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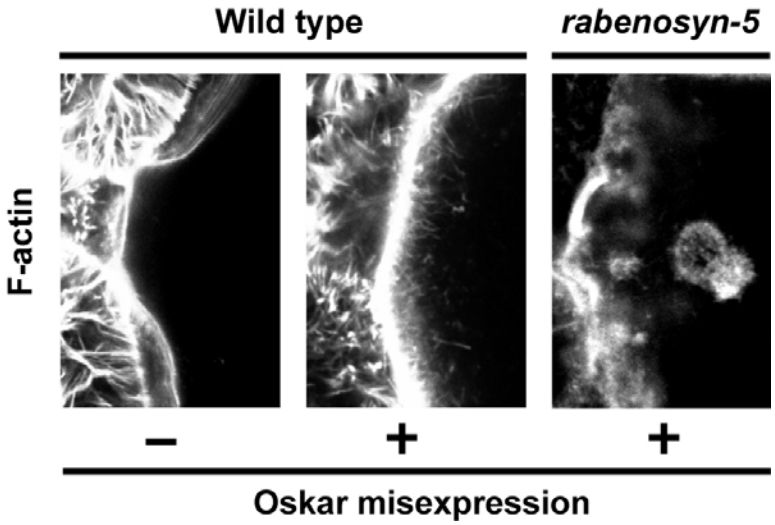
Rbsn-5, a new factor in pole plasm assembly

March 5, 2008 – The *Drosophila* oocyte is a polarized structure, with clearly demarcated anterior and posterior ends showing distinct patterns of RNA and protein localization. The posterior pole is home to the pole plasm, from which the cells of the germline originate, and whose assembly is mediated by the protein Oskar (Osk). Interestingly, *osk* RNA yields two isoforms, long and short Osk, each with distinct functions – short Osk serves as a recruiter of pole plasm components, while long Osk tethers them to the posterior cell cortex through interactions with the actin cytoskeleton governed by some as-yet unknown mechanism.

A new study by Tsubasa Tanaka of the Laboratory for Germline Development (Akira Nakamura; Team Leader) now points to a hitherto unsuspected link between Oskar and the endocytic pathway that may help to explain how this protein fulfills its function. The report, published in *Development*, reveals that the endosomal protein Rabenosyn-5 is required, apparently downstream of Osk, for pole plasm assembly.



F-actin at the anterior pole in wild type or *rbsn-5* oocytes without or with misexpression of Oskar. Anterior misexpression of Oskar promotes long F-actin projections emanating from cortical actin bundles in wild type oocytes (middle image), but induces large aberrant F-actin aggregates in *rbsn-5* oocytes (right image).

The study arose from a mutagenesis screen to identify mutations that affected the formation of pole plasm, a special form of cytoplasm found in the posterior end of the *Drosophila* oocyte. The screen netted one promising candidate factor that was identified as a homolog of the protein Rabenosyn-5 (Rbsn-5), a Rab-effector protein in mouse that functions in endocytosis, a process in which materials are absorbed from the extracellular environment and sorted for processing.

Given the failure of pole plasm assembly in the *rbsn-5* mutant, Tanaka checked for a possible link to Oskar, and found that although the initial recruitment of *osk* RNA to the posterior pole was unaffected, its accumulation was unstable and deteriorated at later stages. Knowing that *osk* RNA is carried to the posterior via a polarized network of microtubules, he investigated a the effects of loss of *rbsn-5* function on this process, and again found that while microtubule polarity was established, it failed to be maintained.

Although this opened up a plausible explanation for the pole plasm assembly defects in *rbsn-5* mutants, the close co-localization of Rbsn-5 and Osk in oocytes suggested that there might be something more to the story. Knowing the

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association of Rbsn-5 with endosomes in other species, Tanaka looked for correspondences with other endosomal markers, and found that all showed polarized (posterior) distributions similar to both Rbsn-5 and Osk. This posterior localization of endosomes appeared briefly but was subsequently lost in an *osk*-null mutant, suggesting that the maintenance of endocytic polarization is dependent on Osk. (The initial establishment of endocytic polarity appears rather to rely on the even earlier oocyte A-P polarization factor, Gurken.)

Experiments in which *osk* was misexpressed at the anterior pole further showed the involvement of Osk in the endocytic pathway. Anteriorly misexpressed Osk recruits endosomal proteins and stimulates endocytic activity. Wondering whether this might be a function specific to one of the two Osk isoforms, Tanaka tried expressing short and long Osk in the anterior and found that, while the ectopic expression of short Osk alone had no noticeable effect, misexpression of long Osk was sufficient to recruit endosomes to the anterior pole of the oocyte.

It has long been suspected that long Osk works by anchoring pole plasm components to the posterior cortex, but the involvement of Rbsn-5 in microtubule polarity in normal oocytes made it impossible to disentangle its known role in this process from a second possible role in Osk-mediated tethering. But as *rbsn-5*$^{-}$ oocytes maintain their competence to localize RNA to the anterior pole, they provide a unique environment for focusing on this question. Looking at Osk recruitment and maintenance in an *rbsn-5* mutant in which Osk was misexpressed at the anterior pole, Tanaka found that the anchoring of Osk and other pole plasm components to the cortex was weak, resulting in their diffusion into the cytoplasm. Although Osk promotes long F-actin projections from the cortex at both anterior and posterior ends of wildtype oocytes, the anterior misexpression of Osk in *rbsn-5* oocytes induced large aberrant F-actin aggregates, which diffuse along with pole plasm components into the cytoplasm, suggesting that long Osk stimulates endocytic activity, which in turn promotes proper F-actin reorganization to anchor pole plasm components to the oocyte cortex.

“Although the genetic approach used in this study reveals that the endocytic pathway acts downstream of long Osk to anchor pole plasm components,” says Nakamura, “further study of the molecular basis of long Osk function in endocytic activity is still needed to develop a better understanding of pole plasm assembly.”

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