Oocyte specification and *orb*

In many invertebrates and vertebrates, oocytes and sperm are derived from a cyst of cells connected to each other by cytoplasmic bridges. These cysts arise from a single cell, the cystoblast, that undergoes a series of synchronous mitotic divisions with incomplete cytokinesis to give to rise to a multicellular cyst. In testes, all cells typically become sperm. In ovaries, this is also true for some vertebrate species; however, in other species only one member of the cyst becomes an oocyte. In *Drosophila* five mitotic divisions give rise to a highly invariant 16-cell cyst. These cell divisions occur in the germarium. In the first division, a germline stem cell (GSC) divides to produce two daughters. One daughter remains a GSC, while the other daughter becomes the cystoblast. The cystoblast undergoes four mitotic divisions to generate the 16-cell cyst. Two of the cells in the cyst, the original daughter of the GSC (the cystoblast) and its daughter, have four ring canals. These two cells correspond to the pro-oocytes, and they are linked to each other by a ring canal which was formed at the first division of the cystoblast (Fig. 1). One of the pro-oocytes becomes the oocyte, while the other assumes a nurse cell fate. The pro-oocytes have three additional ring canals, linking the pro-oocytes to their three successive daughters.

Oocyte specification takes place after the formation of the 16-cell cyst and is a robust symmetry breaking process. As the cysts begin to mature in region 2a of the germarium, cytoplasmic markers of oocyte identity and assemble a synaptonemal complex in their nuclei. One of the pro-oocysts is selected to become the oocyte as the 16-cell cysts transition from region 2a to 2b of the germarium. At this point cytoplasmic markers begin accumulating in only one of the pro-oocytes, which then assume an oocyte identity, while the other pro-oocyte loses cytoplasmic markers of oocyte identity, disassembles the synaptonemal complex, and commits to a nurse cell fate.

The available evidence indicates that nurse cell identity is the default fate. Thus, there must be mechanisms in place that make these two pro-oocytes unique amongst all of the cells in the cyst in that they can potentially acquire oocyte identity. In addition, there must mechanisms that drive oocyte fate in one of the two cells, and conversely ensure that the other cell assumes a nurse cell identity.

Key players in oocyte specification include a tubular membranous structure called the fusome which extends through the ring canals connecting all 16 cells in the cysts and two dynein mRNA cargo adaptors, Bicaudal-D (BicD) and Egalitarian (Egl), Mutations in *BicD* or *egl* block oocyte specification and in both cases mutant egg chambers have 16 nurse cells and no oocyte The fact that oocyte specification depends upon a dynein adaptor for mRNA cargoes suggests that oocyte specification is driven by mRNAs that are localized to the pro-oocytes. Of the mRNAs that are localize in the pro-oocytes in the germarium, the best candidate for the critical specification factor is *orb*. *orb* mRNAs and proteins are amongst the very first gene products that are specifically localized in the pro-oocytes, and subsequently the oocyte. *orb* encodes one of the two fly CPEB (cytoplasmic polyadenylation element binding protein) translation factors. Proteins in this family bind to hundreds of mRNAs and depending on the biological context can negatively or positively regulate their translation. In addition to being able to control the translation of mRNAs required for oocyte differentiation, *orb* has a positive autoregulatory activity in which Orb protein binds to the 3' UTR of *orb* mRNA and activates its on-site translation (Fig. 2: translation of *lacZ-orb* 3' UTR mRNA depends upon *orb*). In other biological contexts, positive feed-forward loops are used to drive fate specification.

Consistent with the idea that *orb* mRNAs function as a critical oocyte determinant, when sequences encoding the *orb* 3' UTR are deleted (*orbΔ3'UTR*) from the endogenous gene, the mutant mRNAs don't localize to the pro-oocytes, the *orb* autoregulatory loop (which drives the translation of high levels of Orb protein in the pro-oocytes) is not activated and oocyte specification fails. The *orb* 3' UTR is also sufficient to drive oocyte specification when linked to the *orb* protein coding sequence: The oocyte specification defects of the *orbΔ3'UTR* mutant can be partially rescued by introducing a sequence, *XN*, containing a part of the *orb* 3' UTR into the *orbΔ3'UTR* deletion. However, the rescuing activity is unusual in that oocyte specification is delayed until stage 2 of oogenesis, which is after the egg chamber exits the germarium. At this point, only half of the chambers successfully specify an oocyte. In these chambers, *orb* mRNA and protein, as well as other markers of oocyte identity accumulate in a single cell and this cell develops as the oocyte. As is the case in wild type, the oocyte is always one of the two cells that have four ring canals. In the chambers that failed to specify an oocyte, *orb* mRNAs and proteins are distributed throughout in all 16 germ cells as are other markers of oocyte identity, and all cells develop as nurse cells.

Ongoing studies are focused on the mechanisms that localize and then anchor *orb* mRNA in the two pro-oocytes, and on the activation of the *orb* autoregulatory loop so that high levels of protein accumulate in these two cells. We find that a critical step for specification of the oocyte in the germarium is the tight association of *orb* mRNA with the fusome. *orb* mRNA association with the fusome requires the *orb* 3' UTR and Orb protein (Fig. 3: *orb* 3' UTR mediates fusome association). When *orb* mRNAs are not linked to the fusome in region 2a/2b, oocyte specification fails. This fusome dependence is bypassed in *XN*; however, oocyte specification doesn't take place till after the egg chambers exit the germarium.

Publications:

1) The Drosophila **orb** RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. Lantz V, Chang JS, Horabin JI, Bopp D, Schedl P.Genes Dev. 1994 Mar 1;8(5):598-613. doi: 10.1101/gad.8.5.598.PMID: 7523244

2) An autoregulatory feedback loop directs the localized expression of the Drosophila CPEB protein **Orb** in the developing oocyte. Tan L, Chang JS, Costa A, Schedl P.Development. 2001 Apr;128(7):1159-69. doi: 10.1242/dev.128.7.1159.PMID: 11245581

3) The Drosophila fragile X protein functions as a negative regulator in the **orb** autoregulatory pathway. Costa A, Wang Y, Dockendorff TC, Erdjument-Bromage H, Tempst P, Schedl P, Jongens TA.Dev Cell. 2005 Mar;8(3):331-42. doi: 10.1016/j.devcel.2005.01.011.PMID: 15737929

4) Cup blocks the precocious activation of the **orb** autoregulatory loop. Wong LC, Schedl P.PLoS One. 2011;6(12):e28261. doi: 10.1371/journal.pone.0028261. Epub 2011 Dec 2.PMID: 22164257

5). The *Drosophila* CPEB Protein **Orb** Specifies Oocyte Fate by a 3'UTR-Dependent Autoregulatory Loop. Barr J, Gilmutdinov R, Wang L, Shidlovskii Y, Schedl P.Genetics. 2019 Dec;213(4):1431-1446. doi: 10.1534/genetics.119.302687. Epub 2019 Oct 8.PMID: 31594794

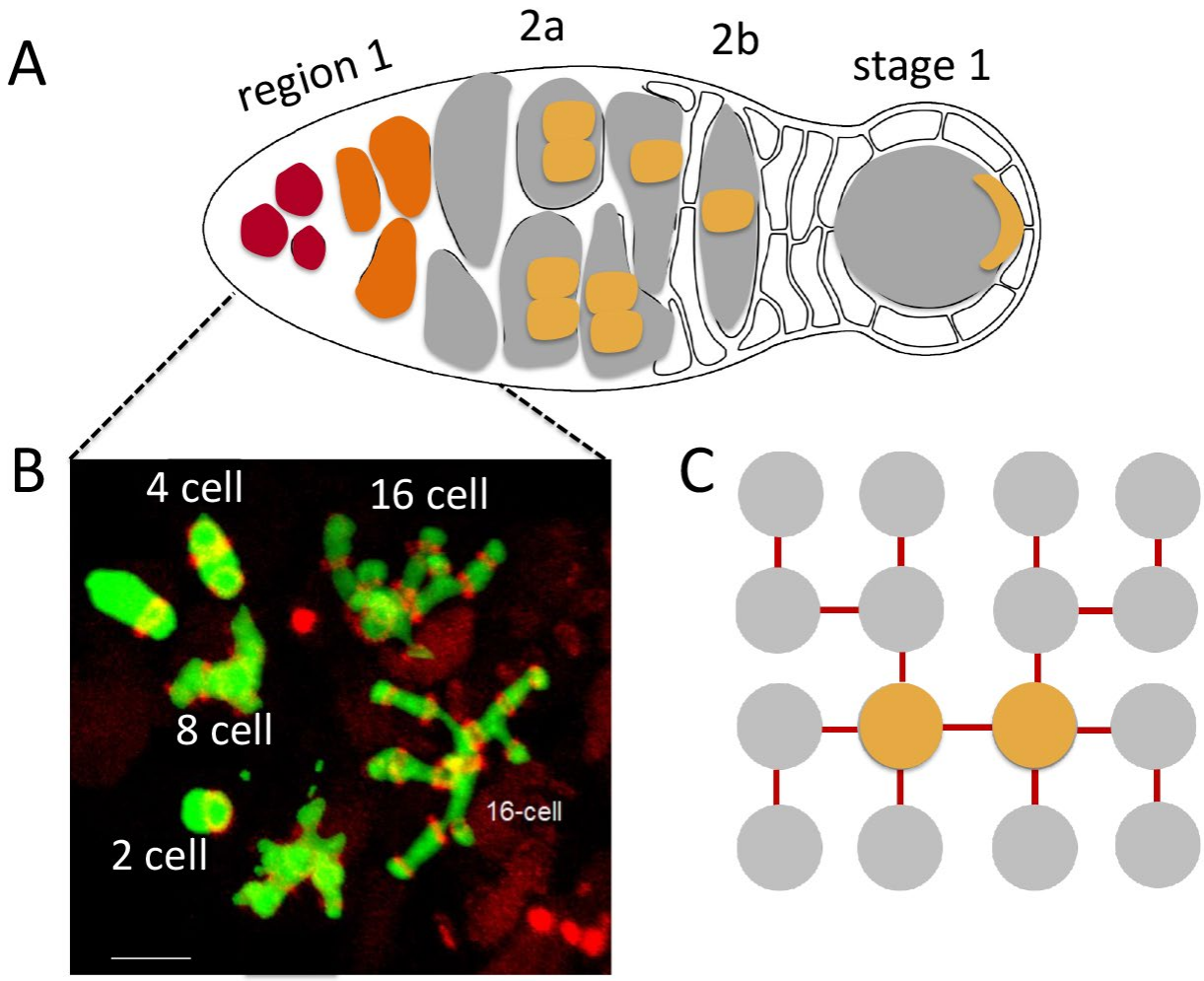

Fig. 1 Formation of the 16 cell cysts plus fusome organization during the mitotic divisions. (orange: pro-oocytes.

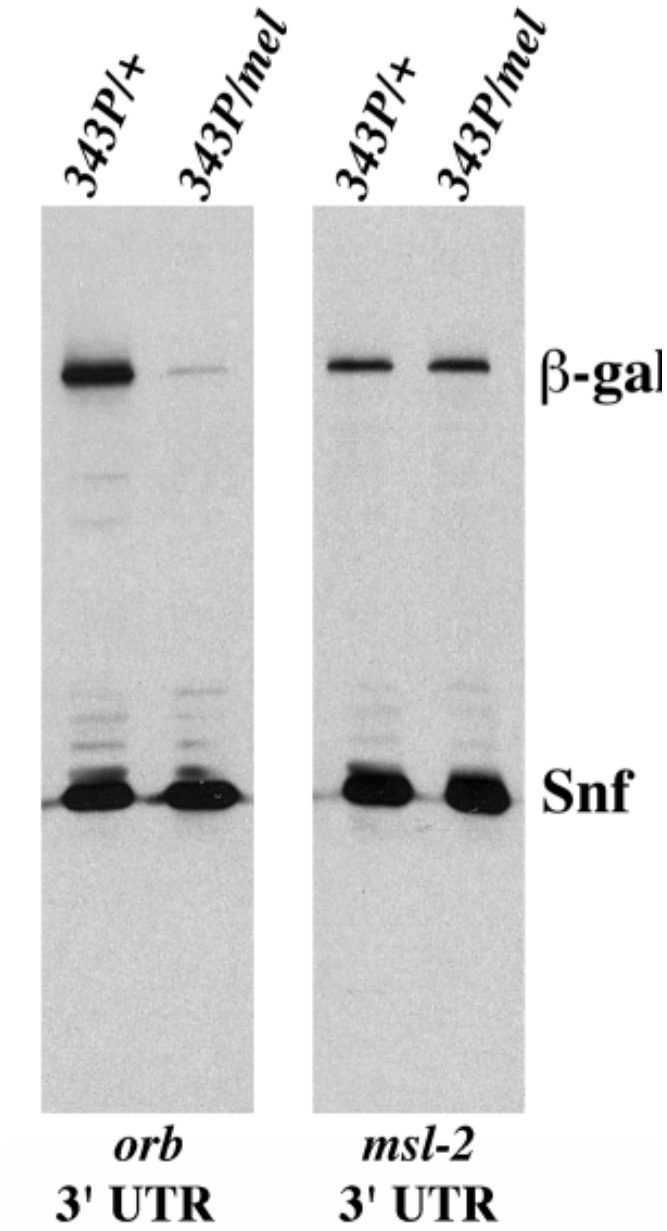

Fig. 2. Orb protein binds to *orb* 3' UTR and regulates its own synthesis. Expression of LacZ in *hsp83 promoter: lacZ orb* 3' UTR and *hsp83promoter:lacZ msl-2* 3'UTR in orb^{343}/+ and orb^{343}/ orb^{mel} female ovaries. orb^{343} is a null allele, while orb^{mel} is a weak hypomorph.

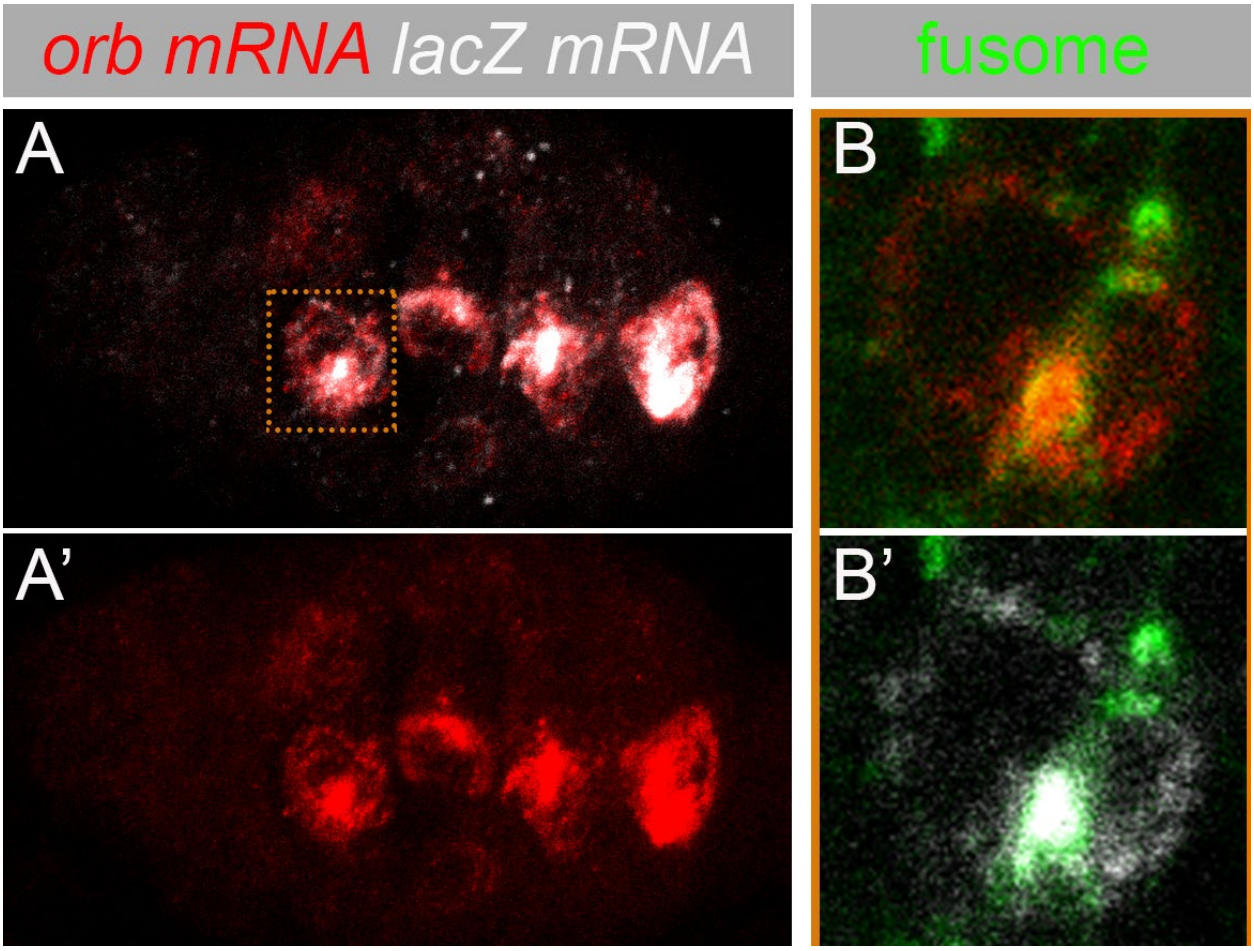

Fig. 3. Association of *orb* mRNA and a chimeric *lacZ orb* 3' UTR with the fusome in regions 2a/2b of the germarium.