Stem Cell Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

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

Nathalie Oulhen, Gary Wessel *

Department of Molecular Biology, Cellular Biology, and Biochemistry, Brown University, Providence, RI 02912, United States

ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 25 October 2017
Accepted 3 November 2017
Available online xxxx

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 (Table 1).

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. 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 (stages 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) Ser2 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 Ser2 phosphorylation through interaction with CycT, the regulatory

* Corresponding author at: Box G, 185 Meeting Street, Brown University, Providence, RI 02912, United States.
E-mail address: rhet@brown.edu (G. Wessel).

https://doi.org/10.1016/j.scr.2017.11.001

Please cite this article as: Oulhen, N., Wessel, G., A quiet space during rush hour: Quiescence in primordial germ cells, Stem Cell Res. (2017), https://doi.org/10.1016/j.scr.2017.11.001

Table 1
Quiescence phenotypes in primordial germ cells.

Organism	Mechanism of germ cell determination	Demonstrated quiescence phenotypes (and mechanisms)	References
Drosophilia 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 Ser2 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)

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 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 (Fig. 1). The cell cycle of this new lineage is immediately slowed so that instead of dividing every 30–60 min 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 (Fig. 2). These changes in the PGCs occur in the midst of their sibling cells rapidly dividing, with dynamic transcriptional changes (McClay, 2011).

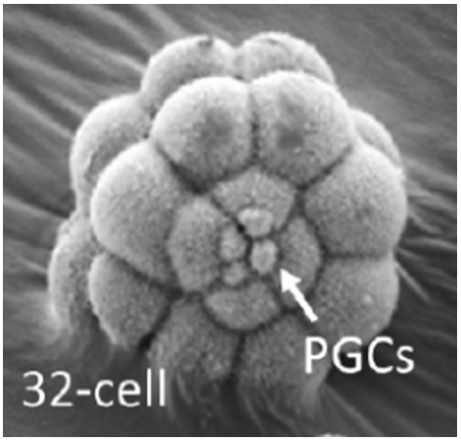

Fig. 1. The primordial germ cells of the sea urchin arise at the 32 – cell stage in the vegetal pole (arrow; vegetal view). JB Morrill, personal communication.

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 (Swartz et al., 2014). 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, 2016;

Please cite this article as: Oulhen, N., Wessel, G., A quiet space during rush hour: Quiescence in primordial germ cells, Stem Cell Res. (2017), https://doi.org/10.1016/j.scr.2017.11.001