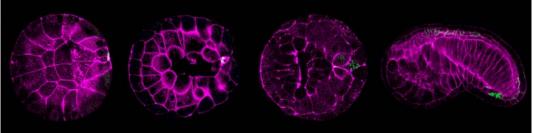
RIKEN Center for Developmental Biology (CDB)

2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

New role for Vasa in *Ciona* germline

July 25, 2006 – All animal cells can be categorized into either somatic or germ lineages. While somatic cells build and maintain the body itself, the cells of the germline differentiate into eggs and sperm, transmit genetic information to the next generation and retain the ability to differentiate into each and every cell type in the body. In many animals, including the research models *Drosophila* and *C. elegans*, the development of the germline founder population of primordial germ cells (PGCs) is characterized by the inheritance of germ plasm, a cytologically distinct assembly of electron-dense granules. These germinal granules later form perinuclear granules, or nuage – a distinguishing feature of the germ cell lineage in these species. Despite their centrality to germline development, however, both the germ plasm and nuage remain poorly understood.



CiVH protein (green) of *Ciona intestinalis* is incorporated into the posterior most blastomeres, called the B7.6 cells, which divide asymmetrically during gastrulation.

Now, in a study published in the online edition of *Development*, Maki Shirae-Kurabayashi (Laboratory for Germline Development; Akira Nakamura, Team Leader) and colleagues shed new light on the mechanism underlying germline formation in the ascidian *Ciona intestinalis*. This remarkable organism has a unique form of cytoplasm known as the postplasm in its developing germline, which is believed to serve as the ascidian germ plasm. Previous studies have shown *Ciona* postplasm to contain a high concentration of maternal RNAs and proteins, as well as electron-dense masses highly similar to the germinal granules found in *Drosophila*; such masses give rise to the centrosome-attracting body (CAB), a specialized cytoplasmic structure.

Ascidian postplasm also contains a high concentration of RNAs and the protein product of the *CiVH* gene (a homolog of the *Drosophila* germline specific gene *vasa*, which is essential for germ cell development and highly conserved in vertebrates and invertebrates). These CiVH-positive cells are subsequently incorporated into juvenile *Ciona* gonads and form the animal's germ cells. Given these similarities with primordial germ cells in other species, many researchers now believe that postplasm serves as the ascidian germ plasm, in which the progeny of a highly specific subset of blastomeres, termed B7.6 cells, develop into PGCs.

A number of postplasmic components, including the centrosome-attracting body itself, are also known to have a role in promoting asymmetric cell division and the regulation of somatic cell differentiation during early embryogenesis. One of these factors, *posterior end mark* (PEM) RNA, appears to control the positioning of cleavage planes, thereby promoting asymmetric division. Intriguingly, the detection of these postplasmic factors in presumptive B7.6 cells opened up the possibility that, in addition to their known function in somatic cell differentiation, they might also play roles in germ cell formation as well.

<u>RIKEN Center for Developmental Biology (CDB)</u> 2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Previous studies were unclear, however, as to the stage at which B7.6 cells directly merge into the gonads, and whether the postplasmic components involved in somatic cell development do in fact contribute to germ cell formation. Shirae-Kurabayashi et al. first established that *Ciona* B7.6 cells undergo an asymmetric cell division to produce two daughter cells, B8.11 (the CAB-containing anterior cells) and B8.12 (CAB-negative posterior cells). As most of the postplasmic components are associated with the CAB, these too are partitioned solely into the B8.11 daughters, subsequently losing CiVH protein expression and associating with the animal's gut wall. The B8.11 cells, however, did retain the remnants of the CAB and other postplasmic components, which were not seen in B8.12 cells, suggesting that B8.11 cells do not function in the specification of germ cells.

The team did find, however that maternal *CiVH* RNA and proteins entered the cytoplasm from the CAB immediately prior to B7.6 division, and were then inherited by the B8.12 progeny. Subsequent upregulation of CiVH protein production in B8.12 cells resulted in the formation of perinuclear CiVH granules, which they tentatively identified as the nuage characteristic of germ cells. Using Dil labeling and CiVH immunostaining, the Nakamura team found that the descendants of the B8.12 cells are incorporated into *Ciona* primitive gonads and go on to form the germ cells. This suggests that the *Ciona* B8.12 cells are indeed early germline progenitors, and that formation of a nuage-like structure occurs immediately after the B7.6 cell division, much earlier than was previously thought.

Shirae-Kurabayashi proposes that the centrosome-attracting body and most postplasmic components involved in somatic differentiation are segregated from the PGCs through a single asymmetric cell division, and that germline development in ascidian embryogenesis requires the diffusion of specific postplasmic components into the cytoplasm prior to the B7.6 division. Studies of other ascidian species showing the localization of postplasmic/PEM RNAs into two distinct embryonic regions indicate that the asymmetric distribution of postplasm components at the B7.6 cell division may be a conserved feature of germ cell specification in ascidians. "In this work, we showed that a postplasm factor participates in the formation of the *Ciona* germline," says Shirae-Kurabayashi, " but we also know that the germline will regenerate itself if it is ablated from a larval ascidian, which suggests that there are two separate mechanisms – embryonic and postembryonic – for constructing the germ lineage in these animals, so of course we're very curious to find out how each of those works."