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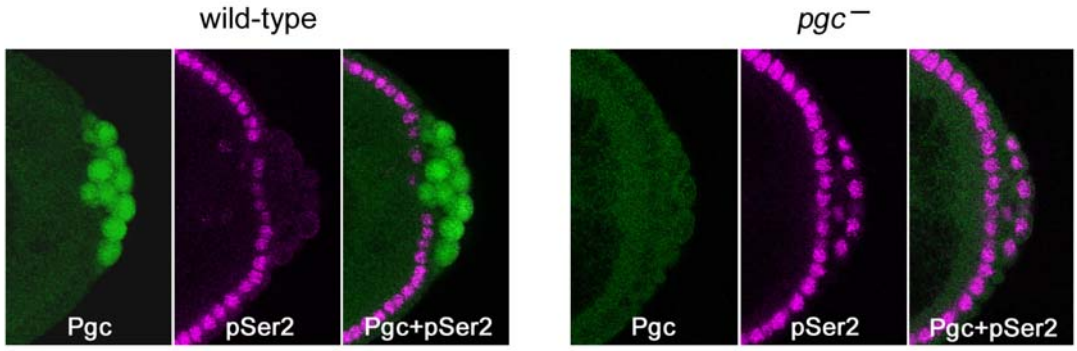
Keeping germline transcription under wraps

January 28, 2008 – The cells of the germline represent the future of a species, as they are the only cells capable of conveying genetic information from one generation to the next. Given this critical function, it is vital that they be protected from the influence of factors that might cause them to differentiate into somatic lineages. In many taxa, this protection is achieved by the transient and global repression of RNA polymerase II (RNAPII)-dependent transcription in germ cell progenitors, which keeps them from deviating from their purpose. The RNAPII enzyme is encoded in all eukaryotic genomes, and catalyzes the transcription of DNA into messenger RNAs (mRNAs). In some invertebrate species, such as the roundworm *C. elegans* and the fruit fly *Drosophila melanogaster*, the repression of transcription in fledgling germline cells has been linked to the absence of phosphorylation (a form of protein modification) of Ser2 residues in a specific domain in the RNAPII protein, but the means by which this is achieved has been obscure.



Posterior pole of the blastoderm-stage embryos immunostained for Pgc (green) and CTD phospho-Ser2 (magenta). Pgc is expressed in newly formed pole cells and CTD Ser2 phosphorylation is repressed in these cells (wild-type, left). In the absence of Pgc, pole cells fail to repress CTD Ser2 phosphorylation (pgc-, right).

Kazuko Hanyu-Nakamura, Hiroko Sonobe-Nojima and colleagues in the Laboratory for Germline Development (Akira Nakamura; Team Leader) have now identified the factor responsible for such transcriptional repression in *Drosophila* germ cell precursors. Their work, published in Nature, reveals that the product of the *polar granule component* (*pgc*) gene represses Ser2 phosphorylation of the RNAPII carboxy-terminal domain (CTD) in germline progenitors known as pole cells by interfering with the recruitment of a second factor, P-TEFb, which is known to play a role in CTD Ser2 phosphorylation in vivo.

The project began somewhat serendipitously when the Nakamura team noted that *pgc* RNA, which was assumed to be non-coding, in fact contains a sequence that suggests a translation start site. Going back to the completed *Drosophila* genome, they found that *pgc* should indeed be capable of encoding a small protein, which they verified by immunostaining; antibodies against the Pgc peptide showed immunoreactivity in early-stage pole cells, before tapering off later in embryonic development.

They next generated flies with various mutations causing losses of *pgc*-function and discovered that, while pole cells did form, there was no repression of Ser2 phosphorylation in the RNAPII carboxy-terminal domain, causing the cells to degenerate by mid-stage embryogenesis. This defect could be rescued by the

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expression of intact Pgc. Moreover, misexpression of *pgc* in somatic cells resulted in the repression of Ser2 phosphorylation, strongly suggesting that the newfound protein plays a central role in transcriptional repression.

The team next turned their attention to a second factor, P-TEFb (Positive transcription elongation factor b), which had previously been implicated in the phosphorylation of CTD Ser2 and promotion of productive transcription elongation. Suspecting that this might be a target of Pgc, they used co-immunoprecipitation and pull-down assay to establish a specific interaction between the two factors, both in vitro and in vivo, in which Pgc interacts with Cdk9, a catalytic subunit of P-TEFb. Overexpression of P-TEFb in pole cells produced a similar effect to that of loss of *pgc* function, suggesting that Pgc represses Ser2 phosphorylation in pole cells by interfering with the function of P-TEFb. But the finding that an MBP-Pgc construct, which interacted with P-TEFb, did not affect CTD phosphorylation by that factor in vitro, indicated that Pgc's function was unlikely to regulate the catalytic activity of P-TEFb. They compared localization of P-TEFb in untreated somatic tissue and tissue in which Pgc was misexpressed, and found a dramatic reduction of their normal localization to active promoter regions on chromosomes. This suggested that Pgc may function by sequestering P-TEFb and preventing its recruitment to promoter sites, a view that was shored up by the finding that Pgc expression inhibited normal P-TEFb recruitment to heat shock genes following heat shock.

"Studies in *C. elegans* have shown that another germline protein, PIE-1, is also involved in regulating the phosphorylation of CTD Ser2 and that it appears to bind to the CyclinT subunit of P-TEFb," says Hanyu-Nakamura. "What's interesting is that Pgc and PIE-1 are non-homologous, which means these germline transcriptional repression systems may well have evolved independently."

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