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# Pining down germline development


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In many animals, the embryonic animal-vegetal axis is established during oogenesis. A key feature in many vertebrate oocytes is a membrane-less compartment, the Balbiani body (Bb), that contains many organelles and ribonucleoprotein (RNP) complexes. The Bb facilitates the organisation of the oocyte into a polarized cell with discrete cytoplasmic domains, including localisation of germ plasm RNP complexes which specify the germline. In zebrafish, the Bb first forms adjacent to the nucleus and subsequently, its position defines the vegetal pole of the oocyte, where germplasm and axis determinants (e.g. Wnt8 and Syntabulin) are anchored. In fertilised embryos, germ granules relocate to the animal blastoderm, and aggregate at the distal ends of cleavage furrows during early cell divisions. The cells that acquire the granules later become germline progenitors or primordial germ cells (PGCs). The molecular mechanisms that govern oocyte polarity, Balbiani body formation and germplasm distribution remain largely unknown.

Through quantitative image analysis of germplasm dynamics and cytoskeletal reorganisation in zebrafish eggs and embryos, we find that germ granule movements commence with furrow formation during early cleavage divisions. Analysis of zebrafish mutants affecting the RNA-binding protein Ybx1 (Y-box binding-protein 1) and a Ybx1 target called *pinchado*, shows that the timing and dynamics of germ granule accumulation in the oocyte and blastoderm is a crucial factor for appropriate distribution of the complex to PGCs. Loss of *pinchado* leads to defects in oogenesis, loss of oocyte polarity and embryonic lethality. Maternal *ybx1* mutant embryos show reduced germplasm and mutant adults show biased adult sex ratios. Germplasm distribution is disrupted in *pinchado* mutant oocytes, and reduced and ectopic aggregates form at the blastoderm margin of maternal *ybx1* mutant embryos. Germline gene expression is altered and there is increased expression of some somatic markers in the ovary. Reporter fusions show dynamic localisation in early zebrafish embryos and Pinchado associates with the Actin cytoskeleton at the cortex. Our findings suggest that *pinchado* functions in maintaining Bb integrity and anchoring of germplasm to the cortex. Thus, Pin and Ybx1 have crucial roles in regulation of oocyte polarity, germplasm distribution and germline development.


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