# Rotating for elongation: Fat2 whips for the race

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Dynamic rearrangements of the actin cytoskeleton are crucial for cell shape and migration. In this issue, Squarr et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201508081) show that the cadherin superfamily protein Fat2 regulates actin-rich protrusions driving collective cell migration during *Drosophila melanogaster* egg morphogenesis through its interaction with the WAVE regulatory complex.

Collective cell migration is a hallmark of tissue remodeling during embryonic development, as well as of tissue repair and cancer invasion (Rørth, 2012). A novel type of collective cell migration has emerged in recent years from studies of *Drosophila* follicles (Haigo and Bilder, 2011), highlighting what seems to be a potentially conserved, intrinsic property of epithelial cells growing in constricted environments. The *Drosophila* follicle or egg chamber is a spherical assembly of germ cells surrounded by an epithelium of somatic follicle cells. The egg chamber elongates to an ellipsoid during oogenesis, thereby conferring the egg its appropriate shape (Fig. 1). Egg chamber elongation is guaranteed by a molecular corset, formed by the follicle cells and the basal ECM, which directs the growth of the egg chamber along the anterior–posterior axis by constraining the central area of the egg (He et al., 2010). In particular, an ordered array of contractile actin filaments within the follicle cells, running perpendicular to the anterior–posterior axis of the egg chamber, contributes to this "corset" (He et al., 2010). Collective migration of follicle cells around the anterior–posterior axis of the egg chamber is required to promote the global polarization of these parallel actin bundles and of the follicular basement membrane (Haigo and Bilder, 2011; Cetera et al., 2014). This is a remarkable type of cell migration as it leads to a rotational movement of a group of cells within a constrained space and without an identified collective leading edge. Nonetheless, it turns out that actin-rich protrusions, which are typical of a leading edge, are present at the basal side of each individual follicle cell. These protrusions point toward the direction of rotation and are necessary to generate the collective rotational movement (Cetera et al., 2014).

Presence of these actin protrusions at the leading edge of follicle cells requires the WASP family verprolin homologous protein (WAVE) and the WAVE regulatory complex (WRC; Cetera et al., 2014), known activators of actin nucleation through the Arp2/3 complex, as depletion of WAVE or of the WRC component Abi eliminates all actin-based protrusions in follicle cells (Cetera et al., 2014).

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The details of the control of WAVE and WRC activation have been under scrutiny for many years (Stradal and Scita, 2006; Ismail et al., 2009; Chen et al., 2010; Mendoza, 2013). Nonetheless, a recent discovery opened the possibility of a novel and conserved mechanism for WRC activation (Chen et al., 2014). A combination of structural and biochemical approaches revealed that the WRC can be recruited to the membrane by an array of membrane proteins sharing a conserved peptide motif, the WRC interacting receptor sequence (WIRS). The binding surface for WIRS is provided by two WRC subunits, including Abi, and leads to activation of WAVE toward the Arp2/3 complex (Chen et al., 2014). Could a WIRS domain-containing molecule be involved in the localized activation of WAVE during the rotational movement of follicle cells? This attractive hypothesis is supported by the observation that disruption of the WIRS interface in the WRC leads to the generation of round eggs (Chen et al., 2014). The round-egg phenotype characteristically results from mutations in genes promoting the rotational movement of the follicle cells or organizing the underlying ECM (Gutzeit et al., 1991; Bateman et al., 2001; Frydman and Spradling, 2001; Deng et al., 2003; Schneider et al., 2006). One of these round-egg genes is the cadherin superfamily *fat2/kugelei* (Gutzeit et al., 1991). Individual follicle cells from *fat2* mutants have parallel-arranged actin filaments. However, these actin filaments are no longer perpendicular to the anterior–posterior axis and their coordinated organization in the tissue is lost (Gutzeit et al., 1991; Viktorinová et al., 2009). Moreover, large clones of *fat2* mutant follicle cells lead to uncoordinated arrangement of actin bundles in the wild-type neighboring cells (Viktorinová et al., 2009). Collectively, these previous results suggested that Fat2 coordinates actin organization in follicle cells.

In this issue, Squarr et al. identified Fat2 as a novel WIRS domain–containing molecule that acts through the WRC to control collective cell migration during *Drosophila* oogenesis. Squarr et al. (2016) elegantly used live in vivo imaging and genetically encoded probes to characterize different types of actin-rich protrusions during egg chamber maturation. Their analysis showed that small filopodial protrusions extend at regular intervals in a polarized fashion at the basolateral cell border, whereas on the apical side filopodial protrusions are not polarized along the migration direction (Cetera and Horne-Badovinac, 2015; Squarr et al., 2016). Stunning time-lapse movies remarkably revealed an additional type of actin-rich whip-like protrusion at tricellular junctions (Fig. 1). All these types of actin protrusions depend on the WRC, as they are largely missing in follicle cells with impaired WRC function.



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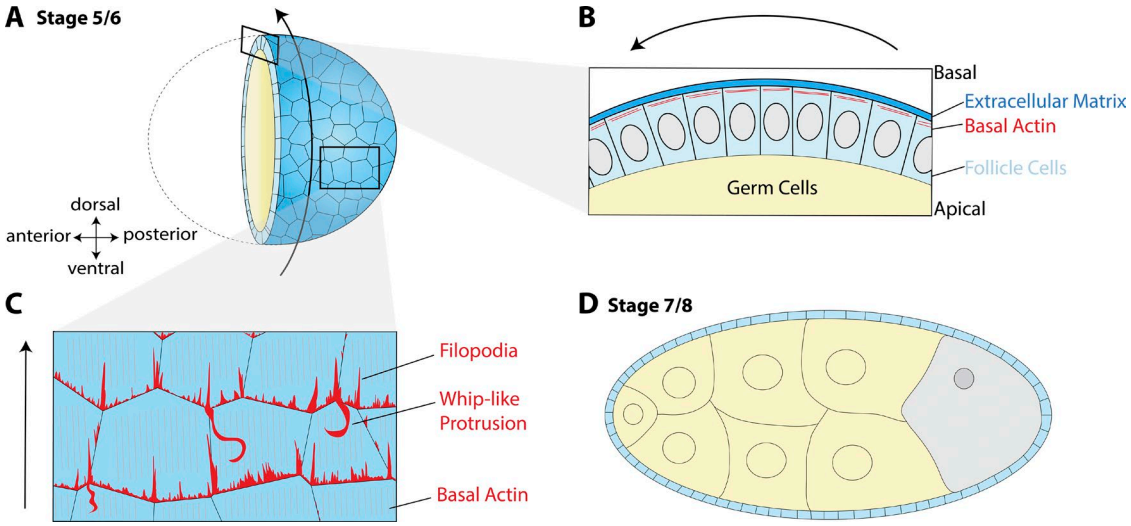


Figure 1. **The actin-rich structures underlying egg chamber elongation.** Maturing egg chambers contain germ cells (yellow), including the oocyte (D, brown), covered by a layer of follicle cells (light blue). During early maturation stages, the follicle cells drive the rotation of the egg chamber over the ECM (dark blue) and pull the germ cells along. This rotation promotes the elongation of egg chambers along the antero–posterior axis, observed from stage 7/8. (A) Schematic representation of an egg chamber at stage 5/6, during the collective rotation movement in the direction indicated by the arrow. The axis cross applies to A, C, and D. (B) A schematic of the follicle epithelium (transversal section) indicating its apical side, oriented toward the germ cells, and the basal membrane, laying over the ECM. Basal actin filaments are depicted in red. (C) Higher magnification of the follicle cells visualized from their basal side. Actin-rich structures are depicted in red. They include actin-rich protrusions at the leading edge of each cell and whip-like protrusions at tripartite junctions between cells. Within each cell, the bundles of actin filaments (basal actin) run parallel to the direction of follicle epithelium rotation (black arrow). (D) Illustration of the elongated egg chamber in the next maturation stages (7/8) in *Drosophila* development.

At defined stages of egg chamber maturation, the WRC is prominently enriched at tricellular junctions, a localization that resembles that reported for Fat2 at the same stages (Viktorinová et al., 2009; Squarr et al., 2016). Motivated by this observation and in search of the molecular mechanisms driving the formation of the actin-rich structures, Squarr et al. (2016) asked whether Fat2 is functionally related to the WRC-dependent organization of actin. Indeed, they identified three conserved WIRS motifs in the cytoplasmic tail of Fat2 and demonstrated a direct interaction of Fat2's cytoplasmic tail with the WRC through in vitro binding assays, establishing Fat2 as a novel WIRS ligand.

They additionally proved that functional WIRS interactions are necessary for the formation of whip-like protrusions and polarized protrusions as well as egg chamber elongation by analyzing flies lacking the conserved WIRS binding surface in the WRC. To test the hypothesis that Fat2 is involved in recruiting the WRC, the researchers examined *fat2* mutant cells, which displayed impaired WRC localization to the basal follicle side and to tricellular junctions as well as reduced actin-rich protrusions at the basal side. Thus, Fat2 contributes to the localization of the WRC in these egg chambers. Interestingly, analysis of additional mutant lines showed that neither loss of the WRC nor loss of the Fat2–WRC interaction affected the distribution of Fat2, suggesting it acts upstream of the WRC to control WRC localization and formation of polarized cell protrusions.

Additional paths of WRC regulation might involve the ECM receptor phosphatase Dlar, as *dlar* mutants also exhibit a round-egg phenotype (Bateman et al., 2001). Indeed, Squarr et al. (2016) observed that Dlar and WRC subunit localization partially overlap during the early stages of egg chamber maturation and provide biochemical evidence for an indirect molecular interaction in vivo between Dlar and the WRC. Importantly, in *dlar* mutants, less WRC localized to the basal side and actin-rich protrusions along the membrane were also reduced. Nonetheless, the localization of the WRC at tricellular junctions is partially maintained and so is actin accumulation at these sites. Collectively, these data establish a model in which Fat2 and Dlar are linked in recruiting the WRC to induce the formation of polarized cell protrusions, contributing to different aspects of WRC regulation for collective follicle cell migration during *Drosophila* oogenesis.

Squarr et al. (2016) combined genetic, biochemical, and live-imaging analyses in *Drosophila* to gain insight into molecular actin dynamics in vivo. Recent data from time-lapse imaging suggested that collective rotational movement is an intrinsic property of epithelial cells and a feature of developing glandular tissues in mammals (Ewald et al., 2008; Tanner et al., 2012). Squarr et al. (2016) confirmed that nonmalignant human breast epithelial cell lines form spheres and undergo multiple rotations (Tanner et al., 2012). To ask whether the actin-rich protrusions that they observed in fly follicle cells represent a conserved structure underlying epithelial rotational migration, they conducted live imaging of mammary epithelial cells expressing the actin marker LifeAct-EGFP to highlight actin organization. In this system, they observed actin accumulation at the basal side of the rotating spheres and actin-rich protrusions at basal intercellular junctions. The function of these actin whips and protrusions across species is not yet clear; Squarr et al. (2016) speculate that whip-like protrusions might interact with the ECM to synchronize directed cell migration and to drive the morphogenetic movement. The similar morphology of the protrusions in *Drosophila* and human epithelial cells additionally suggests that the molecular mechanisms driving tissue rotation might be similar, a hypothesis compatible with the known localization of the human homologue of Fat2 at intercellular epithelial junctions. It will be of great interest to address whether the Fat2-, Dlar-, and WRC-dependent molecular mechanisms described in this study are conserved in other rotational collective cell migration processes.

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