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***Running title:*E-Cadherin dependent anchoring of RCs**

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Intercellular bridges called ring canals (RCs) resulting from incomplete cytokinesis play an essential role in intercellular communication in somatic and germinal tissues. During *Drosophila* oogenesis, RCs connect the maturing oocyte to nurse cells supporting its growth. Despite numerous genetic screens aimed at identifying genes involved in RC biogenesis and maturation, how RCs anchorto the plasma membrane (PM) throughout development remains unexplained.In this study, we report that the clathrin adaptor complex AP-1, although dispensable for the biogenesis of RCs, is required for the maintenance of the anchorage of RCs to the PMduring exponential tissue growth associated with increased membrane tension at the onset of vitellogenesis.Here we unravel the mechanisms by which AP-1 enablesmaintenance of RCs anchoring to the PM during size expansion.We show that AP-1 regulates thelocalization of the intercellular adhesion molecule E-Cadherin and that loss of AP-1 causes the disappearance of E-Cadherin-containing adhesive clusters surrounding the RCs. E-Cad itself is shown to be required for the maintenance of the anchorage of RCs, a function previously missed due to functional compensation by N-Cadherin. Scanning block face electron microscopy (EM) combined with transmission EM analyses reveals the presence of interdigitated, actin and Moesin-positive, microvilli-like structures wrapping the RCs.Thus, by modulating E-Cadherin trafficking, we uncover that the sustained E-Cad-dependent adhesion organizes the microvilli meshwork and ensures proper attachment of RCs to PM thereby counteracting the increasing membrane tensioninduced by exponential tissue growth.

Significance

This work addresses the interplay between membrane trafficking, cell adhesion and tissue integrity maintenance in the *Drosophila* female germline. The Clathrin adaptor complex AP-1 is shown to regulate the trafficking of E-Cadherin to ring canals (RCs), a structure resulting from incomplete cytokinesis and allowing intercellular communication. E-Cad assembles adhesive clusters surrounding RCs, which as revealed by electron microscopy analyses organize a dense microvilli meshwork wrapping around RCs. While dispensable for RCs biogenesis and maturation, AP-1 and E-Cadherin are required for the maintenance of RCs anchoring to plasma membrane at the onset of vitellogenesis, when cells experience exponential growth and increased mechanical stress. Our study unravels a novel unanticipated function for E-Cadherin in the maintenance of RC anchoring to plasma membrane.

\body

E-Cadherin (E-Cad) is a core component of intercellular adhesion complexesin cohesive metazoan tissues. E-Cad assemblesinto clusters that are stabilized by actin filaments via β and α-catenin at the level of adherens junctions(AJ) and forming an adhesive belt mechanically linking cells together.A key feature of AJs is their plasticity enabling tissueremodeling, sustained by a constant endocytosis- and exocytosis-regulated E-Cad turnover (1), critical for various morphogenetic processes in epithelia (2-5).

Drosophila oogenesis consists of a rich, multifaceted developmental process during which E-Cad function is not limited to epithelia as it also regulates intercellular collective migration (6, 7) and stem cell adhesion to their niche (8). Cells derived from two different stem cells populations initially assemble into egg chambers composed of a follicular epithelium surrounding a 16-cell germline cyst (GC), itself composed of one oocyte and 15 nurse cells.During the next 64 hours, GC cells grow by hundreds of times their initial volume. Among these cells, oocyte growth is supported by cytoplasmic connections with nurse cells through RCs (Fig. 1A,B), intercellular bridges that, instead of undergoing abscission are stabilized upon arrested cleavage furrows(9, 10). Recent findings revealed that RCs play a vital role in germlineas well as in somatic tissues(10).RCs are composed of a non-contracting subcortical actin ring (11), the inner rim, attached to an electron-dense PM (12), the outer rim (Fig. 1A). RCs have been mainly studied in *Drosophila* female GC (9) where genetic screens uncovered a variety of actin regulators controlling their establishment at the onset of oogenesis and their growth throughout the entire process (13-17).However, the molecular machinery involved in anchoring the PM to the RC remains unknown. Mutations in several membrane traffic regulators affect the integrity of nurse cells PM, causing multinucleation and giving rise to remnants of detached RCs (18-24) suggesting that an unidentified membrane cargo is required for anchoring RC to PM.

Here, we describe a RC detachment phenotype in mutants for the clathrin-adaptor AP-1, a protein complex regulating polarized membrane protein sorting from the *trans*-Golgi Network and endosomal compartments (25)andprovide direct evidence that polarized membrane trafficking to RCs allows an E-Cad-mediated mechanical strengthening of RCs anchoring necessary to resist the membrane tension generated by cellular growth.

Results

Loss of AP-1 induces multinucleationof nurse cells in female GCs

Here, we have generated homozygous *AP-47^{SHE11}* mutant GCsin female germline(the μ subunit of AP-1, referred to as*AP-1* mutants from this point). Actinstaining revealed that nurse cells of*AP-1*mutant GCs progressively becamemultinucleated,exhibitingfloating RCsorganized in clusters(Fig. 1C-D).Multinucleation was first observedat the onset of vitellogenesis(stage 8, st.8),indicating a membrane stability defect. Loss of AP-1 never caused loss of oocyte membrane integrity, suggesting that this membrane is more robust than that of nurse cells, presumably due to its differential organization and composition(26).

A faster growth rate correlates with higher PM tension

Multinucleation begins at a stage during which the oocyte accumulates yolk andGCs grow 4.6 (st.8) to 34 times (st.10a) faster than at previous stages (Fig. 2A,B). As a faster growth rate could affect mechanical membrane properties, providing a rational for this stage-dependent multinucleation phenotype, we probed PM tension by making 5μm wide holes in nurse cells PM using laser ablation. Such holes did not heal but instead propagated until they reached the PM of neighboring nurse cells, leading to multinucleation (Fig. 2C). We measured PM extremities and vertices retractions following ablation and found that membrane recoil was about four times slower (1μm/min) than in epidermal cellsand that there was no significant difference between slow and fast-growing GCs (Fig. 2D, S1C-E). However, cutting the sheet-like nurse cell/nurse cell interface may not release tension as efficiently as cutting the string-like belt of adherens junction in epithelia.Furthermore, tensions may not be released as efficiently at later stages than at the earlier ones, as we made holes of the same size in nurse cell/nurse cell interfaces of 4.5 fold surface difference between st.5 and 9.

While these two experimental biases prevented us from directly assessing PM tension, we noticed that adjacent nurse cells were subjected to morefluctuationsof cellshapewhen ablation wasperformedatearlycompared to later stages (Fig. S1A,A'), indicating that at early stages PMsare more prone to deformations, possibly due to lower PM tension.Accordingly, we observed that following ablation, tubular PH::GFP-positive deformations appeared on the PM contacting adjacent nurse cells (Fig. 2E, arrowheads). Because such tubular deformations

are reminiscent of those observed *in vitro* at the surface of giant unilamellar vesicles and *in vivo* at the PM of cells upon reduction of PM tension (27, 28), we reasoned that tension release induced by ablation is causal to the appearance of deformations. We found that tubular deformations were frequent in slow-growing GCsbut hardly detectable in fast-growing ones (Fig. 2E,F; S1H; movies 2,3). Strikingly, prior to ablation PM tubular deformations were already present in slow-growing GCsbut not in fast-growing ones (Fig. 2E,F) and could therefore be used as a reliable (as their presence does not rely on laser ablation) readout for PM tension. Thus, we concluded that the PM tension is higher in st.8 to 10a GCs than in earlier stages. Finally, similar recoil velocities and tubular deformations following laser ablation were obtained upon loss of AP-1, suggesting that AP-1 does not significantly regulate PM tension (Fig. S1F-H). PM tension is the sum of the in-plane lipid bilayer tension and the protein-dependent membrane-to-cortex attachment (29). We did not further assess their respective contributions to PM tension changes through oogenesis, but propose that exponential growth that begins at st.8 affects the mechanical membrane properties eventually causing multinucleation in *AP-1* mutants.

AP-1 and Rab11 control the maintenance of RCs anchoring to the PMand E-Cad localization in nurse cells

To follow the dynamics of disappearanceof PM, we monitored the distribution of the PM marker E-Cad using an E-Cad::GFP knock-in line(30)in *AP-1* mutant GCs. Live imaging revealed thatmultinucleation was due toPM detachment from RCs,immediately followed byPM fragmentation (Fig.3A).We never observed fragmentation of portions of PM devoid of RCs, suggesting that multinucleation was exclusively caused by detachment of PM from the RCs. This was further supported by our fixed tissue analysis: according to the stereotyped organization of the GC, loss of allRCsconnecting nurse cells but not of RCs connecting nurse cells to the oocyte should lead to the formation of three syncytia containing two, four and eight nurse cells nuclei.This exact configuration was observed in*AP-1*mutants (Fig. 1B, Fig. 3B), further indicating that nurse cell/nurse cell interfaces devoid of RCs remain stable in AP-1 mutant andthat nurse cells multinucleation is caused by RC detachment.Thus, while dispensable for RC establishment, AP-1 activity is required for maintenanceof RCs anchoring to the PM from st.8.

Live imaging of E-Cad::GFP and immunostaining of endogenous untagged E-Cad revealed that the whole surface of nurse cells PM is decorated by E-Cad-positive clustersvisibly enriched around RCs(Fig.3C). In *AP-1* mutant GCs, this enrichment started disappearing from st.8(Fig.3C,C'). In mutant GCs, E-Cad also localized to cytoplasmic puncta that were absent from controland were already present in non-multinucleated GCsat st.8(Fig.3E), indicating thatcytoplasmic mislocalizationof E-Cad in *AP-1* mutant GCs precedes multinucleation.In mammalian cells, AP-1 controls the subcellular localization and function of the Rab11-positive recycling endosome compartment(31, 32) and E-Cad transits through Rab11-positive compartments(33-35). This raises the possibility that E-Cad mislocalization in *AP-1* mutantsinvolves a Rab11-dependent trafficking defect. Consistent with this proposition, Rab11 localization changed from small endosomes distributed throughout the entire cytoplasm in control nurse cells to enlarged endosomes in *AP-1* mutant nurse cells (Fig. 3D) andthe majority of E-Cad cytoplasmic punctalocalized to Rab11-positive compartments (Fig. 3E). To assess theeffect of Rab11 onE-Cad trafficking, we overexpressed a dominant-negative form of Rab11 ($Rab11^{S25N}$) that was reported toblock entry into recycling endosomes in mammalian cells(36). Overexpression of $Rab11^{S25N}$phenocopied*AP-1* mutants: loss of E-Cad enrichment around RCs (Fig. 3F) and multinucleation of st.8 and older nurse cells (Fig. 3G). Thus, in both *AP-1* and *Rab11* mutant backgrounds, fewerE-Cad clusters surroundingRCs correlates with RCs detachment leading to multinucleation.

E-Cad controls RCs anchoring to the PM

This correlation raises the possibility that E-Cad/Shotgun (Shg)is necessary to anchor RCs. Consistent with this suggestion, GCs mutant for shg^{IG29}loss-of-function allele (37)and null β-*Catenin* (β-Cat)alleles arm^{XP33}(Fig. 4A),arm^{YD35} or arm^{XK22}(6, 37, 38)display nurse cellmultinucleation. However, the amorphicshg^{IH} and the null shg^{R69} mutantalleles do not cause nurse cell multinucleation(6, 39).We reasoned that this apparent discrepancy could be explained by functional compensation by the classical Cadherin N-Cad in E-Cad null mutant GCs, as reported in other tissuesin (40).We found that N-Cad was not detected in control GCs, whereas in *E-Cad* null mutant GCs it was ectopically expressed and localized to the PM (Fig. 4B).We propose that E-Cad somehow negatively regulates N-Cad transcription and/or translation, although we cannot rule out that N-Cad is translated in control GCs but targeted

to degradation, and is below our detection threshold.Nevertheless, in the absence of E-Cad, β-Cat still localized to the PM of nurse cells, albeit at lower levels than in controls (Fig. 4B) consistent with functional compensation. This prompted us to prevent N-Cad ectopic expression by using *N-Cad*^{RNAi} in *E-Cad* null mutant GCs. N-Cad silencing in *shg*$^{+}$GCsdid not cause any detectable phenotype (n=30), whereas N-Cad silencing in *shg*^{R69} mutant GCs, in addition to oocyte mispositioning defects expected from loss of E-Cad(39), induced multinucleation (Fig. 4C). Furthermore, we observed that *shRNA*-mediated E-Cad depletion also caused nurse cellmultinucleation (Fig. 4D). In this situation, we speculate that incomplete E-Cad depletion is sufficient to disrupt E-Cad function in RCs anchoring but not in repressing N-Cad expression. Accordingly, N-Cad was not ectopically expressed and β-Cat was no longer recruited to the plasma membrane in E-Cad-depleted GCs (Fig. S2). Together, our results show that N-Cad is responsible for a functional compensation of E-Cad loss in RCs anchoring, explaining whynurse cellmultinucleationis observed in β-Cat but not in E-Cad null alleles, and enable usto conclude that E-Cad participates inRCs anchoring.

Disruption of a microvillosities-rich PMaround RCs in *AP-1* mutant GCs

How exactly could the Cadherin-Catenin complex participate inthe maintenance of RCs anchorage? Transmission electron microscopy (TEM) analysis of multinucleated *AP-1* mutant GCs revealed that the inner rim of detached RCs remained attached to the outer rim, which was itself still connected to portions of PM surrounding the RC (Fig. 5A). Thus, RC detachment does not result from detachment of the inner rim from the outer rim, but rather from a disconnection of a portion of PM surrounding RCs.TEM further revealed that in controlGCs, the PM surrounding RCs appeared highly convoluted (Fig.5A-B'). In striking contrast, in RCs still anchored in *AP-1* mutant GCs, this region appeared devoid of such convolutions (Fig. 5B,B'). We further examinedthe ultrastructural topology of the nurse cells PM using scanning block face electron microscopy(41). This analysis shows that the complex convolutions surrounding RCs are caused by tightly packed tubular extensions of PM of 65±14nm in diameter and 1500±400nm in length that are protruding into the intercellular space between nurse cells (Fig. 5C, movies4,5).Such protrusions were also observed at lower densityall over the rest of the PM at distance from RCs (Fig.5C,D, movies4,5).We further characterized these structures using light microscopy. We propose that actin-positive filaments at the PM at distance from RCs (Fig 6A) correspond to individual protrusions, and

that the high density of actin (Fig 6A) and the presence of the actin crosslinker α-Actinin (Actn; Fig. 6C,D) (42), the actin regulator Enabled (43), and the microvilli marker phospho-Moesin (Fig. 6D) at the PM surrounding RCs is caused by the local abundance of protrusions revealed by TEM (Fig 5A).*AP-1* mutant cells displayed lower levels of Actn around RCs (Fig.S3A), consistent with the loss of PM convolutions around RCs (Fig.5B,B'), further indicating that AP-1 is necessary forprotrusions organizationaround RCs.

E-Cad organizes microvillosities-like structures in nurse cells

Loss of E-Cad enrichment and loss of protrusions around RCs in *AP-1* mutantsprompted us to analyze the direct requirement for E-Cad in protrusionsorganization. In E-Cad-depleted GCs,lower PH::GFP signals (Fig.6B) and an almost complete loss of Actn signals around RCs indicatethat protrusions surrounding RCs are severely affected (Fig. 6C). Furthermore, protrusions distributed all over the PM of nurse cells were also visibly affected(Fig.S4A,A'). Althoughthis indicates E-Cad controls protrusionsorganization, E-Cad-positive clusters enriched around RCs do not localize to the protrusion-dense region but rather to its immediate periphery (Fig.6A,D) and clusters distributed all over the rest of the PM do not colocalize either with actin-positive linear structures (Fig.6A).Finally, we found that the polarity markers Par-3 and Discs large 1 (Dlg) that are enriched around RCs (Fig.S3B) also localized to the rest of the nurse cellsPM but neither of them localizedtomicrovilli (Fig. S3C,D). Thus, we propose that AP-1-dependent E-Cad clusters organize protrusions independently of a polarized distribution of Par3 and Dlg.

Discussion

In this article, we report that AP-1/Rab11regulatethe polarized trafficking of E-Cad and that E-Cad assembles adhesive clusters that are needed for the maintenance of anchoring RCs to PM at the time of exponential GCgrowth, associated with a change of mechanical membrane properties probably caused byincreased membrane tension.

We show that in*Drosophila* nurse cells,defects in AP-1/Rab11 function lead to the progressive disappearance of E-Cad-positive clusters surrounding RCs, suggesting that AP-1/Rab11 ensure thepolarized delivery of E-Cad to RCs. Several studies in *Drosophila,* C.*elegans*and mammals have already implicated AP-1/Rab11 in E-Cad trafficking (33-35, 44, 45). Mammalian E-Cad carries a tyrosine-based AP-1 sorting signal, but *Drosophila* E-Cad

lacks such motif, arguing against a direct recognition of E-Cad by AP-1. Instead, interactions between E-Cad and membrane trafficking regulators can be mediated by adaptors such as β-Cat (46)and the type I γ phosphatidylinositol phosphate kinase PIPKIγ(34). Alternatively, as AP-1 also controls recycling endosomes position and morphology (31, 32), the E-Cad trafficking defect we describe could result from malfunctioning recycling endosomes.Nurse cells multinucleation has also been described for *Rab6*(18), *Rab11*(24), *PI4KIIIa*(23)and components of the Exocyst(20, 21) and ESCRT (19) complexes. While unknown for PI4KIIIa and ESCRT, Cadherin trafficking requires the activity of Rab6, Rab11 and exocyst complex (46, 47). We therefore anticipate that defective E-Cad intracellular trafficking towards adhesive clusters contributes to multinucleation phenotypes in these trafficking regulators.

Whether AP-1 acts directly or not,this study unravelsnovel E-Cad functions. We show that E-Cad is required for the maintenance of RCs anchorage. E-Cad also organizesmicrovillosities at the surface of nurse cells, intriguingly despite the fact it does not localize to these microvillosities but rather to adhesive clusters interspersed between them. How could E-Cad remotely organize microvilli? One could speculate that close apposition of membranes through E-Cad-dependent adhesion somehow stabilizes protrusions, possibly by allowing specific contacts between protrusions. In epithelia, intermicrovillar adhesion is assured by protocadherins(48-50), and although any requirement for microvilli remains to be demonstrated, we envisage that they reinforce RCs anchorage. This remote action of E-Cad is somehow reminiscent of another E-Cad function during oogenesis: E-Cad clusters at the nurse cells PM control the orientation of filopodia-like actin cables, which themselves position nuclei during later stages of oogenesis. In a similar fashion, these E-Cad clusters are interspersed between the membrane-originating tips of filopodia(51).

Functions for E-Cad in cell adhesion, migration and stem cell maintenance have been extensively studied during *Drosophila* oogenesis. Yet, as illustrated by recent findings (this study, (49)), additional unsuspected roles for E-Cad remain to be identified. One of the reasonsis that, as shown in *Drosophila*follicular epithelium(40),in mammals (52, 53), and as we report in GC, N-Cad compensates for E-Cad function. Our observations also provide a rationale for the previous discrepancy between E-Cad (no multinucleation) versus β-Cat (multinucleation) phenotypes in the GCs (6, 38). We further propose that similar functional inter-compensation betweenclassical Cadherins is likely to occur in a number of other

tissues, developmental stages and organisms, and to similarly bias observations depending on the methods used to affect E-Cad function.

AP-1/Rab11/E-Cad are required for maintenance of RCs attachment throughout vitellogenesis, during which we report that a faster cellular growth is accompanied by an increase of PM tension.We therefore propose that RCs anchoring must be reinforced throughAP-1-mediated delivery ofE-Cad to withstand the increased membrane tensiongenerated by exponential growth during vitellogenesis. Without reinforcement, these forces would be sufficient to physically tear the PM surrounding RCs. Why would the PM only tear at this location, and not anywhere else? We can only suppose that dynamic rearrangements of the PM surrounding RCs, either to organize the microvilli-rich region or to accommodate the growth of RCs somehow destabilizes it. Based on the conservation of the functions of RCs in the germlinesof invertebrates and vertebrates, we anticipate that this novel function of E-Cad is evolutionarily conserved.

Materials and Methods

Materials and methods are briefly described here. Further detailsare in the SI Appendix.

Drosophila stocks and genetics

AP-47^{SHE11}, *shg*^{R69} and *arm*^{XP33} mutant germline clones were generated using the FLP/FRT system. The MTD-GAL4 line was used to drive RNAi and Rab11 dominant-negative expression in the germline.

Immunofluorescence and antibodies

Ovaries from adult flies were fixed in 4% paraformaldehyde and stained with primary antibodies: rat anti-DE-Cad (1:100, DCAD2, DSHB), rat anti-N-Cad (1:500, DSHB), mouse anti-Rab11 (1:100, DSHB), mouse anti-Armadillo (1:200, N27A1, DSHB), rabbit anti-γ-Adaptin (1:1000)(54), rat anti-α-Actn (1:50 DSHB), mouse anti-Hts-RC (1:5, Creative Diagnostics), rabbit anti-P-Moesin (1:100)(55). We then used Cy2, Cy3 or Cy5-coupled secondary antibodies (Jackson's Laboratories) diluted 1:250 and/or Phalloidin-Alexa-647 (Life Technology) diluted 1:500.

Imaging and Laser ablation

Live ovarioles were dissected and maintained in Schneider medium adjusted to pH7.0 after supplementation with 15% fetal calf serum and 200µg.ml^{-1} bovine insulin, as described in (56). Fixed specimens and movies were acquired using LSM Leica SP5 and LSM Leica SP8 microscopes equipped with 63X plan Apo-N.A.1.4 or using a spinning disc confocal microscopy equipped with CSU-X1 disk, a Cool-SNAP-HQ2 camera, a Piezo stage, a 100/3 plan Apo-NA 1.4 lens under the control of the MetaMorphSoftware. All images were processed using ImageJ.

Laser ablation was performed on live GCs using a Leica SP5 confocal microscope. Ablation was carried out on nurse cells membranes, 16,5±0,5µm below the coverslip with a 2-photon laser-type Mai-Tai HP from Spectra Physics set to 800 nm.

Electron microscopy

Ovaries were fixed in 2% paraformaldehyde + 2.5% glutaraldehyde in 0.1M cacodylate buffer for two hours at room temperature, processed for uranyl acetate contrast and embedded in Epon-Araldite mix (57, 58). Samples were observed directly either after ultrathin sectioning using JEOL JEM-1400 electron microscope (Jeol) operated at 80kV, equipped with a GatanOrius SC 1000 camera or with a Gatan 3view® microtome within an FEI Quanta 250 FEG scanning electron microscope as in (57).

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Author contributions

N.L. and R.L.B. designed the study and wrote the manuscript. N.L. carried out the light microscopy work, the genetic and cell biology experiments. N.L. and M.P. carried out the laser ablation experiments. I.K. carried out the electron microscopy work, participated in project data interpretation and in manuscript preparation.

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Figure legends

Figure 1: Nurse cells multinucleation in AP-1 mutant female GCs

(A) Schematic representation of the GC consisting of a single oocyte (Oo, nucleus) connected to 15 nurse cells (blue) via RCs (red), and a surrounding monolayer of about 650 somatic follicle cells (green). (Inset) schematic representation of a transverse section through the RCs composed of an inner rim (Red, containing theAdducin-like Hu-li tai shao (Hts, (13, 15)), and the filamin Cheerio (16)) contacting an electron dense PM (outer rim, black), itself connected to the rest of the nurse cells PM (grey). *(B)*Stereotyped organization of the female GC before and after nurse cells RCs detachment (Gray nucleus: oocyte. Colored nuclei: nurse cells). *(C)*st.8 wild-type and *AP-1*mutant (identified by the loss of nls::GFP, blue) GCs stained for actin (green) and DAPI (red). Arrows: RCs connecting nurse cells in control GCs. Arrowheads: RCs floating in the cytoplasm of multinucleated nurse cells in *AP-1* mutant GCs (at least one floating ring in 29/34 mutant GCs in st. ≥8 GCs), *(C')* Quantitation of multinucleated *AP-1* mutantGCs at st.7 to ≥9. *(D)* Maximal projections of 5µm of anchored and clustered floating RCs in control and *AP-1*mutant GCs.

Figure 2: A faster growth rate correlates with an increase ofPM tension

*(A)*GC volume change after exit from the germarium. Oogenesis stages are indicated over the curve. Arrow: onset of vitellogenesis. *(B)*GC growth rates from st.3 to 10a. Arrow: onset of vitellogenesis. *(C)* Nano-ablation of a PM from the nurse cell of a st.7 GC expressing PH::GFP. The PM progressively regresses after a 5µm long wound has been made. Left panels: confocal planes where the wound has been made (top view). Right panels: orthogonal views to visualize the entire targeted portion of PM.*(D)* Displacement of the free PM extremities (arrows in the inset) generated by PM laser ablation in st.5 and 9 nurse cells. *(E)* Representative cases of ablation of nurse cells PM from st.6 and 9 GCs. As quantified in *(F)*, PM deformations are observed prior to the cut (arrows) in st.6 but not st.9 GCs, and deformations appear after the cut (arrowheads) at the PM contiguous to ablated PM in higher numbers in st.6 than in st.9 GCs. *(F)* Density of deformations at the PM contiguous to the ablated PM before and after the cut (n=14 (st.5-6), n= 26 (st.7), n=23 (st.8), and n=23(st.9-10)).

Figure 3: AP-1 and Rab11 control the maintenance of RCs anchoring and E-Cad localization in nurse cells

*(A)*Time-lapse imaging of st.8 *AP-1*mutant GC. Arrow: RC viewed as a hole (arrow) in the otherwise continuous E-Cad::GFP-positive PM. Top views: single focal planes. Bottom views: orthogonal sections. PM detachment starting from the RC (t=5') is followed by PM fragmentation (t=10', n=5). *(B)*st.9*AP-1* mutant GC stained for actin (green) and Rab11 (red). The two panels correspond to projections of different focal planes. Every nurse cell with the exception of the oocyte (gray asterisk) PM initially bearing a RC collapsed, resulting in three syncitia containing 8, 4 and 2 nurse cells nuclei (as indicated by colored asterisks and the corresponding cartoon in Fig. 1B) and clusters of detached RCs (arrowheads). *(C)* RCs in st.9 GCs stained for E-Cad (green) and actin (red), maximal projections of 5-7µm. E-Cad is enriched around RCs and this enrichment can be lost in st.8 and older *AP-1* mutants (quantified in C',where total numbers of RCs and GCs examined are indicated above columns). *(D)* St.8 control (nls::GFP-positive) and *AP-1* mutant (nls::GFP-negative) GCs stained for Rab11. Enlarged endosomes are observed in *AP-1* mutant GCs(from st.4 to 10, n=17/18 GCs). *(E)* st.8*AP-1* mutant nurse cell stained for E-Cad and Rab11. Left panel: low magnification of the entire GC. Three last panels: magnification of the boxed area in the left panel.Arrowheads: enlarged endosomes positive for Rab11 and E-Cad, which partially colocalized in 12/16 GC st.4 to 10. 145/155 (94%) E-Cad positive endosomes were positive for Rab11 and 145/222 (63%) Rab11-positive endosomes were positive for E-Cad (three st.8 GCs). *(F)* RCs of st.9 GCS overexpressing Rab11WT or Rab11S25N, stained for E-Cad (green) and actin (red), maximal projections of 5-7µm (loss of E-Cad enrichment in 35/83 RCs from 10 st.≥9 $Rab11^{S25N}$-expressing GCs).*(G)*st.10GC overexpressing Rab11S25N. Arrow: floating RCs (seen in 9/13 st.≥8 GCs). Right panel: magnification of the boxed area on left panel.

Figure 4: E-Cad controls RCs anchoring to the PM

*(A)*GCs mutant for *arm* in st.10 GCs stained for Actin. Arrowheads: floating RCs (at least one floating RC in 3/4 arm^{XP33}mutant st.≥8 GCs). *(B)*st.6Control and E-Cad mutant (shg^{R69}) GCs stained for N-Cadherin (green) and Armadillo (red). Ectopic expression of N-Cad was observed in 15/15 st.≥4 *shgR69*GCs. *(C)* E-Cad mutant GC expressing N-Cad $shRNA^{HMS02380}$ in a st.10 GC stained for DAPI (red) and actin (green). Floating RCs (arrowhead) were observed in 5/10 st. ≥8 GCs. Right panel: magnification of the boxed area on left panel.*(D)* GCs

expressing E-Cad *shRNA*^{GL00646} in st.10 egg chambers stained for actin. Arrowhead: floating RC (at least one floating RC in 14/22 E-Cad *shRNA*^{GL00646}and 10/13 E-Cad *shRNA*^{HMS00693}st.≥8 GCs).

Figure 5: Disruption of a microvillosity-rich PM around RCs in *AP-1* mutant GCs

(A) TEM image of anchored and detached RCs in control and *AP-1* mutant st.9 GCs. Left panel: low magnifications; cyan: nuclei; yellow: cytosol. Middle panels: high magnification of the boxed areas in left panels; red: RC actin-rich inner rim. Right panels: interpretative drawings of middle panels;red: inner rim and parallel fibers inside protrusions.The inner rim (red) is attached to the outer rim (arrows, thick line) in anchored and detached RCs. The outer rim of the detached RC is itself still connected to a portion of PM (arrowheads). *(B,B')* TEM image of control and *AP-1* mutant anchored RCs (st.8). Boxed areas in *(B)*: portion of PM surrounding RCs. High magnifications of these areas in (B') show complex PM convolutions in the control and absence of such convolutions in the mutant. *(C)* Projection over 3µm (30 sections) of consecutive scanning block face EM images of a RC (inner rim in red) and the neighboring PM in a control st.8 GC. The RC is surrounded by microvilli-like protrusions also present at lower densities over the rest of the PM. *(D)* Consecutive projections over 1.7µm (17 sections) of TEM images after PM segmentation through the volume of a RC in a control st.8 GC, and over 8.5µm (85 sections) through its whole volume.

Figure 6: E-Cad organizes microvillosity-like structures

(A,A') RC from control st.8 fixed GC stained for E-Cad (green) and actin (red). Actin staining is displayed under two different brightness contrast settings to properly illustrate faint actin-positive filaments (better seen in A') all over the cortex and the intense actin signal (better seen in A) at the periphery of RCs. *(B)* RCs from control and E-Cad *shRNA*^{GL00646} live st.8 GCs expressing the PH::GFP probe. *(C)* Control and E-Cad *shRNA*^{GL00646}fixed st.8 GCs stained for Actn (green) and the inner rim marker Hts-RC (red). *(D)* Control fixed st.9 GC stained for E-Cad (red), P-Moesin (green) and Actn(blue). E-Cad clusters are more peripheral than the portion of P-Moesin/ Actn-positive PM surrounding RCs.