Talin repression of cadherin transcription is required for axis formation in 
Drosophila and functions independent of integrins

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Abstract:

The *Drosophila* anterior-posterior axis becomes polarised early during oogenesis by the posterior localisation of the oocyte within the egg chamber. The invariant position of the oocyte is thought to be driven by an up-regulation of the adhesion molecule DE-Cadherin in the oocyte and the posterior somatic follicle cells, providing the first *in vivo* example of cell sorting specified by quantitative differences in cell-cell adhesion. However, it has remained unclear how DE-Cadherin levels are regulated. Here, we show that talin, known for linking integrins to the actin cytoskeleton, has the unexpected function of specifically inhibiting DE-Cadherin transcription. Follicle cells mutant for talin displayed a strikingly high level of DE-Cadherin, due to elevated transcription of DE-Cadherin in the mutant cells. We demonstrated that this deregulation of DE-Cadherin was sufficient to attract the oocyte to lateral and anterior positions. Surprisingly, we found that this function of talin is independent of integrins. These results uncover a novel role for talin in regulating cadherin-mediated cell adhesion.
**Introduction**

Adhesion between cells is a defining process for the development of multicellular organisms. Two main mechanisms have been found to regulate cell adhesion. The first one allows adhesion between cells of the same type to form layers or epithelia. This form of adhesion is often mediated by members of the cadherin family, which form direct homophilic bonds\(^1\). The second one concerns a higher level of organisation, the adhesion between different layers of cells to form complex tissues. This is mostly mediated by members of the integrin pathway, which link indirectly two layers of cells through their binding to the extracellular matrix\(^2,3\). While the mechanisms of cadherin and integrin adhesion has been extensively studied, relatively little is known about how these two pathways may regulate each other. This may be an important step in the acquisition of invasive behaviour of cancer cells, for example colon cancer cells downregulate E-Cadherin upon activation of integrin-mediated migration\(^4\).

The regulation of cell adhesion is also crucial for dynamic morphogenetic events. For example differential adhesion is used to sort out different cell populations\(^5\). Two possible mechanisms have been proposed for this; one relying on the different cells having different types of adhesion molecule\(^6\), and the other relying on the cells having different amounts of the same adhesion molecule\(^7\). The best characterised example of the latter mechanism \textit{in vivo} is the positioning of the oocyte at the posterior of the \textit{Drosophila} egg chamber\(^8,9\). This localisation is crucial as it provides the first cue to polarise the antero-posterior axis of the egg and of the future embryo\(^10\). It has been shown that this localisation requires DE-Cadherin and the available evidence suggested that it is the higher levels of DE-cadherin in the oocyte and the posterior follicle cells that cause these cells to adhere selectively to each other, positioning the oocyte in the right place for subsequent signaling events\(^8,9\). However, it remains unknown how the levels of DE-Cadherin are regulated.
Three reasons led us to investigate the role of integrins and associated intracellular proteins in the regulation of differential cell sorting during oogenesis. Firstly, the adhesion between cells of different types, here germ cells and somatic cells, to form tissues is mostly mediated by members of the integrin pathway rather than cadherins. Secondly, integrins and associated proteins have been shown to regulate levels of cadherin in vitro in HeLa cell culture and in colon cancer cells. Thirdly, we hypothesised that the elaborate movements which lead to the formation of an egg chamber may require the coordination of both types of adhesion.

Using a genetic approach, we tested recently obtained mutations affecting integrins and integrin-associated proteins for a role during these early steps of Drosophila oogenesis. We found that while integrins are not involved in oocyte positioning, the single Drosophila homologue of the integrin-associated protein talin is required for the positioning of the oocyte by negatively regulating the level of DE-Cadherin transcription. Talin is a cytoskeletal linker protein that may directly link integrins to the actin cytoskeleton. It is a large protein of more than 2500 amino acids, containing a globular N-terminal region of 50 Kda (head domain) composed of a FERM (band 4.1, ezrin, radixin, moesin and merlin) domain, which can bind to the cytoplasmic domain of the integrin β subunit. The rod domain (220kda) contains low affinity integrin binding sites and binding sites for actin and vinculin. Its role as one of the earliest component required for the formation of the integrin adhesion complex has been well documented at the genetic, biochemical and structural levels. In contrast, our findings uncover a novel role for talin that is independent of the integrins receptors.
Results

Talin is required for oocyte localisation

The oocyte reaches its posterior position, at the transition between region 2b and 3, within a specialised structure called the germarium, situated at the anterior of each ovariole (reviewed in 17). At this stage, the germline cyst, formed by an oocyte and fifteen nurse cells, becomes surrounded by somatic cells to form a unit called egg chamber. During this transition, the cyst changes shape from a one cell-thick disc to a sphere, with the oocyte always laying at the posterior pole.

To study the function of talin, encoded by the gene rhea18, we used the Flp/ FRT19 system to generate homozygous mutant clones for two different alleles of rhea: rhea79, a complete deletion of the gene (a genetic and molecular null) and rhea2, an amorphic allele which encodes a protein truncated after amino acid 1279 of 2836. The homozygous mutant clones were identified by the lack of the Green Fluorescent Protein (GFP) and the oocyte by the specific accumulation of the Orb protein. The most striking phenotype caused by loss of talin was a mislocalisation of the oocyte. In contrast to wild type cysts, where the oocyte is always at the posterior pole, in mosaic egg chambers mutant for talin the oocyte localised on the lateral sides or at the anterior of the egg chamber, indicating that talin is required for the posterior localisation of the oocyte. We then asked whether talin is required in the germline or in somatic cells to position the oocyte. We found that germline clones mutant for the null allele rhea79 did not show any phenotype and completed oogenesis normally (n=126) (Fig 1A). However, small follicle cell clones were sufficient to induce a mislocalisation of the oocyte with a high penetrance (Fig 1B and 1C). Mutant follicle cell clones not situated at the posterior of the egg chamber induced a mislocalisation of the oocyte in 75% (n=82) of egg chambers scored for the null allele rhea79 and in 50% (n=35) for rhea2. In contrast, mutant
follicle cell clones at the posterior did not induce a mispositioning of the oocyte (n=80). To analyse this bias further, we checked the correlation between the position of the mutant clones and of the oocyte within the egg chamber. Strikingly, we found that the oocyte adheres with high fidelity to the talin mutant cells (Fig 1B, 1C). We quantified this phenotype and found that 96% (n=61), and 95% (n=18) of the mislocalised oocytes contact rhea79 and rhea2 mutant follicle cells respectively (Fig 1F).

Large clones of talin mutant follicle cells induced a second phenotype, the formation of compound egg chambers containing several cysts encapsulated within one egg chamber. However, even in these conditions, the oocytes preferentially contact follicle cells lacking talin (Fig 1D).

The fact that we obtained identical phenotypes with two independent talin alleles is strong evidence that it is the absence of talin that caused the defect. However, to confirm this we showed that we could rescue the defects by restoring talin expression from a transgene construct (Fig 1E, F).

Follicle cells mutant for talin conserve their polarity and their identity.

Studies in vertebrate cell culture suggest that integrins are required for the establishment of epithelial polarity 20. Therefore, we analysed the apico-basal polarity of the talin mutant follicle cells. We checked the distribution of apical, basal and lateral markers of epithelial polarity and found that apical markers such as Bazooka (Baz)21 and atypical Protein kinase C (aPKC)22 (Fig 2A, data not shown), lateral marker such as α-spectrin (data not shown) and baso-lateral marker such as integrin βPS subunit23 (Fig 2B) localised normally in follicle cells lacking talin in contact with the germline. Thus, the absence of talin did not affect the apico-basal polarity of the follicle cells.
Recently, it has been proposed that a pair of specialised follicle cells, called polar cells, could attract the oocyte to localise it at the posterior of the egg chamber. Thus, we checked if the loss of talin induces ectopic polar cells, which could explain why the oocyte adheres to the cells mutant for talin. Polar cells can be identified by the specific expression of the enhancer trap line PZ80 or the higher expression of Fasciclin 3 (Fas3). We found that follicle cells lacking talin did not overexpress Fas3 (Fig 2C) nor do they express PZ80 (data not shown). Moreover, delocalised oocytes contacted mutant follicle cells which did not have a higher level of Fas3 (Fig 2C’). Therefore, we can conclude that there is no shift in cell fate in follicle cells mutant for talin.

A new function for talin independent of the integrin

The primary role envisioned for talin is to directly link integrins to the actin cytoskeleton, by simultaneously binding to the integrin β subunit cytoplasmic domain and actin filaments. In vivo support for this function comes from the knock-out of talin in C.elegans and Drosophila. In both cases, the loss of talin induces a disorganisation of the actin cytoskeleton, and very similar phenotypes to those seen in the absence of integrins. Therefore, we expected that the role for talin in oocyte positioning would be mediated through integrins. We, thus, examined the phenotype induced by removing integrins from the follicle cells. In Drosophila, there are five α subunits and two β subunits, βPS and βν. As integrin subunits must form an αβ heterodimer to be transported to the cell surface, removing both β subunits will eliminate all integrin function. Therefore, we induced homozygous clones for mutations in the gene encoding the βPS subunit, myospheroid, in flies homozygous for a null mutant in the gene encoding the βν subunit. We found that double mutant follicle cells sometimes formed multiple layers and, rarely, caused the formation of compound egg chambers (data not shown). However, a total loss of integrins
either in the germline or in the follicle cells, did not induce a mislocalisation of the oocyte (n=88) (Fig 3A, 3B). Thus, integrins are not required for the localisation of the oocyte, and talin is functioning in this process independently of integrins.

In other cases where integrins and talin work together, the subcellular localisation of talin to sites of integrin adhesion is directed by the integrins. As a further test of the role of integrins in talin function in the follicle cells, we examined whether talin distribution is altered by loss of integrins. Talin was found localised ubiquitously at the membrane of wild type follicle cells and this cortical location was not altered in cells lacking both integrin β subunits (n=20) (Fig 3C). The specificity of the antibody staining for talin was confirmed by the lack of staining in follicle cell clones mutant for the null allele rhea (Fig 5G). Thus, the general cortical localisation of talin in the follicular epithelium is not directed by integrins. We then checked if the loss of talin affected the organisation of the actin cytoskeleton during early oogenesis. We visualised actin filaments using Rhodamin-phalloidin and the distribution of F-actin in wild type cells and talin mutant cells was indistinguishable (Fig 3D). Thus, the early function of talin in the follicle cell epithelia appears to be distinct from its more general cytoskeletal linker function.

We were able to identify a function for talin with integrins later in oogenesis. An integrin function has been described in the follicle epithelium, much later at stage 11, when integrins contribute to the formation of parallel actin fibers, on the basal surface of the follicle cells surrounding the oocyte, which are required for oocyte elongation. We stained late egg chamber carrying mutant clones for talin and found that, like in clones lacking βPS, loss of talin perturbed the arrangement of the basal actin fibers (Fig 3E) and consequently caused the formation of round eggs. Therefore, at late stages of oogenesis, talin does have a more classical function with integrins to organise the basal actin cytoskeleton.
Cadherin is up regulated in follicle cells lacking talin

As mentioned, the posterior localisation of the oocyte is thought to be directed by cell sorting via DE-cadherin. In support of this hypothesis, in egg chambers containing clones of cells lacking DE-cadherin the oocyte attached to the remaining wild type follicle cells\textsuperscript{8,9}. This is the opposite of what occurred in the absence of talin, suggesting that talin might negatively regulate cadherin function. We therefore analysed the expression and localisation of DE-cadherin in follicle cells mutant for talin. We found that follicle cells lacking talin contained strikingly high level of DE-cadherin at the time when the oocyte normally would reach the posterior pole (Fig 4A). This upregulation was retained until later stages (Fig 4B), as was the strong adhesion between the oocyte and follicle mutant for talin. In contrast, cells mutant for the two integrin $\beta$ subunits showed normal level of DE-Cadherin (Fig 4C). The increase in DE-cadherin levels in the follicle cells lacking talin could thus explain why the oocyte sticks preferentially to talin mutant cells.

We then did the reverse experiment and analysed the distribution of talin in follicle cells homozygous for mutants in the gene encoding DE-cadherin, \textit{shotgun (shg)}. Mutant and wild type cells showed an identical distribution of talin (Fig 4D). This demonstrates that there is not a feedback loop between talin and DE-cadherin.

To test our hypothesis that the higher level of DE-cadherin in follicle cell clones mutant for talin is sufficient to explain why the oocyte adheres to cell lacking talin, we tested if a direct overexpression of DE-cadherin in a group of follicle cells could induce oocyte mispositioning. We used the flip-out technique\textsuperscript{33} to induce group of cells overexpressing DE-cadherin and those cells were positively identified by the expression of GFP. Using precise heat shock conditions (see materials and methods), we were able to induce groups of follicle cells that caused oocyte delocalisation. Furthermore, the mislocalised oocyte was always in contact with a cell or group of cells that overexpressed DE-cadherin (Fig 4E", arrow). Using
the same approach, we found that overexpression of talin in follicle cell clones did not induce any changes in oocyte localisation nor did it cause a detectable decrease in the amount of DE-cadherin (data not shown). Thus, follicle cells mutant for talin express a higher level of DE-cadherin, which can explain why follicle cells lacking talin attract the oocyte, since overexpression of DE-cadherin in follicle cells is sufficient to attract the oocyte.

Finally, to demonstrate that the overexpression of DE-Cadherin in talin mutant clones is the primary cause of the mislocalisation of the oocyte, we induced follicle cells double mutant for null alleles of shotgun and rhea. Double mutant cells were identified by the loss of the two FRT-GFP chromosomes corresponding to the FRTG13-shg^{R69} and FRT2A-rhea^{79} chromosomes (see Material and Methods). We verified the complete absence of talin and cadherin by staining the clones with talin and cadherin antibodies (data not shown). We found that none of the double mutant follicle cells mislocalised the oocyte and that they did not preferentially contact the oocyte (n=17). This is to compare to the 75% of mislocalisation of the oocyte induced by Talin single mutant follicle cells. Thus, removing DE-Cadherin rescues the mislocalisation of the oocyte induced by the loss of Talin (Fig 4F). We conclude that Talin affects oocyte position primarily by affecting DE-Cadherin.

Talin regulates the level of DE-Cadherin transcription

In a wild type germarium, the follicle cells contacting the oocyte express higher amounts of DE-Cadherin, however, it is not known if this regulation occurs at the protein and/or mRNA level. We found that in wild type germarium, follicle cells contacting the oocyte express higher amounts of DE-Cadherin mRNA (Fig 5A). This indicates that at least part of the regulation occurs at the mRNA level. Then, to test for a post-transcriptional regulation, we examined the expression and localisation of a GFP-tagged DE-cadherin, driven by a ubiquitous promoter (tubulin or ubiquitin), which is presumably not sensitive to the
endogenous transcriptional regulation of DE-Cadherin. For both transgenes, we found that DE-Cadherin-GFP was distributed as a gradient with the highest levels at the posterior of the egg chamber (Fig 5B). Thus, a second layer of gene regulation at the protein level was revealed.

We then asked at which step in the synthesis of DE-cadherin (shg) does the talin regulation occur. We analysed the level of DE-cadherin (shg) mRNA in follicle cells lacking talin, identifying mutant clones by the lack of GFP (Fig 5C,D) and shg transcript levels by in situ hybridisation either detected with an histochemical method or by fluorescence (Fig 5C', 5D'). We found that mutant cells expressed a much higher level of shg mRNA than the surrounding wild type cells, including the polar cells (arrowhead, Fig 5D''). Elevated levels of DE-cadherin mRNA were seen early during oocyte positioning and were maintained until later stages. We thus conclude that talin regulates DE-cadherin expression by modulating the level of DE-cadherin transcript. However, these results do not indicate whether talin affects DE-Cadherin transcription in the nucleus or the stabilization of its mRNA in the cytoplasm. To distinguish between these possibilities, we used an enhancer-trap line inserted in the DE-cadherin gene, which reproduces the endogenous mRNA distribution (shotgun P34-1). The LacZ reporter gene is thus under the control of the endogenous shotgun promoter and the lacZ mRNA does not share any sequence with the shotgun mRNA. We found that cells mutant for talin show a clear upregulation of the LacZ expression (Fig 5E and F). This result demonstrates that talin regulates DE-Cadherin at the transcriptional level.

Finally, we checked if talin could also regulate the levels of the DE-cadherin protein independent of mRNA levels. In order to test this, we examined the expression and localisation of a GFP-tagged DE-cadherin, driven by a ubiquitous promoter, which presumably lacks the ability to be regulated by talin. In follicle cell clones mutant for rhea79, identified by the lack of talin (Fig 5G'), the expression and distribution of the fusion protein
was not affected (Fig 5G"), even though the oocyte was misplaced. Therefore, talin cannot regulate DE-cadherin protein levels when it is expressed from another promoter.

**Discussion**

Our analyses contribute three main findings: (1) talin has at least one essential function that does not involve integrins; (2) talin is part of a novel pathway that regulates cadherin transcription; (3) overexpression of DE-cadherin, either directly or by eliminating talin function, is sufficient to induce a delocalisation of the oocyte.

**A new function of talin independent of the integrins**

The current model suggests that talin can bind simultaneously the integrin β subunit cytoplasmic tail and the actin filaments. Recent studies on cells in culture further place talin as one of the earliest components in the formation of integrin adhesion complexes. In *C. elegans* and *Drosophila*, elimination of talin has provided *in vivo* support for talin and integrins acting as a core complex, as loss of talin is essentially indistinguishable from the absence of integrins. Integrins are required for the cortical localisation of talin in the *Drosophila* embryo, however, one exception is the gonadal mesoderm, the precursor of the somatic tissues forming the gonad, where it was noted that talin localised normally at the membrane in embryos mutant for βPS integrins. Our findings indeed reveal that the function of talin during the germline-soma interactions is different from its previously described function.

We have shown that talin is required for normal localisation of the oocyte to the posterior of the egg chamber, whereas integrins are not required for this process. In addition, the localisation of talin to the cell cortex of the follicular epithelium was independent of the integrins. Finally, we have shown that the oocyte delocalisation in talin mutant egg chambers
is not associated with actin defects, although talin is required at later stages to organise the basal actin filaments with the integrins. Altogether, these results demonstrate a novel function for talin independent of integrins.

The localisation of talin to the cell cortex in the *Drosophila* gonad suggests that it might be associated with an alternative transmembrane receptor. In a two-hybrid screen, the transmembrane receptor Layilin was found to bind to the talin head domain \(^36\). However, we could not identify a layilin orthologue in either the *Drosophila* nor *C. elegans* genome sequence. Fear of intimacy (Foi) is a novel transmembrane receptor specifically required in the gonadal mesoderm for the compaction of the embryonic gonad in *Drosophila* \(^37\). It was further shown that Foi functions by positively regulating the level of DE-Cadherin for proper interactions between the germ cells and the somatic cells during the formation of the gonad \(^38\). Foi could thus be a candidate to regulate negatively talin activity. Although it has been shown that talin is not required for gonad compaction \(^18\), a role for Foi in the localisation of the oocyte remains to be tested. It would also be of interest to test whether cytoplasmic binding partners of talin, such as focal adhesion kinase (FAK) and vinculin, contribute to talin function in the follicular epithelium. However, complete loss of function mutants in both vinculin and FAK are viable and fertile, indicating that they are unlikely to be involved \(^39,40\).

**Talin regulates DE-Cadherin transcription**

We showed that follicle cells lacking talin expressed strikingly high levels of DE-cadherin protein and mRNA. Using an enhancer-trap line inserted in the *shotgun* gene, we further showed that in the absence of talin, the *lacZ* reporter gene is strongly overexpressed. In contrast, the expression of a cadherin-GFP fusion, transcribed from a heterologous promoter, was not affected. We thus demonstrated that talin regulates DE-cadherin
expression exclusively by modulating the level of mRNA. This result is unexpected for a
cytoskeletal protein, which has previously not been thought to function in a signalling
capacity. Furthermore, although genes have been identified that are regulated by integrin
signaling in *Drosophila*, reduction of talin did not perturb this signaling pathway\(^{18,41}\). This
suggests that a role for talin in regulating transcription may be specific for its function
independent of integrins. Additional germline-soma interactions also depend on DE-
Cadherin, such as the adhesion of the germline stem cells to the cap cells and the migration
of the border cells through the nurse cells\(^{42-44}\). It will be interesting to test if talin regulates
DE-cadherin in these processes.

Perhaps the key question to arise from this work is how talin is being used in a pathway
that regulates transcription? There are other examples of cytoskeletal linker proteins that are
involved in adhesion also playing a role in transcription (reviewed in\(^{45}\)). A particularly well
characterised example is \(\beta\)-catenin, which not only contributes to the links between DE-
cadherin and the actin cytoskeleton, but can also associate with LEF/TCF transcription
factors and directly translocate to the nucleus to regulate the transcription of several genes
implicated in cancer\(^ {46}\). Talin does not contain any domains shared with DNA binding
proteins or transcriptional regulators. Furthermore, with the antibody we have used, which
recognises the carboxy terminus, we have not seen any evidence of nuclear talin. Talin in
mammalian cells has been found to be cleaved by calpain into a 50 kD head domain and 200
kD tail domain\(^ {47}\), so we have not excluded the possibility that the head domain enters the
nucleus. However, at present it seems more likely that talin acts in the cytoplasm to regulates
the activity of a transcriptional factor rather than controlling gene expression on its own. In
*Drosophila*, *escargot* is a known transcriptional activator of DE-Cadherin, whereas *twist*,
*snail*, and *traffic jam* (\(tj\)) are transcriptional inhibitors\(^ {48-50}\). The Traffic Jam protein is a large
Maf factor and would be an attractive candidate for talin regulation, as it is specifically
required in the somatic cells to inhibit the expression of DE-Cadherin during oogenesis. However, follicle cells mutant for \( tj \) over express not only DE-Cadherin but also two other adhesion molecules, Fas3 and Neurotactin. Thus, talin would have to regulate part of Tj activity, as we found that Fas3 is not upregulated in cells lacking talin.

**Models for oocyte localisation**

The posterior position of the oocyte is crucial as it determines the anterior-posterior axis of the egg and of the future embryo. If this step fails all the subsequent polarisation events are disrupted. In germline clones mutants for \( shg \), the oocyte is randomly localised, whereas in the presence of follicle cell clones the oocyte adheres to the remaining DE-Cadherin expressing cells. Firstly, these results demonstrate that DE-Cadherin is required in the germline and the somatic cells to position the oocyte and secondly, that the oocyte is able to distinguish DE-Cadherin expressing cells from non-expressing cells. It was further noted that even in mosaic egg chambers mutant for \( shg \), the oocyte preferentially localised to the most anterior or posterior follicle cells among the remaining wild type cells. This bias led to the suggestion that a gradient of adhesiveness exists and is sufficient to position the oocyte. Here, we validate this model by demonstrating that the oocyte preferentially adheres to cells expressing higher levels of DE-cadherin among cells that also express DE-cadherin. Indeed, we show that the oocyte is able to discriminate a clone of talin mutant cells, a Flp-out clone overexpressing DE-Cadherin and even a clone of talin mutant cells among cells that also express a Cadherin-GFP transgene driven by a tubulin promoter.

We found that the establishment of the DE-Cadherin gradient involves regulation at both transcriptional and post-transcriptional levels. We showed that the first level depends on talin, whereas the second does not. The post-transcriptional level of regulation seems sufficient to position the oocyte, as a ubiquitously expressed DE-cadherin-GFP protein
reproduces the endogenous gradient and is able to rescue a null allele of DE-cadherin (shotgunR69)\(^{51,52}\). This post-transcriptional regulation remains to be characterised. It is thus not possible to simply remove it to test if the transcriptional regulation is also sufficient to localise the oocyte. However, two lines of evidence emphasize the importance of the transcriptional regulation: firstly, it is likely that a transcriptional gradient would contribute to the formation of a gradient of the corresponding protein; and secondly, cells mutant for talin overexpress DE-cadherin mRNA, which translates into a sufficiently high level of protein to override the post-transcriptional regulation, as the oocyte becomes mislocalise in contact with the mutant cells. Both levels of regulation are thus required for the correct positioning of the oocyte.

The role of talin in positioning the oocyte could be twofold. Firstly, Talin could play an instructive role by directly creating the DE-cadherin gradient. However, using several monoclonal antibodies, we couldn’t find any difference in talin distribution in the posterior follicle cells versus the rest of the follicular epithelium. Therefore, if elevated cadherin expression in wild type posterior follicle cells is caused by downregulation of talin, we have to hypothesise that it is talin activity, rather than levels, that is downregulated. Secondly, talin could play a permissive role by keeping a low level of DE-cadherin transcription to allow the formation of a protein gradient interpreted by the oocyte.

Possible mechanisms for talin regulation include cleavage by the protease calpain, or regulation of phosphoinositides\(^{47,53,54}\). Another attractive hypothesis is that talin is being used in this system as a mechanotransducer, linking force applied to the cytoskeleton to transcription\(^{55}\). The induction of elevated levels of cadherin in the posterior follicle cells occurs as the disc-shaped egg chamber is becoming transformed into a sphere. It is possible that the cell rearrangements lead to the application of maximum force onto the posterior cells, resulting in an inactivation of talin-mediated repression of DE-cadherin.
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Materials and methods:

Fly stocks

The following mutants were used: \textit{rhea}^{79,18}, \textit{rhea}^{2,12}, \textit{rhea}^{17,18}, \textit{shg}^{H1} (Z221, Tubingen), \textit{shg}^{p34-I}^{56}, \textit{shg}^{R69}^{8}, \textit{mys}^{XG43}^{57}, \beta^{1}, \beta^{2}^{31}. Rescue experiments were performed by heat-shocking flies of the following genotype: hs-Flp; ubi-talin; FRT2A-\textit{rhea}^{79}/FRT2AGFPnls. To analyse the distribution of a ubiquitously expressed cadherin-GFP fusion protein, we used two transgenes: \textit{tubulin}-Cadherin-GFP (kind gift of Anne Pacquelet and Pernille Rorth) and \textit{ubiquitin}-Cadherin-GFP^{51}. To analyse the distribution of Cadherin-GFP fusion protein in \textit{rhea}^{79} mutant clones, the following flies were heat-shocked: y,w,hs-Flp; tub-cadh-GFP, FRT2A-\textit{rhea}^{79}/tub-cadh-GFP, FRT2A.

Generation of mutant clones

Mutant clones were generated by the FLP/FRT technique^{58}, using either the FRTG13GFPnls chromosome or the FRT2AGFPnls chromosome^{59}. Clones were induced by heat-shocking third instar larvae for 2 hours on 3 consecutive days. Adult flies were dissected 2 or 3 days after eclosion. We generate double clones for \textit{shotgun} and \textit{rhea} by heat-shocking flies of the
following genotype: y,w,hs-Flp; FRTG13-shg<sup>R69</sup>/FRTG13GFPnls; FRT2A-rhea<sup>79</sup>/FRT2AGFPnls.

**Overexpression**

Somatic overexpression of *shg* was performed by generating Flip-out/Gal4 clones in females y,w,hs-flp/+; act>CD2>Gal4<sup>60</sup>, UAS-GFP/+; UAS-DE-cadherin<sup>5,9,61</sup>. Adult flies were heat shock 2 hours at 37° and dissected 24H after.

**Immuno-staining and in situ hybridisation**

Antibody stainings were performed according to standard procedures<sup>10</sup>. Antibodies were used at the following concentration: mouse anti-Orb (orb4H8 and orb6H4 DHSB) 1/250, rat anti-DE-cadherin (D-CAD2) 1/20, mouse anti-Talin (talin C19) 1/20, mouse anti-integrin βPS (CF.6G11 DSHB) 1/20, mouse anti-Fasciclin III (7G10 DSHB) 1/10, rabbit anti-Bazooka<sup>22,1/500</sup>, mouse anti-GFP (Roche) 1/200, rabbit anti-βgal (ICN Pharmaceuticals) 1/1000. DNA was stained with Hoescht (1/1000) and F-actin was labelled with rhodamine phalloidin (Molecular probes) 1/100. Secondary antibodies conjugated with Cy3 (Jackson laboratories) were used at 1/200. To combine antibody staining and in situ hybridisation, we used a standard procedure for antibody staining except that we used DEPC water and added 1ul RNA guard (Pharmacia) with the first and second antibody. Then, in situ hybridisation was done according to standard protocols (hybridation temperature 55°) using dioxygenin-labelled cDNA of *shotgun* (primer sequences used to synthetise the probe CADH5 (5'-TCAAGTGC GAGGAATCGTGC-3') and CADH3T7 (5'-GAATTGTAATAC GACTCACTA TAGGG TGATGTGCTGATGGCGGATG-3'). In situ staining was stained using either an NBT/BCIP kit or the TSA-Fluorescein system (NEN). Samples were examined either with a Leica DMR microscope or by confocal microscopy using a Leica SP2 AOBS microscope.

**References :**


Figure Legends:

Figure 1 Talin is required in the follicle cells for oocyte localisation

In all the figures posterior is to the right.

A Germline clones mutant for talin marked by the absence of GFP. The oocyte (star), identified by the accumulation of the protein orb (red), is localised at the posterior of the egg chamber.

B-C Follicle cells clones mutant for talin, identified by the absence of GFP, are sufficient to induce a mispositioning of the oocyte (yellow arrow) identified by orb (B’) or DNA staining (C’). White horizontal bars indicate junction points between egg chambers and their stalks, which normally abut the oocyte. The mislocalised oocyte adheres to follicle cells lacking talin (B” and C”).

D Large clones of follicle cells mutant for talin (D) lead to formation of compound egg chambers with several oocytes (yellow arrows) (D’) which all adhere to mutant follicle cells (D”).

E A transgene encoding talin driven by the ubiquitin promoter rescues the mislocalisation of the oocyte induced by follicle cells clones mutant for talin. The oocyte still lies at the posterior despite the presence of a large clone at the anterior.

F Penetrance of the phenotype of oocyte mislocalisation (green) for rhea79, rhea2, (ubi-tal; rhea79). We only considered follicle cells that are not at the posterior of the egg chamber. Percentage of mislocalised oocytes (red) in contact to mutant follicle cell clones for rhea79 and rhea2.

Figure 2 Follicles cells lacking talin conserved their polarity and identity

A-A” Bazooka apical localisation (red) is not affected in follicle cells mutant for talin identified by the lack of GFP.
**B-B”** Integran βPS (red) still localises to the basal cortex of talin mutant follicle cells.

**C-C”** In this egg chamber, the oocyte (yellow arrow) is displaced on one side. In addition, the mispositioned oocyte sticks to mutant follicle cells that do not overexpress the Fas III protein like the wild type polar cells (white arrow).

**Figure 3 Integrins are not required for oocyte localisation**

**A** Germline clones for integrins marked by the absence of GFP. The oocyte (star), identified by the accumulation of the protein orb (red), is localised at the posterior of the egg chamber.

**B** Large (arrow) or small (arrowheads) follicle cells clones mutants for the two β-subunits do not induce a defect in oocyte localisation.

**C-C”** The distribution of talin (red) is not affected in follicle cells mutants for the two β-subunits.

**D-D”** Clones in a stage 6 egg chamber labelled with rhodamine-phalloidin. Talin mutant cells show no defect in F-actin organisation.

**E-E”** Clone in a stage 12 egg chamber stained with rhodamin phalloidin. The optical section is focused on the basal surface, where bundles of actin are perpendicular to the A-P axis. Follicle cell clones mutant for talin identified by the lack of GFP affect basal actin filament organisation (E’,E”).

**Figure 4 Follicle cells lacking talin overexpress DE-cadherin**

**A-A”** Early in oogenesis, follicle cells lacking talin express high levels of DE-cadherin (red) compared to wild type adjacent follicle cells.

**B-B”** Follicle cells lacking talin overexpress DE-cadherin (red) in stage 6 egg-chamber.

**C-C”** DE-cadherin level (red) is not affected in follicle cells mutants for the two integrin β-subunits.
D-D” The distribution of talin (red) is not affected in follicle cells lacking DE-cadherin, compared to wild type follicle cells (D”).

E-E” Using the Flp-out technique, cells overexpressing DE-cadherin are identified by the expression of GFP (D’). The oocyte (yellow arrow) is mislocalised on the lateral side, instead of the posterior. Moreover, the oocyte (yellow arrow) identified by orb (D”’) adheres to cells overexpressing DE-cadherin (white arrow) (D”).

F-F” Follicle cells double mutant for a null allele of shotgun and rhea. The double mutant cells are identified by the complete lack of GFP. Despite the presence of a lateral clone, the oocyte remains at the posterior (Orb in red).

Figure 5 Talin regulates DE-cadherin by transcription

A DE-Cadherin mRNA expression in a wild type germarium. Posterior follicles cells contacting the oocyte express higher levels of DE-cadherin mRNA (arrows).

B Expression of a cadherin-GFP protein fusion driven by the ubiquitin promoter in a wild type germarium. DE-cadherin-GFP is distributed as a gradient with the highest levels at the posterior of the egg chamber (arrows).

C-C’ Follicle cells clones for talin are identified by the lack of GFP with an antibody (C). The level of DE-cadherin transcript, detected by an RNA probe using an histochemical method (C’), is overexpressed exactly in the cells which lack talin in an early egg chamber.

D-D” Follicle cells clones for talin are identified by the lack of GFP (D), immunostaining. The level of DE-cadherin transcript, visualised by fluorescence using an RNA probe (D’), is overexpressed exactly in the cells which lack talin in a late egg chamber (posterior cells of a stage 10 egg chamber).

E-E” Follicle cells mutant for talin in an early egg chamber show a clear upregulation of the lacZ expression, identified by an antibody against the β–galactosidase(red).
F-F" Follicle cells mutant for talin in a late egg chamber show a clear upregulation of the lacZ expression identified by an antibody against the β–galactosidase (red).

G-G” Follicle cells lacking talin, identified by an antibody against talin (red), and wild type cells express the same level of cadherin-GFP protein fusion when expressed under the tubulin promoter.

Figure 1S (Supplementary data)

A Large clones of follicle cells mutant for rhea17 (hypomorphic allele of talin) induce the formation of compound egg chambers containing several oocytes identified by Orb (yellow arrowheads)

B Similarly to talin loss of function, overexpression of cadherin induces the formation of disorganised egg chamber containing more than one oocyte (yellow arrowheads). One of the oocyte adheres to follicle cells overexpressing DE-cadherin identified by the expression of GFP (white arrow)

C Enhancer trap line P34-1 inserted in the shotgun locus. The lac-Z reporter gene reproduces the endogenous distribution of the shotgun gene. Posterior follicle cells contacting the oocyte express higher level of β-gal (yellow arrowhead).
Figure 1
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