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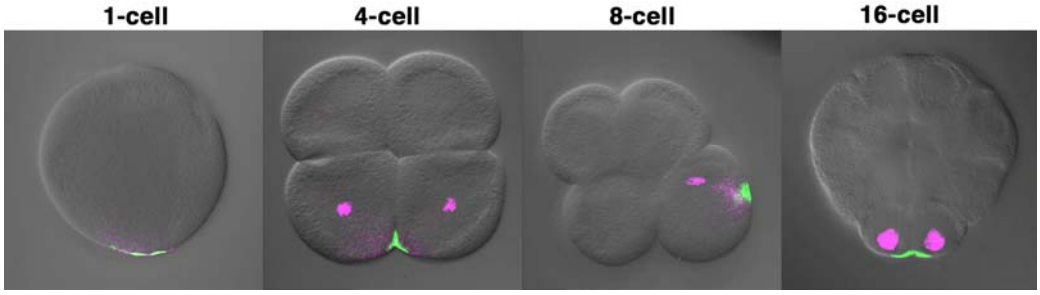
First germline transcriptional repressor in ascidian found

Aug 18, 2011 – In most animal embryos, molecular measures are in place to prevent nascent germ line cells from mistakenly differentiating into a somatic lineage. This is typically achieved by the repression of somatic gene expression by specific genetic factors in the germ plasm, such as Pgc in *Drosophila* and PIE-1 in *C. elegans*. It has been suspected that transcriptional repression is also at work in embryos of the ascidian *Ciona intestinalis*, which also has a germ plasm-like cytoplasmic compartment known as the postplasm, but the factors and mechanisms that might lie behind this process had been unknown.

Now, new work by Maki Shirae-Kurabayashi and colleagues in the Laboratory for Germline Development (Akira Nakamura, Team Leader) has identified what appears to be a key transcriptional repressor in the ascidian, a protein product of *Ci-pem-1* RNA, which is an ascidian specific component of the postplasm. This study, published in the journal *Development*, shows that Ci-Pem-1 interacts with another general co-repressor of mRNA transcription, lending further support to the knockdown findings.



Ciona intestinalis embryos at 1, 4, 8 and 16-cell stage probed for *Ci-pem-1* mRNA (green) and Ci-Pem-1 protein (magenta). In the pair of germline blastomeres, *Ci-pem-1* mRNA is highly concentrated in the postplasm at the posterior cortex, while Ci-Pem-1 protein is enriched in the nuclei.

Transcriptional repressors in other widely studied species, such as fruit fly and nematode, work by inhibiting the phosphorylation of the C-terminal domain in RNA polymerase II, a critical enzyme in mRNA transcription. Shirae-Kurabayashi began this study by examining such RNAPII phosphorylation in *C. intestinalis* blastomeres, at a stage of development in which the germ line begins to emerge as a distinct lineage, as signaled by the inheritance of postplasm. The team analyzed RNAPII phosphorylation in individual blastomeres using anti-phospho-CTD antibodies, and found that while somatic blastomeres showed normal levels of activities, in germline blastomeres this phosphorylation was downregulated.

Familiar with the expression of *Ci-pem-1* from a previous study, Shirae-Kurabayashi used immunostaining to visualize the distribution of its protein product. They previously reported that the postplasmic *Ci-pem-1* RNA was ejected from the germline during gastrulation by an asymmetric cell division (Shirae-Kurabayashi et al., 2006). In the present study, they indicated that the signal of it's protein product also dropped in the germline by the tailbud stage. More intriguingly, she found that it localized to the nuclei in the germline blastomeres in the most posterior region of the cleavage-stage embryo. This nuclear accumulation, however, fluctuated over the course of the cell cycle, appearing only in late telophase when the nuclear envelope reassembles following replication. To eliminate the possibility that this reaction was an artifact of cross-reactivity, they re-tested in blastomeres injected with a morpholino against *Ci-pem-1*, and found, in line with expectations, that MO knockdown prevented its nuclear localization.

Thinking that this protein might play a role in transcriptional control in germline cells, the

team examined the effects of *Ci-pem-1* knockdown on embryonic gene expression. When they knocked down this factor, they detected ectopic expression of a number of genes ordinarily repressed in germline blastomeres. The expression of both beta-catenin and GATA-dependent genes was de-repressed in the knocked-down germline blastomeres, indicating the Ci-Pem-1 exerts a broadly repressive effect on somatic gene expression.

The structure of the Ci-Pem-1 protein includes a motif reminiscent of binding domains in other species that associate with the transcriptional co-repressor Groucho. Shirae-Kurabayashi performed a co-immunoprecipitation assay using tagged constructs for these two proteins, and found that Groucho associated with GFP-labeled Ci-Pem-1, but not with GFP alone, pointing to a physical interaction of potential functional significance.

“Unlike Pgc in *Drosophila* and PIE-1 in *C. elegans*, our findings suggest that Ci-Pem-1 appears to repress somatic gene expression without directly inhibiting RNAP II phosphorylation, indicating that distinct mechanisms and factors are responsible for transcriptional quiescence in different organisms,” says Nakamura. “Interestingly, recent work by Mitinori Saitou’s group, a former CDB team leader, has shown that the silencing of somatic transcriptional programs also occurs during the establishment of mouse germ cells, which are induced through cell-cell signaling without the germ plasm, meaning the active repression of somatic transcriptional programs during the establishment of the germline is a conserved hallmark in animal development, regardless of the mode of germ cell formation.”

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