate. The cytoplasmic acidification in the sea urchin PGCs then may feedback to further repress protein translation, much like is seen in the eggs of this same animal (Epel et al., 1974), where a cytoplasmic pH of 6.8 is sufficient to repress translation for prolonged periods.

What is the function of this broad quiescence? To address the question it might be useful to look at the extremes of how PGCs are determined. The ancient mechanism in PGC determination appears to be by cell communication – a so-called inductive mechanism. Cells of the embryo send and receive signals that somehow instructs a subset to alter their developmental course of a somatic fate, and instead become devoted to the germ line. Consistent with this mechanism is that these PGCs are determined during, or even after, gastrulation - relatively late in development. In some animals, cnidarian for example, the germ line is not specified until adulthood.

Selection mechanisms though perhaps have favored more rapid development in many cases. Getting an egg – with its vast nutritive investments – to a feeding embryo as rapidly as possible may have some benefits for success in fitness. Perhaps establishing axial organization more thoroughly during oogenesis would lead to substantial molecular distinctions between its various regions that direct cell fate early in development, even
during cleavage divisions. This means that the embryo is rapidly able to establish its axial organization, to acquire its germ layers, to feed, be mobile, and perhaps even reach adulthood so that it can reproduce promptly also. Captured in this localized information within the egg may be information for the germ line. After all, rapid development without planning for the germ line is counter to the goals of successful reproduction.

But – with early acquisition of the germ line fate as e.g. in Drosophila, C. elegans, and sea urchin, the tissues, organs, pathways, even migration mechanisms of the embryo are not yet assembled. One strategy in this disparate timing mechanism between the PGCs and the somatic cells is for the PGCs to enter quiescence – wait until the embryo catches up. And this waiting period can be prolonged. Significant cell division of the germ line generally does not occur until the PGCs have completed their migration into the presumptive somatic gonad. There, finally, they begin rapid proliferation to give rise to the many stem cells (gonial stem cells) that will eventually form the gametes. This transition to extensive replication is seen even in organisms that specify their PGCs later in development, and instead by inductive mechanisms (e.g. mouse). Quiescence in the early-formed PGCs though is not just waiting. It is an active mechanism to repress various biological processes selectively in the PGCs.

Our goggles are here focused on the germ line, and by doing so, we can see the lack of cellular activities in these cells, a lack of activity that is otherwise not apparent. Our many assays of differential transcriptome analyses, and antibody assays are usually targeted to identify rapid proliferation, quick changes in transcriptional output, and the expression of new proteins. But maybe other cell types use similar quiescent mechanisms in the developmental transitions they undergo. So then, if the quiescent mechanism in PGCs is so widespread amongst animals, and the process so effective for the success of species propagation, could quiescence mechanisms be a regulator in spatial and developmental coordination of tissue morphogenesis? Perhaps this process might be an effective, and evolutionarily feasible way to coordinate tissue development to come together at the right time, and in the right place. Perhaps a useful, and general developmental strategy is simply to lay off the accelerator and to make use of a quiet space.

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Highlights

- Animals segregate their germ line stem cells in a variety of distinct but overlapping mechanisms.
- When the germ line is segregated early in development, the primordial germ cells may institute quiescence in cellular activities.
- Sea urchins segregate their germ line at the 5th cell division and quickly turn off nearly all measurable activities in the cell – including transcription, translation, and mitochondrial activity.
- These primordial germ cells serve as a model for stem cell quiescence, both initiating quiescence, and later relief from this marked lack of activity.
Figure 1.
The primordial germ cells of the sea urchin arise at the 32 – cell stage in the vegetal pole (arrow; vegetal view).
Figure 2.
Early embryogenesis of the sea urchin (e.g. *Strongylocentrotus purpuratus, Lytechinus variegatus*) highlighting the primordial germ cells (purple) formed from two asymmetric cell divisions at the 4th and 5th cell division (red circles). Changes that occur in this cell type as pertains to the quiescence phenotype are shown temporally.
## Table 1

| Organism                        | Mechanism of germ cell determination | Demonstrated quiescence phenotypes (and mechanisms)                                                                 | References                                                                 |
|---------------------------------|--------------------------------------|---------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Drosophila melanogaster (fruit fly) | Maternal inheritance                 | Cell cycle slowing (nanos inhibition of cyclin B translation)  
Transcriptional repression (the 71 amino acid, polar granule component blocks the transcription elongation factor b, P-TEFb, activation of RNA polymerase II) | (Su et al., 1998)  
(Hanyu-Nakamura et al., 2008) |
| Caenorhabditis elegans (round worm) | Maternal inheritance                 | Transcriptional repression (PIE-1 prevents CTD Ser 2 phosphorylation through interaction with CycT, the regulatory subunit of the cdk9, kinase domain of P-TEFb.) | (Batchelder et al., 1999; Mello et al., 1996; Zhang et al., 2003) |
| Strongylocentrotus purpuratus (sea urchin) | Maternal inheritance                 | Slow cell cycle, translational and transcriptional repression, undetectable mitochondrial activity (nanos is involved in each of these quiescence phenotypes by targeting specific mRNAs for translational repression and/or turnover, e.g. cyclin B, the translation factor EF1A, mitochondrial ADP/ATP translocase. Mechanisms of transcriptional repression are not known.) | (Juliano et al., 2010; Swartz et al., 2014; Oulhen et al., 2017) |
| Mus musculus (mouse)            | Embryonic induction                  | Cell cycle slowing with high variability depending on sex and site of migration (mechanisms unknown, but associated with Wnt-β-catenin signaling pathway) | (Cantu et al., 2016; McLaren, 2003) |