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Germ Cells: Finding Programs of Mass Dispatch Repression

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Transcription is globally silenced in the germline of animals. Recent studies have shown that, in *Caenorhabditis elegans*, this silencing is initially mediated through direct repression, but in *Drosophila*, the factors involved include *pgc*, a non-coding cytoplasmic RNA. Why are these mechanisms so diverse and complex?

Germ cells retain the potential to develop into any tissue, making it critical that they are protected from inappropriate differentiation. The mechanisms that provide this protection are likely to be relevant to understanding how somatic stem cell lineages maintain pluripotency. During early embryogenesis, a widespread strategy through which germ cells avoid differentiation is by transiently and globally silencing mRNA transcription [1–3]. Recent studies have shown that, in the fruitfly *Drosophila* [4,5] and the nematode *Caenorhabditis elegans*, this silencing is effected by mechanisms that are quite distinct, each apparently tailored to the respective biological context.

In *C. elegans*, an embryonic germ cell precursor initially undergoes a series of successive divisions, each of which gives rise to a germline and a somatic daughter cell. In the early germ cell lineage, transcription is blocked by PIE-1, an RNA-binding protein that can interfere with elongation or associated RNA processing steps [6–8]. This model predicts that, in early *C. elegans* germline cells, transcription of individual genes may be initiated, but it either stalls or aborts. Accordingly, these cells have high levels of di-methylated lysine 4 of histone H3 (H3K4), a marker of transcriptionally active chromatin [9]. They also show low levels of phosphorylation of the RNA polymerase II carboxy-terminal domain (CTD) repeat on residue serine 5, a modification that is known to require transcription initiation [10,11].

In *C. elegans*, it thus seems that the transcription machinery is poised and ready in the early embryonic germ cell, so that after each successive division transcription can proceed immediately in the somatic daughter cell. After the cell lineage becomes restricted to the germline, PIE-1 disappears, but a state of seemingly low transcription activity ensues that is characterized by undetectable H3K4 methylation [9]. This apparently quiescent chromatin environment requires *C. elegans* Nanos, a translation regulator involved in germline development in essentially all metazoans.

In *Drosophila*, by contrast, the germ cell precursors — known as pole cells — are segregated from somatic lineages before zygotic transcription begins (Figure 1). Because they do not give rise to transcriptionally active somatic daughters, pole cells do not need to employ a rapidly reversible mechanism to suppress transcription. Accordingly, from the outset, pole cells are marked by an absence of H3K4 methylation [9]. Remarkably though, as in *C. elegans*, this quiescent chromatin structure depends upon Nanos [9]. A Nanos-dependent elevation in levels of a heterochromatin marker (H3K9 methylation) has also been observed in pole cells, but this finding remains controversial [4,9]. As in *C. elegans* embryonic germ cells, in *Drosophila* pole cells RNA polymerase II shows low levels of CTD phosphorylation on serine 5 [10,11], suggesting that some abortive transcription may be occurring.

How is germline transcriptional quiescence mandated in *Drosophila*? Nanos is required to prevent pole cells from expressing certain genes [12,13], but its role in transcription silencing may be limited. In both *C. elegans* and *Drosophila*, embryonic germ cells are distinguished by undetectable levels of RNA polymerase II CTD serine 2 phosphorylation [10], a modification crucial for the elongation and processing phases of transcription [14]. In *C. elegans*, RNA polymerase II CTD serine 2 phosphorylation correlates remarkably well with early embryonic transcription activity [11], which reflects expression of thousands of genes [15]. In *Drosophila*, RNA polymerase II CTD serine 2 phosphorylation is not detectably elevated in *nanos* mutant pole cells [10], indicating that only a limited abrogation of transcription silencing occurs. In contrast, without the nuclear protein Germ cell-less, high levels of RNA polymerase II CTD serine 2 phosphorylation are seen in pole bud nuclei, but these nuclei fail to adopt the germline fate [16].

Two very interesting recent studies [4,5] have shown that, in *Drosophila*, germline transcription silencing depends upon the non-coding RNA encoded by the *polar granule component* (*pgc*) gene [4,5], which is localized to germ plasm (Figure 1). Significantly, *pgc* is not required for germ cell formation [17], indicating that the germ cell fate can be uncoupled from transcriptional quiescence. Without *pgc*, pole cells are characterized by expression of various individual genes and dramatically elevated levels of both RNA polymerase II CTD serine 2 phosphorylation and H3K4 methylation, indicating that transcription silencing is broadly abrogated [4,5]. These changes are not accompanied by elevated H3K9 methylation, suggesting that global heterochromatin effects are not involved [4].

Deshpande *et al.* [5] observed that, in an antisense *pgc Drosophila* strain, the usual dramatic elevation of RNA polymerase II CTD serine 2 phosphorylation and H3K4 methylation in pole cells was only transient. In contrast, Martinho *et al.* [4] additionally analyzed a null *pgc* strain, and observed a persistent loss of transcription silencing in these mutant germ cells [4], suggesting that the latter phenotype more accurately reflects a complete lack of *pgc* function.

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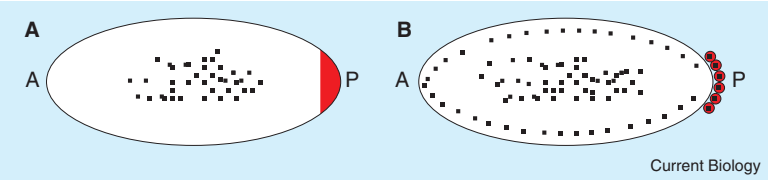

Figure 1. Restriction of the germline fate in *Drosophila*.

(A) Nuclear division cycles 1–8. The *Drosophila* embryo initially develops as a syncytium. The fate of germline precursors is defined when their nuclei migrate into the germ plasm (red), which contains RNA–protein organelles called polar granules. (B) Nuclear cycles 11–13. After first forming buds at the pole, germ cell nuclei cellularize to form pole cells at the end of cycle 10. Discrete somatic cells form later, at cycle 14. Somatic nuclei initiate zygotic transcription during cycle 8, but the pole cells remain transcriptionally quiescent until much later during embryogenesis. (Adapted from [3].)

The two groups also report different findings regarding the effects of *pgc* on the terminal signaling pathway, which involves the receptor Torso. Torso-dependent genes are expressed inappropriately in antisense *pgc* RNA germ cells [4,5]. From the results of staining with a phospho-dependent antibody, Deshpande *et al.* [5] concluded that *pgc* suppresses Torso signaling, and that this is important for its enforcement of transcriptional silence. In contrast, using a different antibody Martinho *et al.* [4] did not detect any Torso signaling in antisense *pgc* germ cells at a later stage.

While this discrepancy should be resolvable by further experiments, substantial evidence suggests that *pgc* has major Torso-independent functions. Martinho *et al.* [4] found that a Torso-independent gene, *Slam*, was expressed robustly in antisense *pgc* germ cells, and removal of Torso-targeted repressors did not prevent their torso-dependent target genes from being silenced in the germline. Finally, the high levels of RNA polymerase II CTD serine 2 phosphorylation and H3K4 methylation in antisense *pgc* pole cells suggest a widespread activation of transcription that is unlikely to derive from a single developmental signaling pathway [4,5]. This issue can be put to rest by determining whether germline expression of Torso-independent genes or RNA polymerase II CTD serine 2 phosphorylation levels are affected when Torso signaling is eliminated in a *pgc* mutant background.

How might a non-coding cytoplasmic RNA inhibit transcription? Martinho *et al.* [4] suggest that the *pgc* RNA might directly sequester a critical transcription factor, particularly one that acts at a post-initiation step. There is an intriguing precedent for this mechanism: the small nuclear RNA 7SK has been shown to bind and inhibit the RNA polymerase II CTD serine 2 kinase P-TEFb [18,19], which may also be targeted by *C. elegans* PIE-1 [8]. Alternatively, polar granules might require *pgc* RNA in order to send a signal directing early germ cells to silence transcription, or *pgc* RNA might have a fundamental role in germ cell function even though it is not required for pole cell formation. A key question raised by these *Drosophila* studies is whether a direct and global *pgc* RNA-responsive mechanism of mass repression lies waiting to be unearthed, or whether germline transcriptional quiescence is enforced by overlapping programs that involve *pgc* RNA, Nanos, Germ cell-less and presumably other factors. The answer will be of relevance not only to stem cell and developmental biology, but also for understanding transcription regulation.

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