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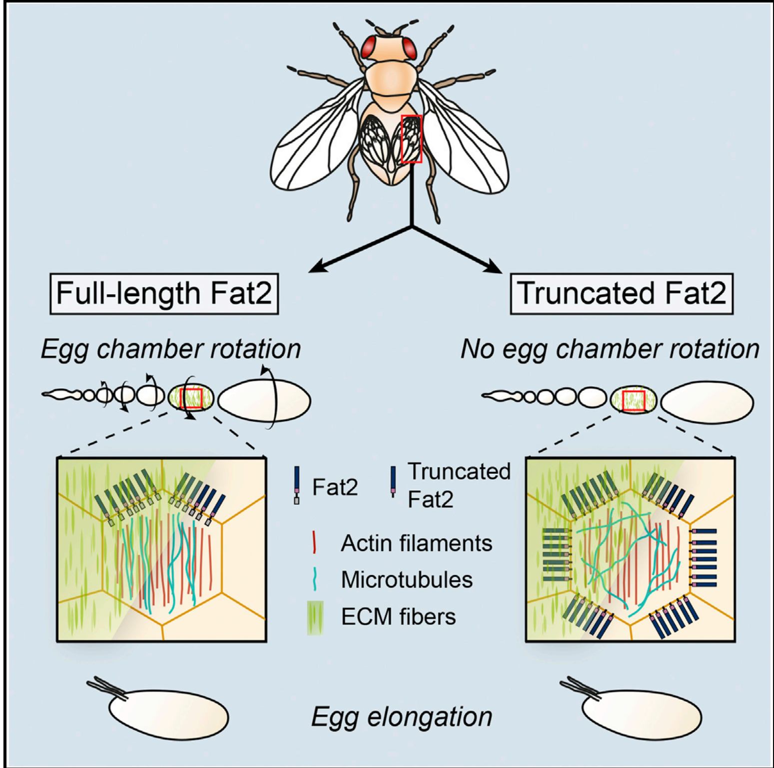
A Mutation in *fat2* Uncouples Tissue Elongation from Global Tissue Rotation

Graphical Abstract



Highlights

- The intracellular region of Fat2 is dispensable for alignment of collagen IV fibers
- The intracellular region of Fat2 is dispensable for egg chamber elongation
- The intracellular region of Fat2 is essential for egg chamber rotation
- Egg chamber elongation can be uncoupled from egg chamber rotation

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In Brief

Aurich et al. show that global tissue rotation, previously proposed to be a morphogenetic process controlling tissue elongation, is not required for the elongation of *Drosophila* egg chambers.

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A Mutation in *fat2* Uncouples Tissue Elongation from Global Tissue Rotation

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SUMMARY

Global tissue rotation was proposed as a morphogenetic mechanism controlling tissue elongation. In *Drosophila* ovaries, global tissue rotation of egg chambers coincides with egg chamber elongation. Egg chamber rotation was put forward to result in circumferential alignment of extracellular fibers. These fibers serve as molecular corsets to restrain growth of egg chambers perpendicular to the anteroposterior axis, thereby leading to the preferential egg chamber elongation along this axis. The atypical cadherin Fat2 is required for egg chamber elongation, rotation, and the circumferential alignment of extracellular fibers. Here, we have generated a truncated form of Fat2 that lacks the entire intracellular region. *fat2* mutant egg chambers expressing this truncated protein fail to rotate yet display normal extracellular fiber alignment and properly elongate. Our data suggest that global tissue rotation, even though coinciding with tissue elongation, is not a necessary prerequisite for elongation.

INTRODUCTION

Tissue elongation is an important morphogenetic process in the development of multicellular organisms (Bilder and Haigo, 2012). The *Drosophila* ovarian egg chamber serves as an ideal system to study tissue elongation (Bastock and St Johnston, 2011; Bilder and Haigo, 2012; Cetera and Horne-Badovinac, 2015; Gates, 2012; He et al., 2011; Horne-Badovinac, 2014). The ovary is composed of strings of egg chambers with each egg chamber consisting of germline cells surrounded by an epithelial sheet of somatic follicle cells (Spradling, 1993). Egg chambers grow and develop through 14 stages to give rise to the mature egg (Figure 1A). During stages 1–4 egg chambers are spherical. From stage 5 onward, egg chambers elongate along their anteroposterior (AP) axis to obtain a final aspect ratio of long–to–short axis of approximately 2.2–2.5 that is a characteristic of the mature egg. Two mechanisms have been proposed to drive this elongation. First, during stages 5–8 of oogenesis, egg chamber elongation correlates with the rotation of the follicle epithelium and the germline cells around the AP axis relative to an underlying extracellular matrix (ECM) (Haigo and Bilder, 2011). During this global tissue rotation, ECM fibers and actin filaments located at the basal side of the follicle cells are aligned perpendicular to the AP axis (Cetera et al., 2014; Haigo and Bilder, 2011). Mutations that disrupt this tissue-level alignment of actin filaments or ECM fibers lead to round rather than elongated egg chambers (Bateman et al., 2001; Conder et al., 2007; Frydman and Spradling, 2001; Gutzeit et al., 1991; Haigo and Bilder, 2011; Horne-Badovinac et al., 2012; Viktorinová et al., 2009). Additionally, a block of rotation correlates with a failure to deposit aligned ECM fibers, to align actin filaments, and elongate egg chambers (Haigo and Bilder, 2011; Lerner et al., 2013; Lewellyn et al., 2013; Viktorinová and Dahmann, 2013). The circumferential alignment of ECM fibers and actin filaments has been proposed to provide a molecular corset that mechanically restricts the circumferential expansion of the egg chamber during growth and thus to promote preferential egg chamber elongation along the AP axis (Gutzeit et al., 1991; Haigo and Bilder, 2011). The second mechanism driving elongation acts during stages 9 and 10 and has been proposed to involve cycles of contraction and relaxation of the circumferentially aligned actin filaments associated with non-muscle myosin II (He et al., 2010). It has been difficult, however, to determine the functional relationship between egg chamber rotation and the formation of the molecular corset and the early egg chamber elongation on the one hand, and between oscillations of the circumferentially aligned actin filaments and later egg chamber elongation on the other hand.

Fat2 (also known as *kugelei* (Gutzeit et al., 1991)) is required for egg chamber rotation, the establishment of tissue-level alignment of ECM fibers and actin filaments, and egg chamber elongation (Gutzeit et al., 1991; Viktorinová and Dahmann, 2013; Viktorinová et al., 2009). Fat2 is a conserved type I transmembrane protein containing 34 cadherin repeats, 6 EGF-like repeats, and 1 laminin G-like domain in its extracellular region, and an intracellular region with two motifs showing homology to WRC interacting receptor sequence (WIRS) motifs (Figure 1B) (Castillejo-López et al., 2004; Chen et al., 2014). Fat2 protein localizes to the basal side of follicle cells and is enriched on cell-cell contact sites oriented approximately parallel to the AP axis of egg chambers (Viktorinová et al., 2009). However, the functions of the different regions of the Fat2 protein for its planar-polarized localization, egg chamber rotation and alignment of ECM fibers and actin filaments, and egg chamber elongation remain unknown.

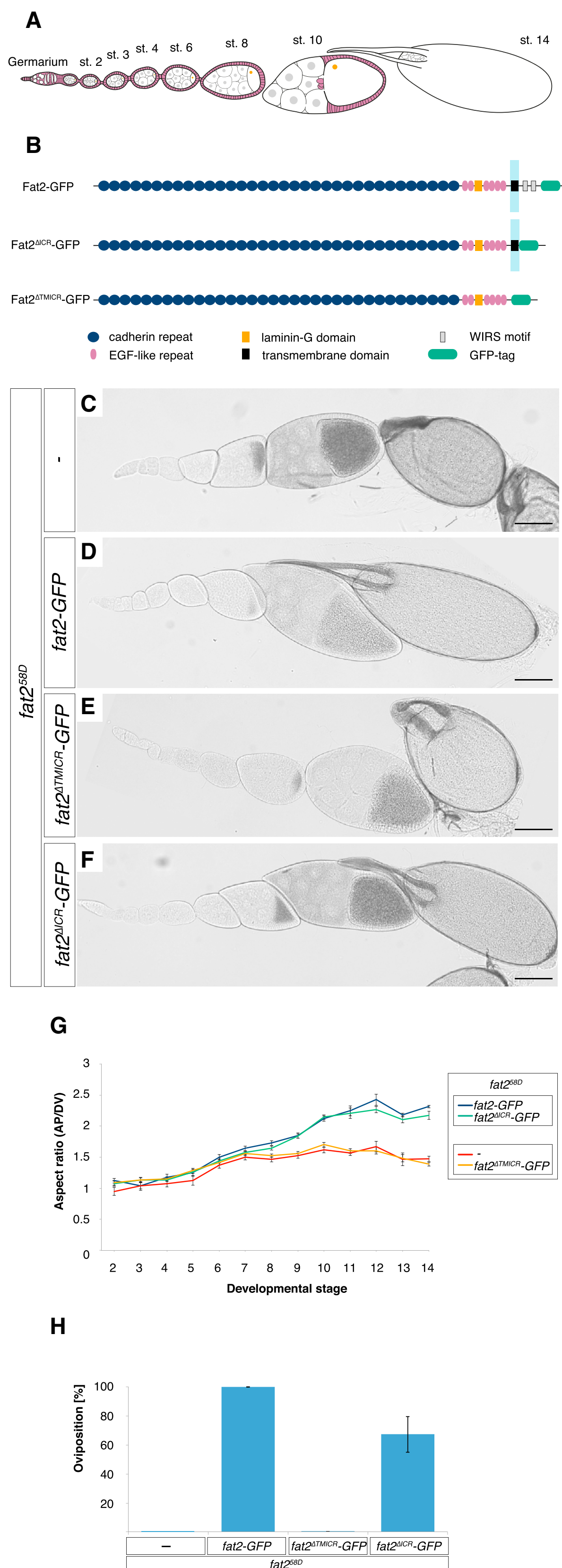

Figure 1. The Intracellular Region of Fat2 Is Dispensable for Egg Chamber Elongation and Oviposition

(A) Scheme of ovariole development. Follicle cells are colored in magenta. The oocyte nucleus is depicted in yellow. Developmental stages are shown on the top.

(B) Structure of full-length Fat2-GFP and of the truncated forms Fat2$^{\Delta ICR}$-GFP and Fat2$^{\Delta TMICR}$-GFP. Blue shaded region indicates plasma membrane.

(C–F) Morphologies of egg chambers in ovarioles of flies with the indicated genotypes are shown. In these, and all subsequent images, anterior is to the left. Scale bars represent 100 μm.

(G) Ratio of long to short axis of fixed egg chambers of the indicated genotypes for the shown developmental stages. Mean and SEM are shown (n = 20 egg chambers per stage and genotype). Comparing Fat2-GFP and Fat2$^{\Delta ICR}$-GFP, p = 0.07-0.9 for the different stages; comparing Fat2-GFP and Fat2$^{\Delta TMICR}$-GFP, p = 0.18-0.92 for stages 2-7 and $p < 0.001$ for stages 8–14 (Student's t test).

(H) Rate of oviposition of flies with the indicated genotypes normalized to the rate of oviposition of *fat2*^{58D} flies expressing full-length Fat2-GFP as a percentage. Mean and SEM are shown (n = 5 experiments). Approximately 10 female flies were used per experiment. Comparing Fat2-GFP and Fat2$^{\Delta ICR}$-GFP, p = 0.04; comparing Fat2-GFP and Fat2$^{\Delta TMICR}$-GFP, $p < 0.001$ (Student's t test).

See also Figure S1.

Here, we demonstrate that the intracellular region of Fat2 is critically required for the planar-polarized localization of Fat2, for the timely establishment of tissue-level alignment of actin filaments, and for egg chamber rotation, yet it is dispensable for the alignment of ECM fibers and egg chamber elongation. Our data show that egg chamber elongation can be uncoupled from egg chamber rotation.

RESULTS

The Intracellular Region of Fat2 Is Dispensable for Egg Chamber Elongation

We sought to generate mutants of Fat2 lacking various regions of the protein to dissect the various functions of Fat2 during egg chamber development and to gain insights into the mechanisms of its planar-polarized localization. We have previously generated a fosmid-based, GFP-tagged transgene of *fat2* that fully rescues *fat2* mutant flies (Figure S1) (Viktorinová et al., 2009). We have now used recombineering (Sarov et al., 2006) to delete specific *fat2* sequences on this fosmid. In one fosmid, the sequence coding for the intracellular region of Fat2 was deleted (Fat2$^{\Delta ICR}$-GFP) (Figure 1B). In a second fosmid the sequence coding for the transmembrane region and the intracellular region of Fat2 was deleted (Fat2$^{\Delta TMICR}$-GFP) (Figure 1B). Both truncated forms of Fat2 carried the GFP-tag to assess their expression and subcellular localization in cells. Both fosmids carrying the *fat2-GFP* deletions were integrated at the same site of the genome as the original fosmid carrying the full-length *fat2-GFP* using *phiC3*-mediated integration into the genome (Venken and Bellen, 2012), thus avoiding possible differences in transgene expression due to position-effect variegation (Elgin and Reuter, 2013). Two independent fly lines per fosmid were established. We obtained indistinguishable results with the two transgenic lines for each transgene (data not shown) and report here the results of one line for each fosmid.

To test the ability of the truncated Fat2-GFP proteins to functionally substitute for full-length Fat2 protein, we generated fly stocks in which the fosmids carrying the deletions were present

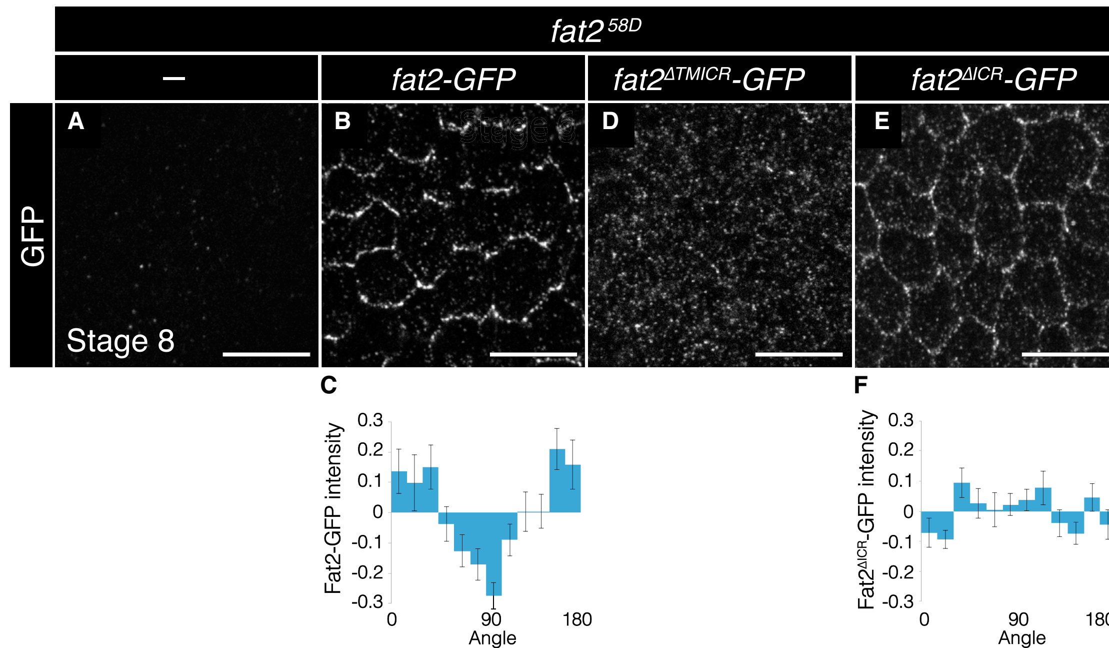

Figure 2. The Intracellular Region of Fat2 Is Required for the Planar-Polarized Localization of Fat2 Protein

(A, B, D, and E) Basal views of stage 8 egg chambers of *fat2*^{58D} flies carrying no transgene (A) full-length *fat2-GFP* (B), *fat2*$^{\Delta TMICR}$*-GFP* (D), or *fat2*$^{\Delta ICR}$*-GFP* (E), and stained for GFP are shown. Scale bars represent 10 μm.

(C and F) Values of average Fat2-GFP (C) or Fat2$^{\Delta ICR}$-GFP (F) pixel intensities for cell membranes oriented in 15° intervals normalized to the average pixel intensity for all membranes and subtracted by "1" are shown. Membranes oriented parallel to the AP axis of the egg chamber are denoted as 0°/180°. Mean and SEM are shown (n = 10 egg chambers per genotype).

in a *fat2*^{58D} (a null allele of *fat2*) (Viktorinová et al., 2009) homozygous mutant background.

Egg chambers of *fat2*^{58D} mutant flies initially elongated until stage 7 but then failed to further elongate and remained almost spherical in shape (Figures 1C and 1G) (Gutzeit et al., 1991; Viktorinová et al., 2009). Expression of full-length Fat2-GFP restored normal elongation to *fat2*^{58D} mutant egg chambers (Figures 1D, 1G, and S1A–S1C) (Viktorinová et al., 2009). We first assessed the ability of the truncated Fat2-GFP proteins to restore normal elongation to *fat2*^{58D} mutant egg chambers. Expression of Fat2$^{\Delta TMICR}$-GFP did not restore egg chamber elongation in *fat2*^{58D} mutants (Figures 1E and 1G). Interestingly, *fat2*^{58D} mutant egg chambers expressing Fat2$^{\Delta ICR}$-GFP elongated with the same kinetics compared to egg chambers expressing full-length Fat2-GFP and obtained the characteristic elongated shape at stage 14 (Figures 1F and 1G). These data demonstrate that the intracellular region of Fat2 is dispensable for egg chamber elongation.

The Intracellular Region of Fat2 Is Dispensable for Egg Deposition

fat2^{58D} mutant female flies fail to lay eggs (Figure 1H) (Gutzeit, 1991; Viktorinová et al., 2009), presumably because the spherical shape of the eggs prevents their transport through the oviduct. Therefore, we tested next the ability of the different Fat2-GFP proteins to restore egg deposition to *fat2*^{58D} mutant females. Full-length Fat2-GFP restored a normal rate of egg deposition (Figures 1H and S1D) (Viktorinová et al., 2009). Expression of Fat2$^{\Delta TMICR}$-GFP did not restore egg deposition (Figure 1H) and expression of Fat2$^{\Delta ICR}$-GFP restored egg deposition to ~70% of the rate of egg deposition of *fat2*^{58D} mutant flies expressing full-length Fat2-GFP protein (Figure 1H). Thus, the intracellular region of Fat2 has only a minor role for egg deposition, consistent with the observation that expression of Fat2$^{\Delta ICR}$-GFP restores normal elongation to *fat2*^{58D} mutant egg chambers.

The Intracellular Region of Fat2 Is Essential for the Planar-Polarized Localization of Fat2

Fat2 protein is enriched in follicle cells at cell-cell contact sites that are oriented approximately parallel to the AP axis of the egg chamber (Viktorinová et al., 2009). This planar polarization of Fat2 is observed during stages 6–8 and depends on intact microtubules (Viktorinová and Dahmann, 2013; Viktorinová et al., 2009). Alignment of microtubules in a feedback-amplification mechanism depends on the planar-polarized localization of Fat2 (Viktorinová and Dahmann, 2013). Therefore, we tested next the subcellular localization of the different Fat2-GFP proteins. No signal was detected when no GFP-tagged transgene was expressed (Figure 2A). As previously reported, full-length Fat2-GFP was enriched on cell contacts oriented roughly parallel to the AP axis (Figure 2B and 2C) (Viktorinová et al., 2009). Fat2$^{\Delta TMICR}$-GFP did not obviously localize to cell contacts but was rather distributed throughout the cytoplasm (Figure 2D), consistent with the lack of a transmembrane domain that could anchor the protein at the plasma membrane. Fat2$^{\Delta ICR}$-GFP did

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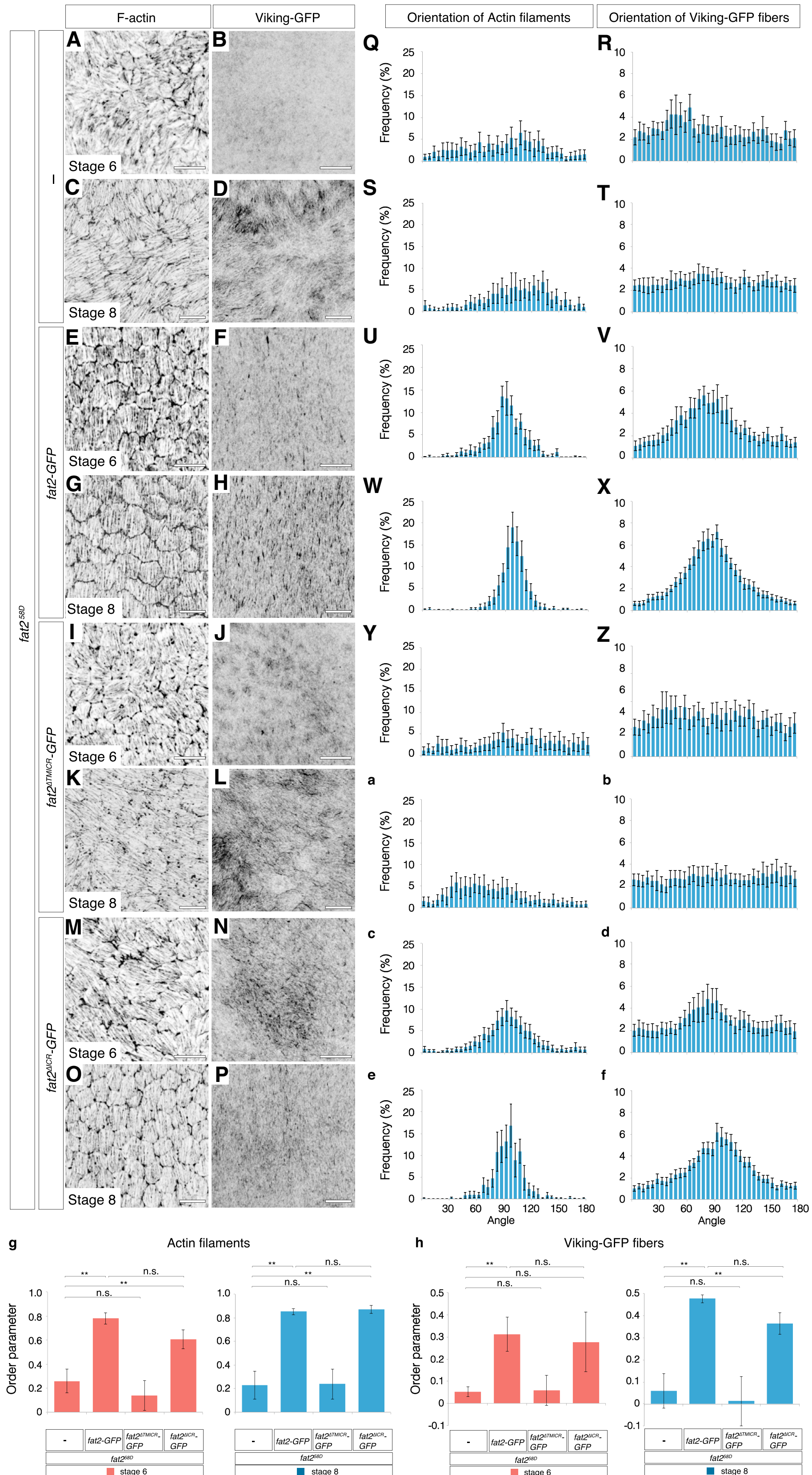

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localize to cell contacts, but was uniformly distributed on all cell contacts and was no longer enriched on cell contacts oriented parallel to the AP axis of the egg chamber (Figures 2E and 2F). Thus, the intracellular region of Fat2 is critically required for the planar-polarized localization of the protein in follicle cells.

The Intracellular Region of Fat2 is Dispensable for Tissue-Level Alignment of Actin Filaments and Collagen IV Fibers at Stage 8

Actin filaments and microtubules at the basal side of the follicle epithelium and collagen IV fibers in the ECM are temporarily aligned perpendicular to the anteroposterior axis of egg chambers (Gutzeit, 1990; Viktorinová and Dahmann, 2013). Alignment of actin filaments is observed from stages 1–10A (Cetera et al., 2014; Gutzeit, 1990), microtubules are aligned from stages 4–8 (Viktorinová and Dahmann, 2013), and laminin and collagen IV fibers in the ECM are aligned from stage 6 onward (Cetera et al., 2014; Gutzeit, 1990). The tissue-level alignment of microtubules, actin filaments and laminin and collagen IV fibers in the ECM depends on Fat2 (Figures 3A–3D and 3Q–3T, g and h; Figures S2A–S2D) (Gutzeit et al., 1991; Viktorinová and Dahmann, 2013; Viktorinová et al., 2009). Therefore, we next tested the ability of the different Fat2 proteins to restore proper alignment of actin filaments, microtubules, and collagen IV fibers in *fat2*^{58D} mutant stage 6 and stage 8 egg chambers. Collagen IV fibers were visualized using a GFP-protein trap in the collagen IV $\alpha2$ chain, Viking-GFP (Buszczak et al., 2007). Tissue-level alignments of actin filaments, microtubules, and collagen IV fibers were separately quantified by an alignment parameter S_{AP}. The alignment parameter is based on (Cetera et al., 2014) and characterizes the alignment of structures relative to the AP axis of the egg chamber (Supplemental Experimental Procedures). S_{AP} can vary from -1 (alignment parallel to AP axis) to +1 (alignment perpendicular to AP axis). Full-length Fat2-GFP fully restored normal actin filament, microtubule, and collagen IV fiber alignment at both stages (Figures 3E–3H and 3U–3X, g and h; Figures S2E–S2H and S2Q). Fat2$^{\Delta TMICR}$-GFP did not restore alignment of these structures (Figures 3I–3L and 3Y–3b, g, and h; Figures S2I–S2L and S2Q). Fat2$^{\Delta ICR}$-GFP failed to restore microtubule alignment (Figure S2M–S2Q). Expression of Fat2$^{\Delta ICR}$-GFP only partly restored tissue-level alignment of actin filaments and collagen IV fibers in stage 6 egg chambers (Figures 3M and 3N, c, d, g and h). Actin filaments were only normally aligned in 5 out of 12 stage 6 egg chambers. Moreover, normal actin filament alignment was not observed in stage 5 (n = 10 egg chambers, Figure S3A–S3C). Actin filaments still tended to co-align with collagen IV fibers within cells in egg chambers in which actin filaments did not show a normal tissue-level alignment (Figure S3D–S3O). Actin filament and collagen IV fiber alignment, however, were independent of each other (Figures S4A–S4F). At stage 8, expression of Fat2$^{\Delta ICR}$-GFP fully restored the alignment of actin filaments (Figure 3O, e and g). Likewise, expression of Fat2$^{\Delta ICR}$-GFP restored normal oscillating F-actin contractions in *fat2*^{58D} mutant egg chambers (Figure S4G–S4O). Fat2$^{\Delta ICR}$-GFP also fully restored the tissue-level alignment of collagen IV fibers at stage 8 (Figure 3P, f and h), although the collagen IV fibers appeared to be shorter and thinner in these egg chambers compared to controls (Figure 3P). We conclude that the intracellular region of Fat2 is critically required for the tissue-level alignment of microtubules and the early tissue-level alignment of actin filaments. This region of Fat2, however, is dispensable for the late tissue-level alignment of actin filaments and collagen IV fibers.

Uncoupling Egg Chamber Elongation from Egg Chamber Rotation

Egg chamber rotation is observed from stage 1–8 of egg chamber development (Cetera et al., 2014; Haigo and Bilder, 2011). Early rotation during stages 1–5 is slow and is required to maintain tissue-level alignment of F-actin (Cetera et al., 2014). Late rotation during stages 6–8 is fast and coincides with the elongation of the egg chamber (Haigo and Bilder, 2011). Previous data showed that mutants that stalled egg chamber rotation also resulted in the failure of egg chamber elongation (Haigo and Bilder, 2011; Lerner et al., 2013; Lewellyn et al., 2013; Viktorinová and Dahmann, 2013). It has been proposed that egg chamber rotation is a necessary prerequisite for egg chamber elongation (Bilder and Haigo, 2012; Haigo and Bilder, 2011). To test this proposal further, we determined whether the ability of the different Fat2-GFP proteins to restore egg chamber elongation also correlated with their ability to restore egg chamber rotation in *fat2*^{58D} mutants. Egg chambers of *fat2*^{58D} mutants did not elongate and did not rotate during early and late stages (Figures 1G, 4A, and 4E; Movie S1) (Viktorinová and Dahmann, 2013; Viktorinová et al., 2009). Full-length Fat2-GFP fully restored egg chamber elongation (Figure 1G) and egg chamber rotation in *fat2*^{58D} mutants (Figures 4B and 4E; Movie S1; 0.44 ± 0.05 μm/min during stages 6-8, consistent with previous data on wild-type egg chambers) (Haigo and Bilder, 2011). Fat2$^{\Delta TMICR}$-GFP did not restore egg chamber elongation (Figure 1G) and it also did not restore egg chamber rotation (Figures 4C and 4E; Movie S1). In these two scenarios, the ability of egg chambers to rotate correlated with their ability to elongate, similar to previous mutant analyses (Haigo and Bilder, 2011; Lewellyn et al., 2013; Viktorinová and Dahmann, 2013). Surprisingly, this correlation was no longer the case in *fat2*^{58D} mutant egg chambers

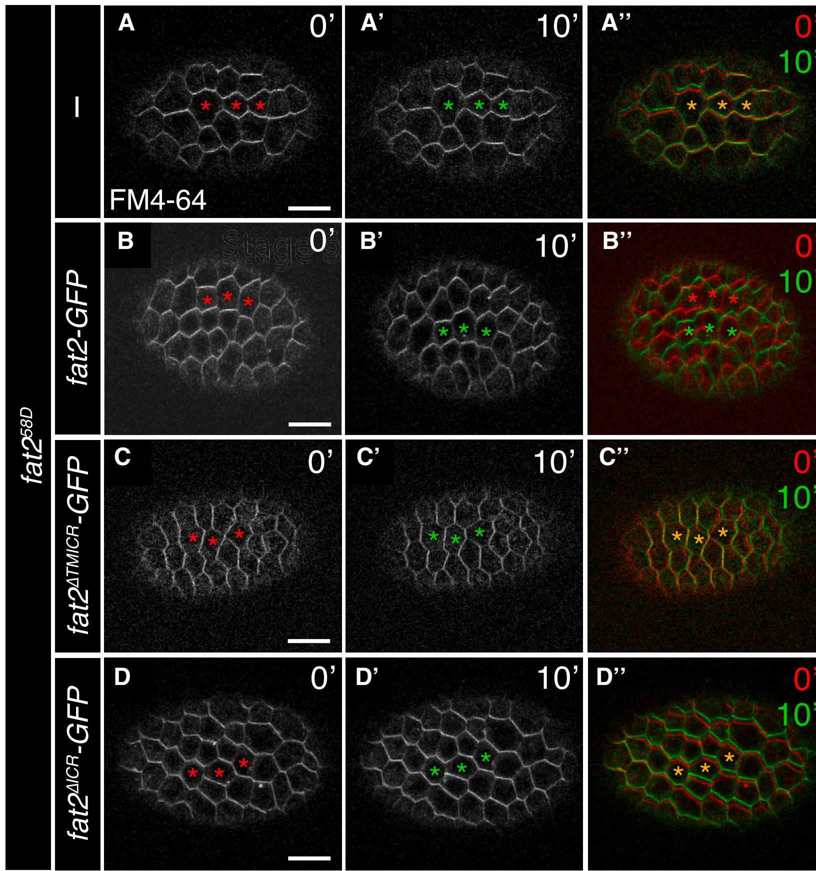
Figure 3. The Intracellular Region of Fat2 Is Dispensable for Tissue-Level Alignment of Actin Filaments and Collagen IV Fibers at Stage 8

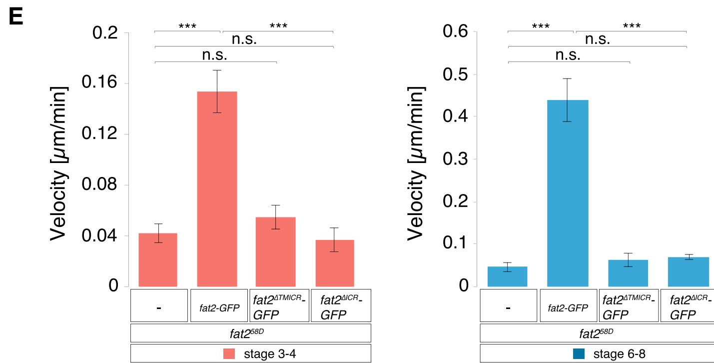
(A–P) Basal views of follicle cells of egg chambers of indicated stage and genotype stained for F-actin (A, C, E, G, I, K, M, and O) or Viking-GFP (B, D, F, H, J, L, N, and P). Scale bars represent 10 μm.

(Q–f) Frequency of actin filaments (Q, S, U, W,Y, a, c, and e) and Viking-GFP fibers (R, T, V, X, Z, b, d, and f) oriented within a 5° interval relative to the AP axis of egg chambers of the indicated stage and genotype. Actin filaments or Viking-GFP fibers oriented parallel to the AP axis of the egg chamber are denoted 0°/180°. Mean and SEM are shown (n = 5 egg chambers per stage and genotype, except for stage 6 *fat2*$^{\Delta ICR}$*-GFP* egg chambers where n = 12).

(g and h) Alignment parameter describing the alignment of actin filaments (g) and Viking-GFP fibers (h) relative to the AP axis of egg chambers of the indicated genotypes and stages. Mean and SEM are shown (n = 5 egg chambers per genotype). n.s. not significant, *p < 0.05, **p < 0.02, ***p < 0.005 (Wilcoxon test).

See also Figures S2–S4.





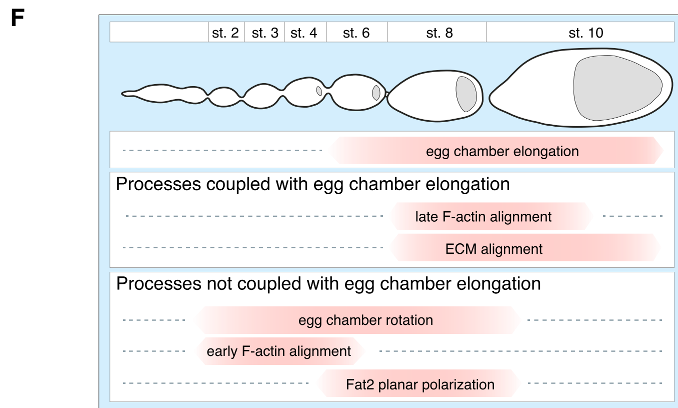


Figure 4. The Intracellular Region of Fat2 Is Required for Egg Chamber Rotation

(A–D) Time-lapse analysis (0' and 10') of ex vivo cultured stage 6–8 egg chambers of the indicated genotypes stained with FM4-64 to label cell membranes. In (A'')–(D'') red and green asterisks mark identical cells at the two time points. Scale bars represent 10 μm.

(E) Velocity of egg chamber rotation for egg chambers of the indicated genotypes and stages. Mean and SEM are shown (n = 10 egg chambers per stage and genotype). n.s. not significant, ***$p < 0.001$ (Student's t test).

(F) Scheme showing that egg chamber rotation, early tissue-level alignment of F-actin, as well as Fat2 planar-polarized localization can be uncoupled from egg-chamber elongation. Hence, these processes are not essential for egg chamber elongation. Alignment of actin filaments and collagen IV fibers (ECM) during later stages cannot be uncoupled from egg chamber elongation in *fat2* mutants expressing Fat2$^{\Delta ICR}$-GFP, indicating that these structures are important for egg chamber elongation (Gutzeit et al., 1991; He et al., 2011).

See also Movie S1.

expressing Fat2$^{\Delta ICR}$-GFP. These egg chambers did not rotate in early and late stages (Figures 4D and 4E; Movie S1), yet they elongated with normal kinetics (Figure 1G). Thus, egg chamber rotation is not a necessary prerequisite for egg chamber elongation.

DISCUSSION

We have addressed the mechanisms driving egg chamber elongation in *Drosophila* ovaries. The elongation of the egg chamber during stages 5–8 coincides with egg chamber rotation and the tissue-level alignment of ECM fibers and actin filaments. Previous work has not been able to disentangle these processes (Haigo and Bilder, 2011; Lewellyn et al., 2013; Viktorinová and Dahmann, 2013). Models have been proposed wherein egg chamber rotation leads to the tissue-level alignment of ECM fibers and actin filaments that in turn build a molecular corset promoting egg chamber elongation (Bilder and Haigo, 2012; Haigo and Bilder, 2011). We have now generated a truncated form of Fat2 that allows us to uncouple some of these processes. First, egg chamber elongation proceeds normally until stage 7 in the absence of aligned ECM fibers and actin filaments. Second, collagen IV fibers show tissue-level alignment at stage 8 in the absence of egg chamber rotation. And third, egg chambers elongate with normal kinetics in the absence of egg chamber rotation.

Fat2 protein is required for the tissue-level alignment of ECM fibers and actin filaments, for egg chamber rotation, and for egg chamber elongation (Gutzeit et al., 1991; Viktorinová and Dahmann, 2013; Viktorinová et al., 2009). We have now mapped out some of these functions to distinct regions of the Fat2 protein. The intracellular region of Fat2 is required for the planar-polarized localization of Fat2 protein and the early tissue-level alignment of actin filaments. The Fat2 intracellular region contains two WIRS motifs (Figure 1B). WIRS motifs are involved in connecting transmembrane proteins with the actin cytoskeleton (Chen et al., 2014). It will be interesting to determine whether the WIRS motifs of Fat2 are essential for Fat2′s function to align actin filaments. The intracellular region of Fat2 is also required for the tissue-level alignment of microtubules and for the planar-polarized localization of Fat2 protein. The observed coupling between these two processes is consistent with the proposed feedback amplification mechanism between Fat2 localization and microtubule polarity (Viktorinová and Dahmann, 2013). The requirement of the intracellular region of Fat2 for early tissue-level actin filament alignment and egg chamber rotation is in line with prior work showing that early tissue-level alignment of actin filaments requires egg chamber rotation (Cetera et al., 2014). Moreover, in the absence of the intracellular region of Fat2, egg chamber elongation parallels the proper tissue-level alignment of actin filaments and collagen IV fibers during late egg chamber development, consistent with the previously proposed role of a molecular corset in egg chamber elongation (Gutzeit et al., 1991). The ability of *fat2*^{58D} mutant egg chambers expressing Fat2$^{\Delta ICR}$-GFP to align collagen IV fibers in the absence of egg chamber rotation, however, is inconsistent with the proposed role of egg chamber rotation to align collagen IV fibers (Haigo and Bilder, 2011). The observation that collagen IV fibers are shorter and thinner in the absence of rotation (Figure 3P) indicates that egg chamber rotation may play a role in the deposition of collagen IV fibers. The mechanisms by which collagen IV fibers align during egg chamber development remain to be further explored, but may involve anisotropic mechanical stresses exerted on the ECM (Frantz et al., 2010).

We propose a model in which egg chamber elongation until stage 7 is independent of the tissue-level alignment of actin filaments and ECM fibers and of egg chamber rotation (Figure 4F). The mechanisms of egg chamber elongation during these stages need to be identified and may include oriented cell divisions and convergent-extension-like cell rearrangements in the follicle epithelium, as has been described for other elongating tissues (Vichas and Zallen, 2011). Alternatively, the contractile muscle sheath surrounding the follicle (King, 1970) may exert mechanical forces onto the egg chambers that contribute to their elongation. From stage 8 onward, as proposed previously, aligned ECM fibers may form a molecular corset that contributes to egg chamber elongation by physically constraining the expansion of the growing egg chamber perpendicular to its anteroposterior axis. The oscillating contractions of the aligned actin filaments, generated by the activity of non-muscle myosin II, further contribute to egg chamber elongation during stages 9 and 10 (He et al., 2010).

Coordinated rotational movements correlate with several morphogenetic processes (Cetera et al., 2014; Haigo and Bilder, 2011; Tanner et al., 2012; Wang et al., 2013). Our work suggests that even though concomitant, these rotational movements may not be essential for the morphogenetic process.

EXPERIMENTAL PROCEDURES

Fly Stocks

Fly stocks used were *fat2*^{58D} (Viktorinová et al., 2009), *fat2-GFP* (Viktorinová et al., 2009), *fat2*$^{\Delta ICR}$*-GFP*, *fat2*$^{\Delta TMICR}$*-GFP*, *sqh-utABD*::*GFP* (Rauzi et al., 2010), and *vkg-GFP* (Buszczak et al., 2007). Wild-type flies were *y w*. Flies were reared at 25°C on standard food.

Fixation and Immunohistochemistry

Adult fly ovaries were dissected in 1xPBS and fixed with 4% *p*-formaldehyde for 20 min. Immunostaining followed standard protocols. Primary antibodies used were mouse anti-acetylated Tubulin (Santa Cruz Biotechnology; 1:400), rabbit anti Laminin (Gutzeit et al., 1991) (no. 329; 1:100), and rabbit anti-GFP (Clontech; 1:2000). Secondary antibodies, all diluted 1:200, were goat anti-rabbit Alexa 488 (Molecular Probes) and goat anti-mouse Alexa 633 (Molecular Probes). Rhodamine-phalloidin was used at a dilution of 1:200. Images were acquired on a Leica SP5 MP inverted confocal microscope.

Statistical Analysis

Student's t test and the Wilcoxon signed rank test were used for statistical analysis as indicated. The Wilcoxon test was used when sample values were not normally distributed.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one movie can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.02.044.

AUTHOR CONTRIBUTIONS

F.A. conducted and analyzed the experiments. F.A. and C.D. designed the experiments and wrote the manuscript.

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