Developmental regulation of oocyte lipid uptake via 'patent' tricellular junctions in *Drosophila* epithelium

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Abstract

Epithelia form protective permeability barriers that selectively allow the exchange of material while maintaining tissue integrity under extreme mechanical, chemical, and bacterial loads. Here, we report in the *Drosophila* follicular epithelium a developmentally regulated and evolutionarily conserved process, 'patency', wherein a breach is created in the epithelium at tricellular junctions during mid-vitellogenesis. Crucial for lipid uptake by the oocyte, patency is also exploited by endosymbionts. Our findings in this study reveal a novel developmentally regulated trans-epithelial transport mechanism in a classic model system.

One sentence summary

Follicular Patency for Oocyte lipid uptake

Main text

Epithelial integrity is maintained through cell division, apoptosis, and morphogenetic movements *(1,2)*, and the preservation of tricellular junctions (TCJs) is key to its physical and electrochemical barrier function *(3)*. Failure to maintain TCJs can disrupt epithelial integrity and

have catastrophic consequences such as metastatic cancer *(4,5)* and Crohn's disease *(6)*. The epithelium is also permeable, allowing selective passage of material via transcellular and paracellular routes *(7)*. The *Drosophila* follicular epithelium (FE) surrounding developing egg chambers provides an attractive model to study the balance between barrier function and permeability in epithelia. The FE monolayer protects and supports germline development while going through a series of spatiotemporally regulated morphogenetic movements *(8)*, and also secretes the chorion, or eggshell *(9)*. Vitellogenic stages (stage (St) 8-12) are characterized by the trans-epithelial movement of hemolymph-borne yolk proteins (YPs) into the oocyte. YPs are synthesized primarily in the fat body, secreted into the hemolymph, and travel between the follicle cells to reach the oocyte membrane, and are internalized via receptor-mediated endocytosis *(10-12)*. The FE also synthesizes YPs in small amounts until St11 when it switches to chorion secretion *(13, 14)*. Other materials carried by the hemolymph have been postulated to enter the oocyte, such as lipophorins and endosymbionts *(15,16)*, but the mechanistic details of trans-epithelial FE transport remain unclear.

While examining wildtype (WT) FE, we noticed an anomalous but consistent feature — the opening of tricellular junctions (TCJs) in mid-vitellogenic FE extending from the basal to the apical domain of the epithelial monolayer [Fig.1(A-D"), Fig.S1(B)], effectively creating a breach in the epithelium. We confirmed the presence of TCJ gaps using a milder detergent-free immunostaining protocol, and electron microscopy [Fig.S1(A-A"), Fig.1(E-E')]. A phenomenon termed 'patency' has been described in other insect species, characterized by the opening of intercellular junctions between follicle cells for yolk uptake in vitellogenic stages *(17)*. We therefore deduced that the *Drosophila* FE also exhibits follicular patency.

Unlike in other insects (e.g., *R. proxilus*) where patency is present in most vitellogenic stages *(18)*, we found that patent TCJs in *Drosophila* FE are present only during mid-vitellogenic stages (St10a and 10b), suggesting a more limited temporal range [Fig.2(A-E")]. Immunostaining with various epithelial markers revealed a dynamic pattern of TCJs in the FE over the course of patency [Fig.S2(A-P")]. Septate junctions, marked by Discs large (Dlg), lose their connections at TCJs during patent stages and are reconnected at stage 11 when the gaps disappear [Fig.S2.(A-D")]. Adherens junctions, represented by E-cadherin (E-cad), are removed from TCJs in patent epithelia, and reappear when patency is terminated [Fig.S2.(E-H")]. In contrast, cortical F-actin remains intact and continuous through mid-vitellogenesis, indicating maintenance of tissue integrity even in patent FE [Fig.S2.(I-L")]. The TCJ septate junction protein Gliotactin (Gli), however, only appears at the TCJs starting at late stage 10b [Fig.S2.(M-P")], coincident with the cessation of patency. Gli is required at TCJs for epithelial barrier function *(19)*, and we found that FE expressing *gli-RNAi* presented patent TCJs even at St11 [Fig.S2.(Q-Q"")]. The accumulation of Gli at TCJs at the termination of patency therefore suggests reinforcement of the TCJs at the end of patency, tipping the functional balance of the FE away from trans-epithelial transport, and in favor of barrier function for the remainder of oogenesis.

Next, to determine how patency is temporally regulated, we performed an RNAi screen for transcription factors known to be active during mid-oogenesis, and identified Tramtrack69 (Ttk69) - a zinc-finger transcription factor that coordinates FE morphogenesis by regulating the expression levels and localization of adhesion proteins such as E-Cad *(20,21)*. Ttk69, previously reported to be expressed starting at St10a, is also expressed during pre- and early vitellogenic stages [Fig.S3.(A-D')], and knocking down *ttk69* resulted in a larger temporal range of patency,

with ectopic TCJ gaps ranging from St9-11 [Fig.2.(F-I'')]. Similar to normal patent FE [Fig.S2.(F-G")], *ttk69*-knockdown FE with premature patency exhibited a lack of E-cad at TCJs [Fig.S3.(E-F'')], suggesting that Ttk69 plays a role in restricting the temporal range of patency in the FE.

Patency also shows a spatial pattern in the FE - specifically, the dorsal-anterior region has intact TCJs while the rest of the FE TCJs are patent at St10b [Fig.3.(A-B'', G-G')]. The *Drosophila* FE is patterned along the anteroposterior and dorsoventral axes by the BMP (Bone Morphogenetic Protein) or Decapentaplegic (Dpp), and Epidermal Growth Factor Receptor (EGFR) pathways, creating a well-characterized subset of cells in the dorsal anterior with elevated levels of adhesion proteins (eg., E-cad, Fasciclin 3 (Fas3)) *(22-24)* [Fig.S4.(A-B'')]. Ectopic expression of Dpp in the whole FE resulted in elevated E-cad and Fas3 levels across the FE [Fig.S4.(F-G")], and all TCJs remained intact at St10a [Fig.3.(C-D",H-H')]. In contrast, removal of EGF signaling by expressing a dominant negative form of EGFR in the FE resulted in the loss of E-cad and Fas3, and ectopic patency in the dorsal anterior FE, thus eliminating the spatial pattern of patency [Fig.S4.(H-I"), Fig.3.(E-F", I-I')]. Together, our data indicate that the spatial distribution of patency in the FE is largely regulated by Dpp and EGFR signaling.

During oogenesis, lipid levels show a marked increase in the oocyte at St10a *(25)*, which, incidentally, is also when TCJ gaps appear in the FE, and we therefore hypothesized that lipids are transferred across the FE into the oocyte via patency. Indeed, our TEM pictures showed material that could possibly be lipids in the gaps of patent FE [Fig. S1.(C)]. Further investigation using neutral lipid dyes Nile red and BODIPY revealed that lipids were in fact present in the TCJ gaps, and appeared to be moving across the epithelium through the gaps [Fig.4.(A-C'')]. Additionally, in egg chambers with FE lacking patency, the oocyte lipid levels were significantly

reduced at St10a [Fig.4.(D-E")], suggesting that follicular patency is necessary for oocyte lipid uptake. Recently, the hormone ecdysone has been shown to induce lipid uptake at St10a by an unknown mechanism *(25)*. When we expressed a dominant negative form of the ecdysone receptor EcR B1, the FE showed no signs of patency [Fig.S5]. Along with our lipid data, we thus propose that patency is the mechanism by which the St10a oocyte accumulates lipids under ecdysone regulation.

Endosymbionts also enter the oocyte from the maternal hemolymph in mid-vitellogenesis (*16),* and we asked whether patency assists in this vertical transfer. Staining egg chambers for the endosymbiont *Spiroplasma pulsonii*, we found that some bacteria are present between the follicle cells prior to the onset of patency, while heavily populating the TCJ gaps at St10a and 10b *(16)* [Fig.S6.(A-B")]. In FE lacking patency, *S. poulsonii* was still detected between the follicle cells as in earlier stages of vitellogenesis [Fig.S6.(C-D")]. These data suggest that patency is an advantage rather than a dependence for vertical transmission.

In summary, the FE, whose primary function is to maintain an intact shield around the egg chamber, is developmentally perforated with TCJ gaps essential for lipid transport. We illustrate spatiotemporal regulation of patency on a global scale by ecdysone signal, at a local level by the axial patterning signals of the FE (Dpp and EGFR), and at a transcriptional level by Ttk69. These signals together regulate TCJ openings to allow trans-epithelial transport of lipids [model in Fig.S7]. Although Ttk69 is placed directly downstream of Ecdysone activation in later oogenesis *(21)*, our data suggests a more complex interaction for the regulation of patency. The presence of the extracellular matrix protein Laminin in the gaps [Fig S8] indicates the possibility of basement membrane components also being transported across the epithelium, perhaps to aid in the closing or maintenance of the TCJs post-patency. Tissue remodeling via a non-canonical

115 secretion pathway aids in closing 'open Zones of contact (ZOCs)', and we propose that these
116 open ZOCs are indeed the patent TCJs we have characterized here *(26)*. The closely related
117 species *Drosophila simulans* also exhibits the same spatiotemporal patterns of patency,
118 indicating evolutionary implications [Fig.S9]. Overall, our studies reveal that patency is not only
119 a significant non-typical epithelial function that needs detailed characterization, but also a novel
120 process in an established model system that must be accounted for in future studies.

121 **References and Notes**

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Author contributions

Conceptualization, methodology, validation, investigation, visualization, project administration: S.R., W.-M.D.; Writing - original draft: S.R.; Writing - review & editing: S.R., W.-M.D.; Supervision, funding acquisition: W.-M.D.

Competing interests

The authors declare no competing interests.

Data and material availability

All data is available in the manuscript or the supplementary materials.

Supplementary Materials

Materials and Methods

Figures S1-S9

References *(27-34)*

Fig. 1. Patency in *Drosophila melanogaster*. (A-A') Stage 10a oocyte-associated FE. White box is expanded in (B-D"). (B-B", C-C", D-D") Basal, lateral, and apical view, respectively, of the TCJ gaps in oocyte associated FE at St 10a. Hts (Hu li tai shao, adducin) marks the membrane, DAPI marks the nuclei. (E-E') TEM images of the surface of a St10a FE. TCJ gaps indicated by red arrowheads.

Fig. 2. Temporal range of patency and regulation by Ttk69. (A-D) Projections of St9-St11 egg chambers. Regions in the white box are expanded in (A'-A'" to D'-D'"). Intact TCJs at stages 9 and 11 (yellow arrowheads in A" and D" respectively), and patent TCJs at stages 10a and 10b (red arrowheads in B" and C" respectively). (E-E'") Illustrations of FE at stages 9 through 11 depicting intact and patent TCJs. (F-I"") *UAS-ttk69-RNAi* expressing FE under *Tj>Gal4*, marked by *UAS-GFP*. *ttk69* KD St9 (F-F") and St11 (H-H") egg chambers; white boxes are enlarged in (G-G"") and (I-I"") respectively, both showing ectopic gaps (white arrowheads).

Fig. 3. Spatial pattern of follicular patency and regulation (A-A") Dorsal view of a St10b egg chamber. Box#1 in the dorsal-anterior is enlarged in (B-B') showing intact TCJs (yellow arrowheads); box#2 in the dorsal posterior is enlarged in (B"-B"') showing patent TCJs (red arrowheads). (C-C') Dorsal view of a *UAS-Dpp* expressing FE. White box is enlarged in (D-D") showing absence of patency (yellow arrowheads) (E-E') Dorsal view of *UAS-EGFR^{DN}* expressing FE. White box is enlarged in (F-F") showing ectopic patency in the dorsal anterior (directly above the oocyte nucleus) (red arrowheads). (G-I') Illustration of the spatial pattern of patency, with yellow dots marking intact TCJs, and red dots marking patent TCJs in WT (G-G'), *UAS-Dpp* (H-H'), and *UAS-EGFR^{DN}* (I-I') expressing FE.

Fig. 4. Patency for lipid uptake by the oocyte at St10a. (A-C"") Lipids are present in the gaps in patent FE. (A-B"") Nile red staining shows lipids in the TCJ gaps, and spanning the FE. Basal

and cross section views (A'-A'''', B' – B'''' respectively). CD8-GFP marks the membrane. (C-C'''') BODIPY493/503 (green) confirms presence of lipids in the gaps. Membrane is marked by Hts (red). BODIPY staining of control WT St10a egg chamber (D-D") and *UAS-Dpp* expressing FE lacking patency showing reduced levels of oocyte lipids at St10a (E-E"). Note FE lipid globules are present in both. DAPI marks the nuclei.

Fig. 1.

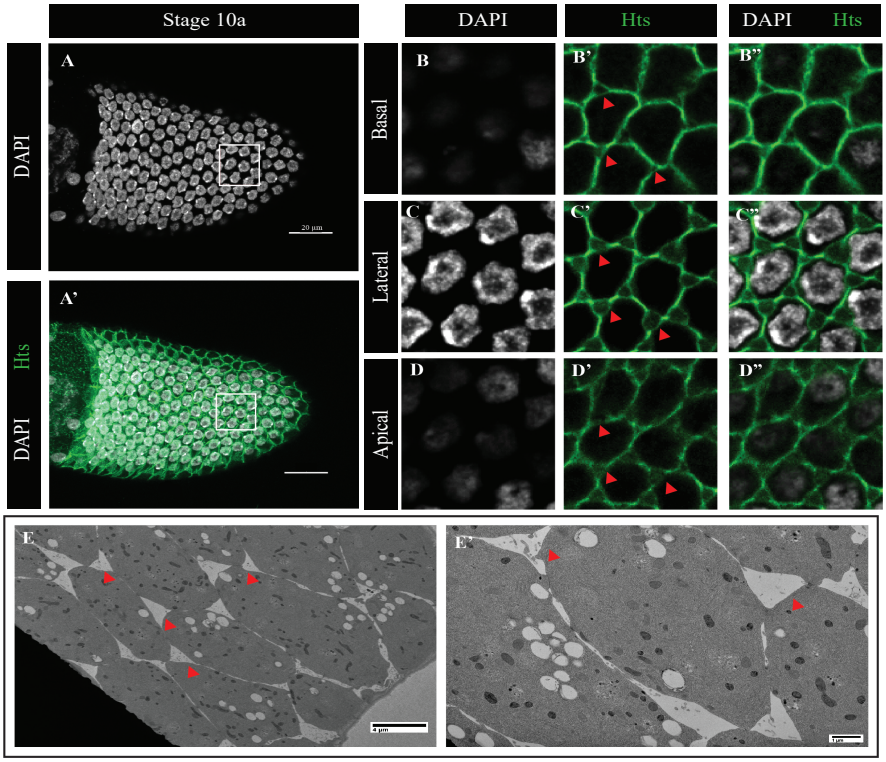

Fig. 2.

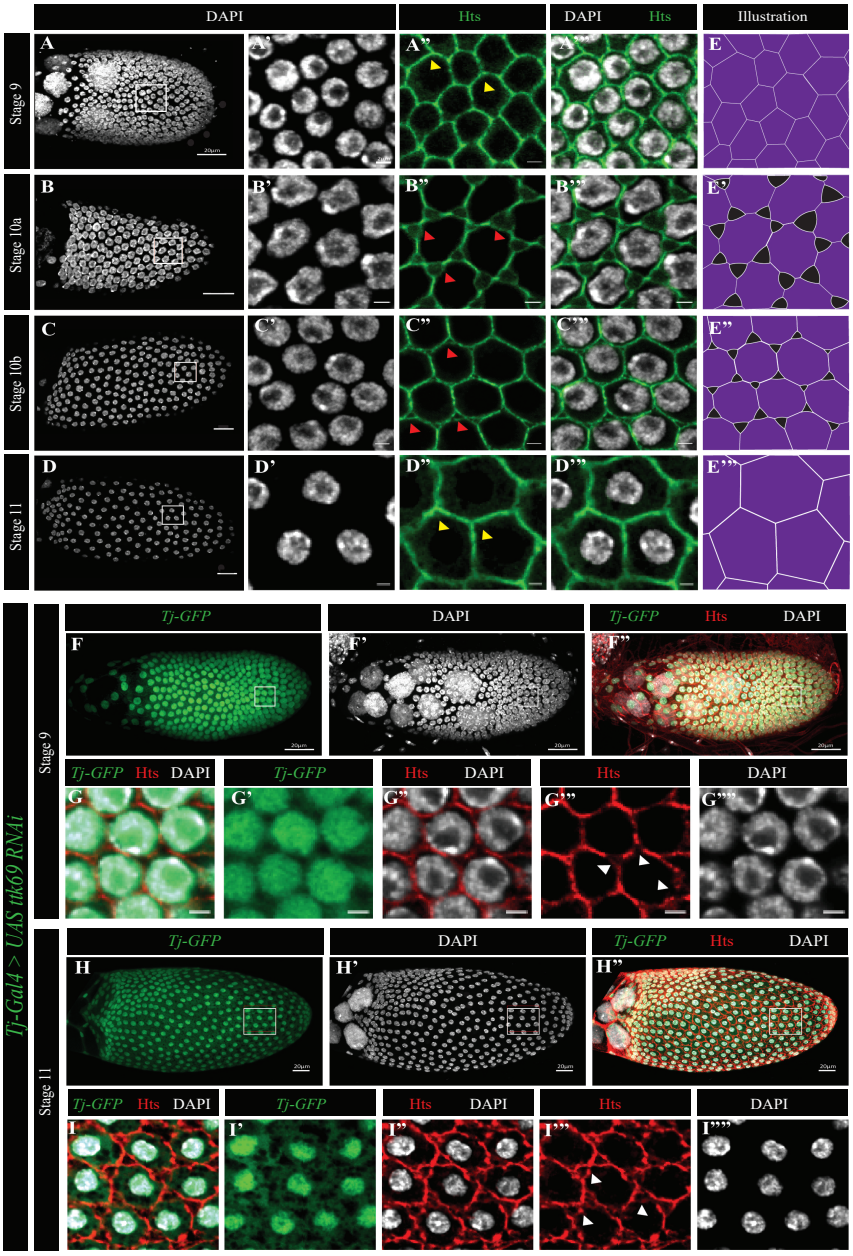

Fig. 3.

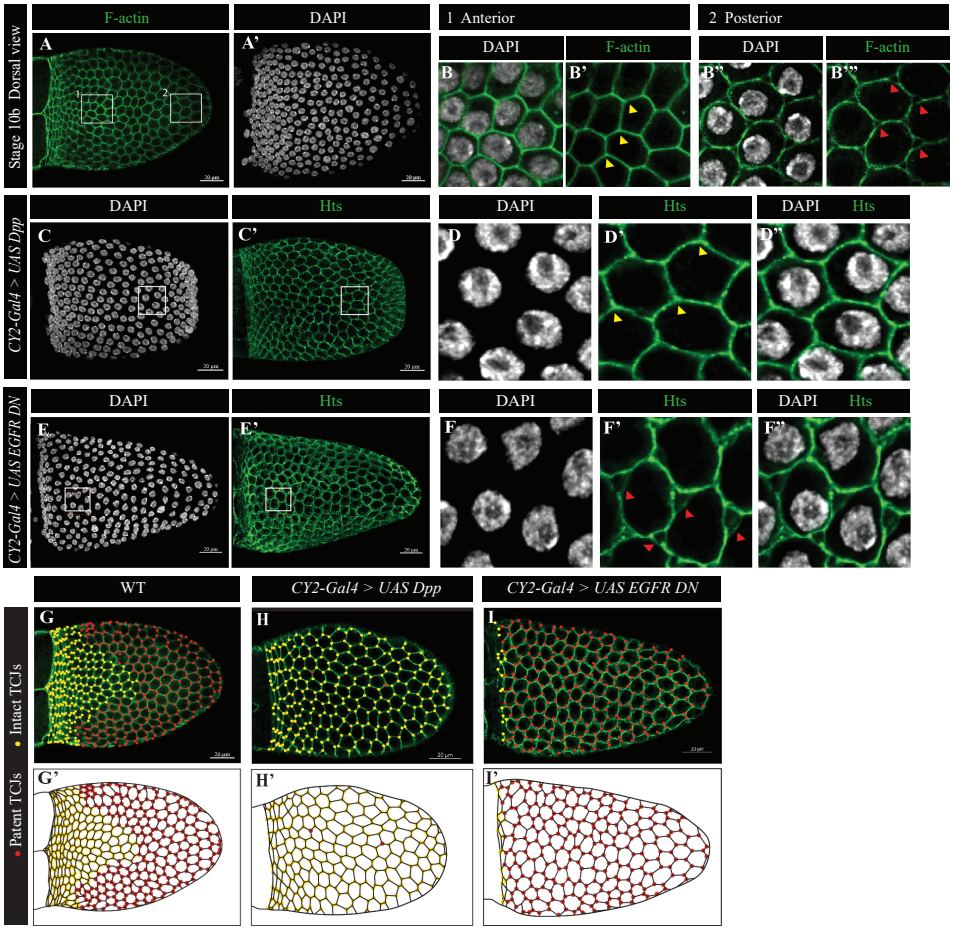

Fig. 4.