Zasp52 strengthens whole embryo tissue integrity through supracellular actomyosin networks

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ABSTRACT

During morphogenesis, large-scale changes of tissue primordia are coordinated across an embryo. In Drosophila, several tissue primordia and embryonic regions are bordered or encircled by supracellular actomyosin cables, junctional actomyosin enrichments networked between many neighbouring cells. We show that the single Drosophila Alp/Enigma-family protein Zasp52, which is most prominently found in Z-discs of muscles, is a component of many supracellular actomyosin structures during embryogenesis, including the ventral midline and the boundary of the salivary gland placode. We reveal that Zasp52 contains within its central coiled-coil region a type of actin-binding motif usually found in CapZbeta proteins, and this domain displays actin-binding activity. Using endogenously-tagged lines, we identify that Zasp52 interacts with junctional components, including APC2, Polychaetoid and Sidekick, and actomyosin regulators. Analysis of zasp52 mutant embryos reveals that the severity of the embryonic defects observed scales inversely with the amount of functional protein left. Large tissue deformations occur where actomyosin cables are found during embryogenesis, and in vivo and in silico analyses suggest a model whereby supracellular Zasp52-containing cables aid to insulate morphogenetic changes from one another.

KEY WORDS: Actomyosin, Supracellular cable, Junctions, Cytoskeleton, Tension, Epithelium, Morphogenesis

INTRODUCTION

Morphogenesis, the generation of shape in development, is the basis of organ formation and major topological changes in development. Within individual cells, changes in shape are driven by the cytoskeleton. Over the last decade, enormous progress has been made in deciphering which type of cell shape changes drive changes at the tissue scale (Blanchard et al., 2009; Guirao et al., 2015; Herrera-Perez and Kasza, 2018). Cell shape changes in most cases depend on the actomyosin cytoskeleton underlying the plasma membrane, the actomyosin cortex. This cortex is linked to cell surface receptors such as cadherins or integrins, thereby allowing modulation of the actomyosin cortex during cell-shape changes through cell-cell and cell-matrix adhesion (Chugh and Paluch, 2018; Mege and Ishiyama, 2017).

Crucially, cells do not change shape in isolation, rather a close coordination between all cells in a tissue is essential for productive changes at the tissue scale. Such coordination is based on different molecular mechanisms. Many morphogenetic events in embryos occur in epithelial tissues, where cell-cell adhesion between neighbouring cells allows for physical coupling of behaviours. The degree of turnover and stability of adhesion sites between neighbouring cells determines the extent of physical coupling between the neighbours, thereby, for example, controlling the number of neighbour exchanges (Song et al., 2013). Mechanical coupling through cell-cell adhesion connects the actomyosin cortices of neighbouring cells, which can also tie into cytoskeletal structures such as actin stress fibres or the microtubule cytoskeleton. Within epithelial cells, actomyosin is enriched near apical adherens junctions in what has been historically described as the ‘actin belt’ or ‘adhesion belt’ (Furukawa et al., 2017). Interestingly, this cell-cell-adhesion-associated actomyosin can be coordinated between neighbouring cells into seemingly ‘supracellular’ assemblies. These were first observed during wound healing in embryonic epithelia (Martin and Lewis, 1992), termed actomyosin ‘cables’ or ‘purse strings’, and subsequently found during morphogenetic processes across the evolutionary tree (Rodriguez-Diaz et al., 2008).

Actomyosin cables have been intensively studied in model processes such as dorsal closure and salivary gland invagination in the Drosophila embryo (Jacinto et al., 2002; Röper, 2012; Sidor et al., 2020), neurulation in both Ciona (Hashimoto and Munro, 2019) and mouse (Galea et al., 2017), and wound healing (Wood et al., 2002). Actomyosin cables can show different properties and provide different functionalities: they can be stable, or dynamic and short lived, be involved in active morphogenetic changes or stable delineation of differently fated populations of cells (Röper, 2013). In a few instances, the upstream molecular cascade leading to the assembly of the cable has been elucidated (Hashimoto and Munro, 2019; Röper, 2012; Sidor et al., 2020).

We have previously analysed the morphogenesis of the tubes of the salivary glands from a flat epithelial primordium in the Drosophila embryo, termed the salivary gland placodes (Fig. S1), as well as the mechanism that leads to the assembly of supracellular actomyosin cables surrounding each of these two bilateral placodes (Röper, 2012; Sanchez-Corralles et al., 2018, 2021; Sidor et al., 2020). The salivary gland placodes are specified on the ventral side of the embryo half-way through embryogenesis at late stage 10, and cells start to invaginate through a focal point termed the invagination pit in the dorsal-posterior corner, forming a narrow lumen tube on the inside while cells continuously invaginate...
(Girdler and Röper, 2014; Sidor and Röper, 2016). Cells at the invagination point undergo apical constriction driven by apical-medial actomyosin prior to internalisation, while cells further away from the invagination pit undergo directional cell intercalations driven by polarised junctional actomyosin, to continuously feed cells towards the invagination point (Fig. S1A-I; Sanchez-Corrales et al., 2018, 2021). Furthermore, the assembly of the supracellular actomyosin cable at the boundary of each placode with the surrounding epidermis is dependent on the anisotropic localisation of the transmembrane protein Crumbs and its downstream effectors Pak1 and aPKC (Röper, 2012; Sidor et al., 2020). The cable is under increased tension compared with nearby junctions that are not part of the cable, as shown by laser-ablation of junctions and measurements of initial recoil of junction vertices (Röper, 2012). The function of the circumferential cable surrounding the placode is not clear, but it could help the cell internalisation by exerting an inward-directed boundary force, or it could help to insulate cell behaviours within the primordium from the surrounding epidermis.

Zasp52 is a member of the Alp/Enigma family of proteins, containing an N-terminal PDZ domain and four LIM domains (Fig. 1A). Drosophila Zasp52 was originally identified as a component of striated muscles, localised to the Z-line, where it interacts with its binding partner α-actinin and is important to link actin-barbed ends into the Z-disc (Jani and Schöck, 2007). More recently, Zasp52 was also found to localise to the actomyosin cable that assembles at the leading front of epidermal cells during dorsal closure in the fly embryos (Stronach, 2014). These cells move towards the dorsal side of the embryo to cover the non-embryonic tissue of the amniotrophia. Zasp52 was shown to assist in providing a straight leading-edge front, thereby assisting the correct matching of segments between left and right sides of the embryo (Ducuing and Vincent, 2016).

Here, we show that Zasp52 is in fact a component of many, though not all, embryonic actomyosin cables or supracellular actomyosin assemblies. Zygotic loss of Zasp52 leads to a reduction of junctional F-actin in the salivary gland placodal cable. We identify a novel F-actin binding motif (ABM) in the coiled-coil domain of Zasp52 that is related to the motif usually found in the actin-capping protein CapZbeta. In addition, we identify several ubiquitous cell-cell-adhesion-associated proteins as interaction partners of Zasp52, in particular APC2, Polychaetoid (Pyd; ZO-1) and Sidekick (Ahmed et al., 2002; Choi et al., 2011; Finegan et al., 2019; Letizia et al., 2019). Embryos lacking all maternal and zygotic Zasp52 function show major defects in morphogenetic events in tissues associated with the presence of supracellular actomyosin cables such as the salivary glands, the ventral midline, the head and the leading edge/amnioserosa interface. We propose that the supracellular actomyosin cables containing Zasp52 serve as mechanical insulators, preventing morphogenetic changes from ‘spilling over’ into neighbouring regions or otherwise interfering with nearby events, a model which is further supported by in silico investigations.

RESULTS

Zasp52 is a component of embryonic supracellular actomyosin cables

In order to analyse Zasp52 protein localisation in the Drosophila embryo we made use of two protein-trap insertion lines in the zasp52 locus (Fig. 1A). Zasp52-GFP (using either the Zasp52[ZCL423] or Zasp52[G00189] lines) within the embryonic epidermis of Drosophila was particularly strongly localised to junctions that were part of supracellular actomyosin assemblies or cables (Fig. S1F-H). Both GFP-exon trap lines label most isoforms of Zasp52, including the longest possible one (Fig. 1A, protein isoform F) and both lines in the embryo show indistinguishable patterns (Fig. S2C-H′). Zasp52-GFP was enriched in junctions of the ventral midline (Fig. 1B,B′), the cable surrounding the salivary gland placode (Fig. 1C,C′), the leading edge of epidermal cells during dorsal closure (Fig. 1D,D′) as well as several large-scale cables found on the ventral anterior side of the embryo at the start of head involution (Fig. 1E,E′; Röper, 2013). These ventral anterior cables in particular appeared to be linked into a large-scale network that spans the whole anterior epidermis at this stage (arrows in Fig. 1E′). We could not detect Zasp52-GFP in cables found at parasegmental boundaries in the early embryo before stage 11 or in the more dynamic short cables found in tracheal placodes during tracheal morphogenesis (Fig. S2). In addition to supracellular cables, Zasp52-GFP localised at lower levels to some bicellular junctions and was already slightly more enriched at tricellular junctions (highlighted in Fig. 1C′ and E′, magenta arrows in insets).

We analysed in more detail the localisation and levels of Zasp52-GFP in the forming salivary gland placode. Zasp52-GFP was only present at low levels in the embryonic epidermis at early stage 10 before placode specification (Fig. 1F–F′), but showed a patchy expression in the salivary gland placode at late stage 10, with accumulation at circumferential cable commencing at this stage (Fig. 1G–G′). During stages 11 and 12, Zasp52-GFP quickly accumulated in the forming cable at the placode boundary (Fig. 1H–H′). In fact, by late stage 11 Zasp52-GFP was strongly enriched in the placodal cable compared with E-Cadherin (also known as Shotgun, Shg) (Fig. 1I), showing a clear anisotropy in the boundary cells that was as prominent as the anisotropy of Rok or myosin (Röper, 2012; Sidor et al., 2020). The enrichment in the ventral anterior cables at stage 14 was of similar magnitude (Fig. 1I). Zasp52 therefore appears to be an enriched component of many, though not all, supracellular actomyosin cables in the Drosophila embryo.

Zasp52 contains an actin-binding motif related to the CapZbeta-actin binding motif

The strong enrichment of Zasp52-GFP in supracellular actomyosin structures and especially the circumferential cable around the salivary gland placode prompted us to investigate whether Zasp52 played a role in the establishment or function of this cable. In wild-type embryos, not only myosin II (Fig. S1K-K′) but also F-actin (Fig. 2A–A′; Röper, 2012) accumulates strongly at the boundary of the placode. In embryos zoologically lacking Zasp52, using a deletion of zasp52 called zasp52Δ (Jani and Schöck, 2007), this F-actin accumulation at the placode boundary was strongly reduced, as it was in transheterozygous embryos of the zasp52Δ allele combined with an allele of a deficiency spanning the zasp52 locus (Fig. 2B–C).

Zasp52 has been suggested to interact with F-actin directly through its N-terminal region, containing the PDZ domain and Zasp motif (Liao et al., 2020). Using a purified Zasp52 fragment of this region (PDZ+ZM) we confirmed in vitro that this binding is direct and does not require additional factors (Fig. S3). Furthermore, in the Z-disk of muscles, Zasp52 binding to actin is mediated by its interactor α-actinin (Jani and Schöck, 2007). However, using a GFP-protein-trap insertion into the α-actinin locus we found that α-actinin-GFP in embryos did not localise to junctions of epidermal cells and was not enriched in the cable surrounding the salivary gland placode (data not shown). As actin-binding through the PDZ+ZM part of Zasp52 appeared weak, we analysed the amino acid.
acid sequence of the longest isoform of Zasp52 (isoform PF) for further possible conserved actin-binding motifs that might have been overlooked. We identified a motif in the central unstructured region of Zasp52 that closely resembles the highly conserved actin-capping-motif usually found in the actin-capping protein CapZbeta (Fig. 2D-G; Hug et al., 1992; Kim et al., 2010). In CapZbeta, the conserved residues are part of a flexible C-terminal domain shown to be able to flip in and out of the hydrophobic furrow of the F-actin analogue Arp1-A. This interaction is integral to the capping of the barbed end of F-actin (Narita et al., 2006;
Fig. 2. Zasp52 contains a novel F-actin binding site related to CapZbeta and affects supracellular F-actin localisation. (A-B′) F-actin accumulates at the boundary of the salivary gland placode where a supracellular actomyosin cable forms (A-A′). In zasp52Δ zygotic mutants less F-actin accumulates (B-B′). E-Cadherin, magenta; Phalloidin (F-actin), green. Asterisks mark the invagination point. The dotted lines indicate the boundary of the placode.

(C) Quantification of Phalloidin intensity at the boundary of the placode in the wild-type compared with zasp52Δ homozygous mutants or zasp52Δ/Df308 transheterozygous mutants (Df308 uncovers the zasp52 locus). Data are mean±s.d. *P<0.05, ****P<0.0001 (two-tailed Mann–Whitney test). (D) Pairwise alignment of CapZbeta (Gallus gallus) and Zasp52 (Drosophila melanogaster), conserved motif highlighted in pink. This motif in Zasp52 is near identical to the actin-capping motif in CapZbeta. (E) Amino acid sequence alignment of Zasp52-PF across members of the Drosophila genus. Conserved, putative ABM highlighted in pink. (F) Amino acid sequence alignment of CapZbeta across metazoan species with conserved leucines of CapZbeta highlighted in yellow (Hug et al., 1992; Kim et al., 2010) and the complete QLQRELS motif (pink letters). (G) Phylogenetic tree of the Drosophila genus with D. melanogaster subgroup (yellow) in which species show the QLQRES motif in Zasp52. In D and F, the asterisk indicates positions which have a single, fully conserved residue; the colon shows conservation between groups of strongly similar properties; and the period shows conservation between groups of weakly similar properties. (H) Secondary structure predictions about local disorder in the stretch of amino acids surrounding the ABM in Zasp52-PF, generated with Quick2D toolkit (NeetSurfP2 (Klausen et al., 2019), DISOPRED3 (Jones and Cozzetto, 2015) and SPOT-Disorder (Hanson et al., 2017), suggesting this region to be disordered (D=disorder). (I-I′) Ectopic expression of the Zasp52-ABM-GFP in S2 cells, where it localises to the cell cortex (I′, green) and colocalises with F-actin (I; Phalloidin, magenta). (J-J′) Ectopic expression of the long Zasp52-PF-GFP isoform in S2 cells, where it localises to the cell cortex (J′, green) and colocalises with F-actin (J; Phalloidin, magenta). Both constructs also show small non-specific aggregates in the centre of the expressing cells (green arrowheads in I′ and J′). (K) Ultracentrifugation-based co-sedimentation assay of F-actin and Zasp52-ABM: 0.8 µM Zasp52-ABM was incubated with increasing concentrations of pre-polymerised F-actin (2 µM, 4 µM, 6 µM, 8 µM). Blots of supernatant and pellet were analysed by staining with Instant Blue (Coomassie) dye. The 2 µM and 8 µM Zasp52-ABM were centrifuged without F-actin as control. (L) Pull-down of bound Zasp52 isoforms using GST-Zasp52-PDZ-ZM as bait, labelled with anti-Zasp52-N antibody, indicating that this domain likely participates in dimerisation of Zasp52. Scale bars: 20 µm. See also Fig. S3.
Purified recombinant Zasp52-ABM and performed also colocalised with F-actin labelled by Phalloidin in S2 cells the Zasp52-PF isoform, containing the ABM in its central part, specific aggregates, likely due to the overexpression. Furthermore, labelled using Phalloidin (Fig. 2I-I′).

GFP in S2 cells, where it colocalised with cortical F-actin structures higher order organisation of F-actin filaments, thereby possibly could also assist in actin cross-linking, actin bundling or other expression and its general ability to bind F-actin and junctional structures, suggesting that the localisation to and incorporation of Zasp52 into such structures is likely controlled by its pattern of expression and its general ability to bind F-actin and junctional components, rather than a unique membrane-associated binding partner in these supracellular assemblies. In agreement with this, overexpression of different Flag-tagged Zasp52 isoforms in stripes in the embryonic epidermis, using enGal4, led to junctional localisation of these isoforms (Fig. S4G-H′), confirming that Zasp52 binding partners appear to be present in epidermal junctions throughout the embryo.

Zasp52 associates with junctional proteins

In order to identify whether and how Zasp52 was recruited specifically to supracellular actomyosin structures and uncover what function it fulfils there, we set out to identify potential further interaction partners in addition to F-actin. We generated lysate from embryos before formation of muscles (pre stage 14), either from embryos expressing endogenously-tagged Zasp52, Zasp52[ZCL423], or from wild-type embryos, or from embryos expressing an endogenously-tagged Armadillo[β-Catenin, armadillo-YFP, and performed anti-GFP co-immunoprecipitations (note that as soluble cold lysates were used, no F-actin or polymerised microtubules are present in the input material). Anti-GFP immunoprecipitation of Zasp52-GFP lysates compared with wild-type lysates showed a specific enrichment of many potential interactors with links to both actomyosin and cell-cell adhesion (Fig. 3A; i.e. APC2, Sidekick, Polychaetoid, Patj, Raskol, Canoe, Cindr, Bazooka, p120-Catenin, α-Catenin, G-beta13F, Scribble), whereas the anti-GFP immunoprecipitation from Armadillo-YFP lysates compared with wild-type lysates identified the components of adherens junctions known to interact with Armadillo as enriched (Fig. 3B; i.e. Armadillo, α-Catenin, Shotgun, APC2, p120-Catenin). The identification of partly distinct sets of interactors for Zasp52-GFP and Armadillo-YFP indicated that Zasp52 is not just a direct interactor of adherens junctions (Fig. 3A-C; for a full list of interactors see Tables S1 and S2).

We analysed the localisation of potential interaction partners in comparison with Zasp52-GFP, in particular the localisation of the junctional components APC2, Polychaetoid and Sidekick (Ahmed et al., 2002; Choi et al., 2011; Finegan et al., 2019; Letizia et al., 2019). APC2, one of the two APC tumour suppressor proteins in Drosophila with functions in Wnt signalling and cell adhesion (Ahmed et al., 2002; Hamada and Bienz, 2002), was localised to junctions in the embryonic epidermis and was particularly enriched at tricellular junctions, but was not specifically enriched in supracellular actomyosin structures (Fig. 3D-E′). However, it colocalised with Zasp52-GFP in the junctions where Zasp52 was enriched. Similarly, Sidekick, a protein especially enriched in tricellular junctions in the embryonic epidermis and important for epithelial integrity during morphogenesis (Finegan et al., 2019; Letizia et al., 2019), also colocalised with Zasp52-GFP in these junctions (Fig. 3F-G′). Lastly, Polychaetoid, a protein with a variety of functions in apical junctions including linkage to the actin cytoskeleton (Choi et al., 2011), localised to apical junctions in the embryonic epidermis and was enriched in placodal junctions, again colocalising there with Zasp52-GFP (Fig. 3H-I′).

Thus, Zasp52-GFP appeared to associate with many junctional proteins at bi- and tricellular junctions. When analysing its localisation along the apical to basal extent of the epithelial junctions, Zasp52 in actomyosin cables colocalised with E-Cadherin at the level of adherens junctions, but also partially overlapped with the more apically localised Crumbs protein within the apical marginal zone of the lateral sides (Fig. 3C; Fig. S4A-F).

The associated proteins analysed above do not usually show a specific enrichment in supracellular junctional actomyosin structures, suggesting that the localisation to and incorporation of Zasp52 into such structures is likely controlled by its pattern of expression and its general ability to bind F-actin and junctional components, rather than a unique membrane-associated binding partner in these supracellular assemblies. In agreement with this, overexpression of different Flag-tagged Zasp52 isoforms in stripes in the embryonic epidermis, using enGal4, led to junctional localisation of these isoforms (Fig. S4G-H′), confirming that Zasp52 binding partners appear to be present in epidermal junctions throughout the embryo.

Partial and complete loss of Zasp52 affects epithelial morphogenesis

In order to uncover the molecular role of Zasp52 in supracellularly coordinated junctions, we analysed the embryonic phenotypes, first in the zygotic mutant zasp52Δ, in more detail: zasp52Δ embryos, in addition to the reduction in F-actin accumulation at the salivary gland placode boundary shown above (Fig. 2A-C), showed a slight disorganisation of the placode in that some groups of cells appeared to be over-constricted (Fig. 4A′,B′, magenta arrows) compared to control where there is a clear gradient of constriction from the invagination point (Fig. 4C-C″; Sanchez-Corrals and Röper, 2018). Invaginated glands often showed an aberrant lumen (Fig. 4B″ inset, compared with control in Fig. 4C″ inset, Fig. 4D-E′). Such a widened invagination pit is indicative of defects in coordination of cell behaviours such as apical constriction during invagination (Sanchez-Corrals et al., 2021). At mid to late stages of embryogenesis, zasp52Δ embryos showed occasional holes within the epidermis where the salivary glands previously invaginated into the embryo (Fig. 4F compared with wild type in G, magenta arrow).

As zasp52 mRNA is provided maternally (https://insitu.fruitfly.org/cgi-bin/ex/insitu.pl; Tomancak et al., 2002), in order to observe the most severe possible phenotypes, we generated embryos lacking both maternal and zygotic Zasp52 contribution, zasp52m/zΔ embryos. These embryos completely lacking Zasp52 displayed numerous defects, including a strong loss of patterned cell shapes in the early salivary gland placode compared with control placodes.
(Fig. 5A-A’ versus C-C’)) or even holes or tissue disruption where the placode is located (Fig. 5B-B’, arrows in B’, compared to D-D’), as well as aberrant lumens of glands that managed to invaginate (Fig. 5B’ inset versus D’ inset; E,E’ at stage 14 compared with control in J,J’). Later stage embryos often showed wider epidermal disruption, in particular in regions where supracellular actomyosin cables were present in wild-type embryos such as the head region and ventral midline, whereas the posterior epidermis and segmental pattern appeared to be unaffected. Defects were visible as the disruption or lack of ventral midline structures (already visible at

Fig. 3. Zasp52-GFP interacts with many junctional proteins. (A,B) Volcano plots comparing spectral intensity values generated from anti-GFP co-immunoprecipitations from embryo lysates of Zasp52-GFP, Armadillo-YFP and wild-type embryos. Three experiments each for Zasp52-GFP and wild-type embryos were performed and two for Armadillo-YFP embryos. A shows enriched interactors for Zasp52-GFP compared with wild type (highlighting hits related to cell adhesion and regulation of junctional cytoskeleton), and B shows enriched interactors for Armadillo-YFP compared with wild type. The x-axis is the difference score for each protein as calculated in MaxQuant, and y-axis is the probability score calculated between Zasp52-GFP or Armadillo-YFP and wild type. The significance threshold was set to 0.75. (C) Schematic of protein complexes and interactions in marginal zone, apico-lateral and lateral junctions in *Drosophila* epithelial cells. (D-I’) Localisation of three possible Zasp52-interaction partners within the embryonic epidermis. Localisation around the salivary gland placode and in the leading edge epidermal cells and amnioserosa during dorsal closure is shown, two processes with prominent supracellular actomyosin cables containing Zasp52. (D-E’) APC2 localises to apical junctions, both bicellular and tricellular, but is more enriched at tricellular junctions. (F-G’) Sidekick is particularly enriched in tricellular junctions. (H-I’) Polychaetoid is localised homogeneously to apical junctions throughout the epidermis. APC2, Sidekick and Polychaetoid are not enriched in actomyosin cables. Zasp52-GFP, green; APC2, Sidekick and Polychaetoid, magenta. Dotted lines indicate the boundary of the salivary gland placode; asterisks mark the position of the invagination point; arrows indicate the position of the cable during dorsal closure. Scale bars: 10 µm. See also Fig. S4 and Tables S1 and S2.
stage 10 in Fig. 5A, arrows, and loss of the ventral midline at stage 15 in Fig. 5F). Furthermore, head involution, a process whereby the anterior-most structures of the embryo are internalised in major morphogenetic movements, appeared to partially fail, with structures that should have been internalised remaining on the outside along with significant epidermal tears visible in these
regions (Fig. 5F-I, white arrows pointing to non-internalised structures, magenta arrows pointing to epidermal holes and tears, compare with control in K). These phenotypes are reminiscent of disruption seen in weak alleles of shg (E-Cadherin) or alleles of α-Catenin that cannot interact with E-Cadherin and thus disrupt the link between E-Cadherin and actin (Orsulic and Peifer, 1996; Tepass et al., 1996), in line with Zasp52 also playing a role in the linkage of actin to junctions.

Therefore, loss of Zasp52 appeared to impair morphogenesis of tissues that displayed prominent supracellular actomyosin cables during their morphogenesis and led to large-scale disorganisation of embryonic tissues. The observed phenotypes of major aberrant tissue deformations as well as epidermal ruptures suggest that these cables could have served to coordinate major movements or protect tissue integrity.

**Genetic interaction suggests cooperation between Zasp52 and APC2**

We identified above that Zasp52 is able to physically associate with several junctional components. We therefore generated a double
mutants. suggest a weakened adherens junction-actomyosin link in these nearby, in order to prevent undue physical interference between

ventral anterior cables present during head involution, have not yet been elucidated. However, the function of the cable around the salivary gland (Monier et al., 2010), or for the cable during dorsal closure in ensuring a taut and mechanical insulators

Supracellular actomyosin cables containing Zasp52 act as mechanical insulators

Zasp52 as a component that is specifically enriched in embryonic supracellular actomyosin assemblies, in cooperation with other more widespread junctional components, appears to be important for the persistence of these cables and the morphogenetic events associated with them. It is still difficult, however, to pinpoint the collective function of such supracellular actomyosin cables during embryogenesis. Functions for certain cables have been proposed, for example for parasegmental cables in maintaining compartment identity against challenges such as cell divisions (Monier et al., 2010), or for the cable during dorsal closure in ensuring a taut and coordinated advancing front of epithelial cells (Ducuing et al., 2015). However, the function of the cable around the salivary gland placode, the cables at the ventral midline, as well as the network of ventral anterior cables present during head involution, have not yet been elucidated.

We suspect that one function of supracellular actomyosin cables is to insulate certain morphogenetic processes from others occurring nearby, in order to prevent undue physical interference between different morphogenetic events. Such an effect could in fact be observed when we analysed the movement of cell nodes or vertices in the early salivary gland placode, when apical constriction at the position of the future pit is only just beginning at late stage 10 (Fig. 7A-D; Fig. S6). At this stage the actomyosin cable near the forming pit at the dorsal-posterior boundary of the placode is already visible (Fig. 7A-B′; Fig. S6A-B′,D-E′). Time-lapse movies were collected for embryos expressing a ubiquitous membrane-tethered RFP (Ubi-RFP) as well as a GFP-myosin regulatory light chain (sqhGFP) transgene (Movies 1-3). Cell vertices that are inside the placode and near, but not part of, the forming invagination pit move towards the pit by about 2 μm over 10 min of apical constriction at the future pit position (Fig. 7A′,B′,C,D; Fig. S6; vertices of actively constricting cells marked in green in A and B were excluded from the analysis as these moved due to the apical constriction). By contrast, cell vertices equally close to the forming pit but posterior to the actomyosin cable, and hence outside the placode, barely move at all (Fig. 7A′,B′,C,D; Fig. S6). These observations indicate that one function of the actomyosin cable surrounding the salivary gland placode might be to serve as a barrier to insulate morphogenetic movements within the placode from the surrounding epidermis.

We set out to explore whether ‘morphogenetic insulation’ could be a general role of actomyosin cables containing Zasp52 and, in particular, what properties of such cables could confer such functionality. We already knew that the cable surrounding the salivary gland placode is under increased tension (Röper, 2012). We now employed a simplified in silico 2D vertex model of the salivary gland placode to test whether a tensed boundary could behave as a morphogenetic insulator (Fig. 7; Durney and Feng, 2021). This model considers a centre of hexagonal cells surrounded by a barrier of increased contractility and stiffness to model the actomyosin cable surrounding the placodal cells, with a further two coronae of cells surrounding the barrier that represent the surrounding epidermis (Fig. 7E). In this model, the central cells constrict, thereby pulling on and deforming other cells on the inside and outside of the barrier (Fig. 7F). The starting configuration takes into account that junctions at the barrier in vivo are more aligned and hence vertices at tricellular junctions deviate drastically from 120°. This is reflected in the model by the contribution of a bending energy coefficient (k) on edges representing the supracellular barrier. The central cells are allowed to actively constrict and the average nodal movement towards the centre is measured, while the spring constant (μ) of the barrier is varied. For a set value of the bending energy coefficient k (1, 10 or 100) an increase in spring constant μ consistently led to a decrease in the nodal movement of cells outside the barrier towards the centre (Fig. 7G,H), highly reminiscent of the reduced or absent movement of nodes we observed in vivo.

Thus, the in vivo and in silico data suggest that during mid to late embryogenesis in the fly the many large-scale actomyosin cables observed could act as insulators, thus ensuring that morphogenetic movements do not spread beyond a defined primordium, or act to prevent neighbouring regions undergoing different morphogenetic processes from unduly influencing each other.

DISCUSSION

The formation of organs during development requires coordination at many different time and length scales. Cell behaviours within a tissue primordium need to be coordinated but, equally, at boundaries between differently fated embryonic regions or organ primordia, such coordination has to cease so as not to inadvertently
Fig. 6. Zasp52 and APC2 interact genetically to strengthen supracellular actomyosin assemblies. (A-C) Salivary gland placodes in zasp52Δ; apc2D40 mutant embryos are disorganised (A-B') with aberrant patterns of apical constriction (green arrow), a disorganised boundary and ventral midline (magenta arrows) and the maxillar-mandibular fold invading the placodal area (white arrows), in comparison with control placodes (C,C'). Dotted lines mark the position of the boundary of the placode. Asterisk indicates the position of the invagination pit. (D-G) In zasp52Δ; apc2D40 mutants at stage 12 (mid-embryogenesis; D,D') the epidermis near the ventral midline is often disrupted by tears (magenta arrows in D'), compared with the smooth intact epidermis in control embryos (E). At late stage 16, the anterior head region in zasp52Δ; apc2D40 mutants shows tears and protruding internal tissues (F,F'; magenta arrows) compared with the intact epidermis in control embryos (G). The invaginated salivary gland of the embryo shown in F,F' also has an aberrant lumen shape (inset in F'). (H-I) Before dorsal closure commencing, zasp52Δ; apc2D40 mutants at early stages can fail to develop a clear boundary between epidermis and amnioserosa (magenta arrows in H') compared with control embryos (I). (J-K) During dorsal closure zasp52Δ; apc2D40 mutants (J,J') show uneven accumulation of F-actin in the leading edge cable and deformation of the cable indicative of uneven tension (magenta arrows in J') compared with the homogeneous accumulation in control embryos (K). E-Cadherin, magenta; Phalloidin, green. Scale bars: 10 µm (A-C'); 30 µm (D-H',J-K); 40 µm (I). See also Fig. S5.
affect the neighbouring processes. Coordination between groups of cells is often achieved through coordination of the cell cytoskeletal systems, via alignment of actin or microtubules, and is transmitted through cell-cell adhesion complexes usually located at adherens junctions (Röper, 2013; Sánchez-Corrales and Röper, 2018).

An intriguing cytoskeletal coordination is observed during numerous morphogenetic events in both invertebrates and vertebrates in the seemingly supracellular arrangement of actomyosin into supracellular cables. In Drosophila, numerous actomyosin cables are formed throughout embryogenesis, from early parasegmental cables and cables flanking the midline to cables in these structures, means that they span an enlarged region along the lateral membrane compared with general junctional actomyosin. (E-H) A vertex model of a simplified symmetrical 2D version of the placode was implemented similar to Durney and Feng (2021). (E) The starting configuration assumes individual cells as hexagons, apart from cells next to a barrier or supracellular cable (thick black line) where, due to an implemented contractility and hence rigidity, as well as a bending energy, cell junctions of the barrier are aligned (as is seen in vivo) with tricellular junctions showing 90°, 90°, 180° angles. A central group of seven cells as well as some selected edges (green) construct to represent the forming invagination pit, and movement of nodes outside the barrier (representing the epidermis surrounding the salivary gland placode) is assessed. (F) An example of a final configuration. (G) Decrease in radial movement for the outside nodes, normalised by the movement without the barrier, for different values of a bending energy coefficient k (that reflects a penalty for the nodal angles deviating from 120° at the boundary) observed for increasing spring constants, μ. (H) A comparison of the starting configuration compared with two different final configurations, with k=100 for both and μ=1 (green configuration) or μ=100 (magenta configuration). Scale bars: 10 µm. See Fig. S6.

But are these junctions in any way different to regular adherens junctions? Furthermore, are there specific components of such supracellular cables that set them apart from ubiquitous cortical actomyosin?

Zasp52 as a core component of Z-lines in muscles is the first component of supracellular actomyosin cables that distinguishes these from regular junctional actomyosin. We identified an actin-binding-motif in the Zasp52 central domain, as well as a bending energy, cell junctions of the barrier are aligned (as is seen in vivo) with tricellular junctions showing 90°, 90°, 180° angles. A central group of seven cells as well as some selected edges (green) construct to represent the forming invagination pit, and movement of nodes outside the barrier (representing the epidermis surrounding the salivary gland placode) is assessed. (F) An example of a final configuration. (G) Decrease in radial movement for the outside nodes, normalised by the movement without the barrier, for different values of a bending energy coefficient k (that reflects a penalty for the nodal angles deviating from 120° at the boundary) observed for increasing spring constants, μ. (H) A comparison of the starting configuration compared with two different final configurations, with k=100 for both and μ=1 (green configuration) or μ=100 (magenta configuration). Scale bars: 10 µm. See Fig. S6.

Zasp52 as a core component of Z-lines in muscles is the first component of supracellular actomyosin cables that distinguishes these from regular junctional actomyosin. We identified an actin-binding-motif in the Zasp52 central domain, as well as a host of junctional proteins interactors, and furthermore its N-terminus, known for binding actin (Liao et al., 2020), also promotes Zasp52 dimerisation and multimerisation. These results suggest that, upon binding to Zasp52, junctional actomyosin likely takes on a new and higher-order structure. It is noteworthy that the junctional interaction partners localise to the marginal zone (Crumbs, Patj), the adherens junctions (armadillo, α-Catenin, p120-Catenin, E-Cadherin, APC2, Sidekick, Bazooka, Polychaetoid, Canoe) and even basally to adherens junctions (Scribble). This suggests that the increased size of actomyosin cables, which can be inferred visually from the strongly increased intensity of F-actin and myosin labelling in these structures, means that they span an enlarged region along the lateral membrane compared with general junctional actomyosin that is usually restricted to adherens junctions only (Desai et al., 2013; Röper, 2015). Such lateral expansion of actomyosin accumulation could be a defining feature of supracellular actomyosin cables, with a possible actomyosin organisation across the epithelial junctions similar to lateral stacking of thin filaments in sarcomeres, though this requires future investigation.

The phenotypes observed in the absence of Zasp52, as well as in the combined absence of Zasp52 and APC2 (but not APC2 alone),
in particular the epidermal tears and holes, are reminiscent of phenotypes observed when adherens junctions and their linkage to the actin cytoskeleton are compromised by the lack of α- catenin (Sarpal et al., 2012), or by hypomorphic mutations in E-Cadherin itself (Tepass et al., 1996). These mutant embryos also show epidermal holes, especially in the head and ventral region, as well as anterior disorganisation that might be a result of head involution defects. Thus, impairment of the linkage of adherens junctions to the junctional actin cytoskeleton, occurring in α-catenin, E-Cadherin and also Zasp52 mutants, appears to lead to a common set of phenotypes.

Interestingly, several of the junctional components identified in the complex with Zasp52 are either enriched at or are exclusive to tricellular junctions (APC2, Canoe, Sidekick), and Zasp52 is enriched in these tricellular junctions itself. These cell vertices are the points at which individual segments of actomyosin cables require to be connected to the next cable segment one cell on. The presence of Zasp52 in precisely these junctions could help to reinforce them against the increased tension that actomyosin accumulation at cable sites in the morphogenesis (Ebrahim et al., 2013). The loss of actin and myosin assembly than general actomyosin, imposed by even more profound, as its role in Z-lines of sarcomeres could trigger by other mechanisms, and second, the expression of Rok accumulation; Röper, 2012; Sidor et al., 2020), increased junctional F-actin at a position where a cable is forming, through a positive feedback loop. Initial Zasp52 recruitment to these crosses, virgin females were picked and crossed to stock. Progeny of this cross was heat shocked for 1 h at 29°C before imaging. Embryos for immunoprecipitations were pre-laid on 90 mm apple juice agar plates at 25°C overnight or for durations as indicated. Embryos used for live imaging were collected at 25°C and aged 2 h at 29°C before imagining. Embryos for immunoprecipitations were pre-laid on 90 mm apple juice agar plates for h 1 to avoid collecting older retained embryos, the plate was discarded and collections started for 9, 10, 11 or 12 h. Overnight collections without pre-lay were collected in the same manner. SeeTable S3for key resources, and Table S4 for genotypes used in figure panels.

**Materials and Methods**

**Fly husbandry and genetics**

*Drosophila melanogaster* embryos were collected for staining on apple juice agar plates at 25°C overnight or for durations as indicated. Embryos used for live imaging were collected at 25°C and aged 2 h at 29°C before imaging. Embryos for immunoprecipitations were pre-laid on 90 mm apple juice agar plates for h 1 to avoid collecting older retained embryos, the plate was discarded and collections started for 9, 10, 11 or 12 h. Overnight collections without pre-lay were collected in the same manner. SeeTable S3for key resources, and Table S4 for genotypes used in figure panels.

**Generation of zasp52Δ germine clones**

To generate embryos devoid of maternally deposited Zasp52, the FLP-DFS strategy was employed, based on the work of Chou and Perrimon (1992). To this end, FRT G13 zasp52Δ virgins were crossed to males of hsFLP; FRT G13 ovoD1/TTP stock. Progeny of this cross was heat shocked for 1 h at 37°C when reaching first instar stage and again the following day. From these crosses, virgin females were picked and crossed to zasp52ΔCyO twi:: GFP males and embryos collected and analysed.

**Immunofluorescence staining of whole-mount embryos**

Embryos of desired stages were collected from apple juice-agar plates with a brush and tap water and transferred into a sieve. After rinsing with tap water, embryos were dechorionated with 50% of thick bleach/water for 3 min. Embryos were washed several times with water to remove bleach solution and residual water absorbed with paper wipes. Embryos were then taken up in 800 µl heptane and placed in a small glass screw-cap vial. Then 400 µl PBS and 400 µl 8% paraformaldehyde (PFA; EM-grade)/PBS solution and residual water absorbed with paper wipes. Embryos were washed several times with water to remove bleach and fixed embryos were simultaneously permeabilised and blocked with PBT [0.3% Triton X-100, 2.5% bovine serum albumin (BSA) in PBS] for 1-3 h at 4°C. Primary antibodies were diluted in PBT and embryos incubated with antibodies overnight at 4°C on a shaker. Embryos were washed three times with 500 µl PBT for 20 min on shaker at room temperature (RT). Secondary antibodies were diluted 1:200 in PBT or according to the manufacturer and added to washed embryos to incubate for 2.5 h at RT or overnight at 4°C. Embryos were subsequently washed 3× with PBT for 20 min on a shaker at RT. Embryos were mounted in Vectashield mounting medium (H-1000).
Live imaging of whole-mount embryos
For live time-lapse experiments, embryos of the genotype sqh-GFP; UbiRFP were dechorionated in 50% bleach and extensively rinsed in water. Stage 10 embryos were manually aligned and attached to heptane-glue-coated coverslips and mounted on custom-made metal slides; embryos were covered using halocarbon oil 27 (Sigma-Aldrich) and viability after imaging after 24 h was controlled prior to further data analysis. Time-lapse sequences were imaged under a 40×/1.3NA oil objective on an inverted Zeiss 780 Laser scanning system, acquiring z-stacks every 0.58-3 min with a typical voxel xyz size of 0.22×0.22×1 µm. Z-stack projections to generate movies were assembled in ImageJ or Imaris. The membrane channel images from time-lapse experiments were denoised using nd-safr software (Boulanger et al., 2010).

Ectopic expression of Zasp52 in striped embryos
Zasp52 truncations were expressed with the UAS-GAL4 system using the enGal4 driver. To this end, virgins of w/+; enGal4/Cyo. UAS-GFP were crossed with w*/Y; UAS-Zasp52-PK-FLAG/TM3 twi::GFP or w*/Y; +/+; UAS-Zasp52-PRP-PDZ-FLAG/TM3 twi::GFP, and the resulting F1 embryos were dechorionated in 50% bleach and extensively rinsed in water. Extensive rinsing in water was essential since Zasp52-PK-6xHis-FLAG was unstable within a short period of time, and thus prior to muscle development, which commences at 9:20 hpf. Embryos fixed and stained with anti-Cadherin and anti-FLAG antibodies as described above. The subcellular localization of Zasp52 truncions was determined using the confocal microscope.

Quantification of F-actin signal intensity at the supracellular actomyosin cable in the placode
Whole-mount embryos were fixed and stained as described above. Higher resolution images of placodes were acquired as z-stacks using the Olympus Fluoview 1200 microscope. Equivalent slices (eight slices corresponding to 400 nm, starting apically at first-observed intensity of E-Cadherin) were used to create a SUM projection. Three rectangular areas were defined over the whole placode to measure background intensity and calculate average background intensity. Phalloidin signal intensity of individual junctions at the placode border was measured, leaving out the two rows of cells at the ventral midline and the junctions closest to the invagination pit because of distortion of the tissue at these points. Junction intensity was normalised to background [IJunction/AreaJunction]/(Ibgf/Areabgf).

Transfection and staining of S2 cells with Zasp52 truncations
S2 cells (University of California, San Francisco, CA, USA; mycoplasma-free judged by DAPI staining) were grown at 25°C in Schneider’s Medium (Thermo Fisher Scientific) supplemented with 1% Pen/Strep (Gibco) and 10% (vol/vol) fetal bovine serum (Gibco; heat-inactivated for 1 h at 70°C). Stable cell lines were obtained by transfection with pMT puro vectors (Thermo Fisher Scientific) supplemented with 1% Pen/Strep (Gibco) and 10% (vol/vol) fetal bovine serum (Gibco; heat-inactivated for 1 h at 70°C). Transfection and staining of S2 cells with Zasp52 truncions were expressed with the UAS-GAL4 system using the enGal4 driver. To this end, virgins of w*/Y; UAS-Zasp52-PK-6xHis-FLAG/TM3 twi::GFP or w*/Y; +/+; UAS-Zasp52-PRP-PDZ-FLAG/TM3 twi::GFP, and the resulting F1 embryos were dechorionated in 50% bleach and extensively rinsed in water. Extensive rinsing in water was essential since Zasp52-PK-6xHis-FLAG was unstable within a short period of time, and thus prior to muscle development, which commences at 9:20 hpf. Embryos fixed and stained with anti-Cadherin and anti-FLAG antibodies as described above. The subcellular localization of Zasp52 truncions was determined using the confocal microscope.

Recombinant expression and purification of the central Zasp52 ABM
The Zasp52 ABM was expressed as a GST fusion protein in Escherichia coli and purified via affinity and size exclusion chromatography. The plasmid was generated via synthetic assembly of aa1131–aa1318 of the Zasp52-PF cDNA (synthetic assembly of Zasp52-PF cDNA, Twist Bioscience) with Ascl and Fsel restriction enzyme overhangs and was digested and ligated in pGEX F/A vector cut with Ascl and Fsel. The pGEX plasmids containing truncations were transformed into the Rosetta (DE3) strain via heat shock transformation and selected via antibiotic selection. Colonies were picked the next day to inoculate pre-cultures. Precultures were used to set up large cultures grown to OD600=0.6 and expression was induced by addition of 0.5 mM IPTG. Cultures were grown overnight at 18°C and harvested the next day via centrifugation at 4000 g for 30 min. Supernatant was discarded and pellets lysed on ice by resuspending in lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM KCl, 1% Triton X-100, 10 mM MgCl2, 5% glycerol, 1 mM DTT, 1× complete protease inhibitors, 0.7 mg/ml lysozyme and 10.00 µg/ml DNAse I in water] and then sonicated on ice, followed by clearing of lysate via centrifugation at 60,000 g for 30 min at 8°C. Protein-containing supernatant was incubated with 1-5 ml of GSH-beads equilibrated in lysis buffer on a shaker for 2 h at 4°C. Flow-through was collected and the column was washed once with 45 column volume (CV) of wash buffer [50 mM Tris-HCl (pH 7.4), 150 mM KCl, 5% glycerol, 1 mM DTT]. To remove chaperones, an additional wash step with 1 CV ATP wash buffer [50 mM Tris-HCl (pH 7.4), 150 mM KCl, 5% glycerol, 10 mM NaATP, 10 mM MgCl2, 1 mM DTT] was carried out. GST-fusions were eluted with 10 ml glutathione [100 mM Tris-HCl (pH 7.4), 150 mM KCl, 10 mM GSH, 5% glycerol, 1 mM DTT] in 1 CV fractions. Fractions rich in GST-fusion protein were pooled and cleaved using TEV enzyme during dialysis [50 mM Tris-HCl (pH 7.4), 150 mM KCl, 5% glycerol, 1 mM DTT] overnight at 4°C. In order to remove free GST from the solution, the dialysed protein was incubated with equilibrated GSH resin for 2 h at 4°C and the flow through collected, concentrated and injected in a Superdex 200 (16/100, GE Healthcare) exclusion chromatography column. The column was equilibrated with gel filtration buffer [20 mM K-HEPES (pH 7.5), 150 mM KCl, 5% glycerol, 1 mM DTT]. Fractions collected were analysed by staining on SDS PAGE and fractions chosen, pooled and concentrated. Aliquots were frozen in liquid nitrogen and stored at −80°C until used for experiments. See Table S5 for the DNA sequences of cloned Zasp52 fragments or isoforms.

F-actin binding assay
To investigate protein binding to filamentous actin, an F-actin pelleting assay was employed. To this end, rabbit muscle actin (Akl99, Cytoskeleton) was prepared according to the manufacturer’s instructions and Ca2+-actin exchanged to Mg2+-actin with exchange buffer (0.2 mM EGTA, 0.02 mM MgCl2 final) in G-buffer [5 mM Tris-HCl (pH 8.0), 0.2 mM ATP, 0.1 mM CaCl2, 0.5 mM DTT] on ice for 10 min. Mg2+-G-actin stock solution was diluted to 8 µM, 6 µM, 4 µM and 2 µM and co-incubated with Zasp52-ABM fragments or isoforms. Fractions collected were analysed by staining on SDS PAGE and fractions chosen, pooled and concentrated. Aliquots were frozen in liquid nitrogen and stored at −80°C.

Immunoprecipitation of GFP-labelled proteins from Drosophila embryos
To identify proteins associated with Zasp52 in epithelia, co-immunoprecipitations against GFP were performed using either the Zasp52-GFP[2CL423] or Zasp52-GFP[GI00189] endogenous protein trap lines as well as wild-type w1118 flies (Morin et al., 2001). Armadillo-YFP[CP7001198S] was used as a control for co-immunoprecipitation of a junctional cytoplasmic protein. To prevent the contamination of the sample with muscle tissue, the collection protocol consisted of a 1 h pre-lay collection to eliminate embryos retained in the mother (discarded), and was then followed by a 9 h collection, resulting in embryos between 0 and 9 hpf and thus prior to muscle development, which commences at 9:20 hpf. Dechorionated frozen embryos were placed on ice and immediately covered with 100 µl chilled lysis buffer [50 mM Tris (pH 7.4), 150 mM KCl, 0.5 mM EDTA, 0.1% Glycerol, 0.01% Triton X-100, 1200 µg/ml benzamidine,
40 µg/ml chymostatin, 40 µg/ml antipain, 2 µg/ml leupeptin, 0.96 µg/ml pefabloc, 0.5 mM PMSF). Each sample contained roughly the same mass of embryos, as different samples were used per experiment in order to observe differences between sample types, e.g. wild-type versus Zasp52-GFP, and quantitative analysis of triplicate samples used. If necessary, different collections of equivalent laying conditions were pooled to create one sample. Per sample, 500 µl of lysis buffer were added and total sample transferred into a glass dounce-homogeniser. Embryos were lyzed with 20 strokes and a subsequent 30 min incubation on ice. To separate the protein-containing aqueous phase from lipids and cell debris, samples were centrifuged at maximum speed in a tabletop centrifuge for 10 min at 4°C. The aqueous phase was carefully collected and added to equal amounts of GFP-nanobody buffer for subsequent analysis by western blotting, or the wash buffer washed 3× with wash buffer (10 mM Tris (pH 7.4), 150 mM KCl, 0.5 mM EDTA, 0.1% Glyceral, 0.5 mM PMSF) and then either eluted in sample buffer for subsequent analysis by western blotting, or the wash buffer decanted, beads resuspended in 50 mM ammonium bicarbonate and stored at –20°C before mass spectrometry analysis.

Mass-spectrometry processing of immunoprecipitation samples

Proteins were prepared for enzymatic cleavage from magnetic beads by submersion in 50 mM ammonium hydrogen carbonate (pH 8.0). The solution containing bead-bound protein was digested with 0.5 µg of trypsin for 60 min at 37°C in a thermomixer, shaking at 800 rpm. This was followed by another overnight digestion step for which an additional 1 µg trypsin was added and the samples digested under the same conditions as above. The reaction was terminated by adding formic acid to a final concentration of 2% v/v. The output of the digestion reaction was analysed using a nano-scale capillary liquid chromatography with tandem mass spectrometry (LC-MS/MS) in an Ultimate U3000 HPLC setup (Thermo Fisher Scientific Dionex) to deliver a flow of ~300 nL/min. A C18 Acclaim PepMap100 5 µm, 100 µm × 20 mm nanoViper (Thermo Fisher Scientific Dionex), trapped the peptides before separation on a C18 Acclaim PepMap100 3 µm, 75 µm × 150 mm nanoViper (Thermo Fisher Scientific Dionex). The Peptides were eluted from the column via an acetonitrile gradient. The analytical column outlet was immediately interfaced by a modified nano-flow electrospray ionisation source, coupled to a hybrid dual pressure linear ion trap mass spectrometer (Orbitrap Velos, Thermo Fisher Scientific). The data acquisition was carried out using a resolution of 30,000 for the full MS spectrum, then followed by ten MS/MS spectra in the linear ion trap. All MS spectra were collected over an m/z range of 300-2000 and MS/MS scans were collected with a threshold energy of 35 for collision-induced dissociation. All raw files were processed with MaxQuant 1.5.5.1 (Cox and Mann, 2008) using standard settings and searched against the UniProt KB with the Andromeda search engine (Cox et al., 2011) integrated into the MaxQuant software suite. Enzyme search specificity was Trypsin/P for both endopeptidases. One or two missed cleavages for each peptide were allowed. Carbamido-methylation of cysteines was selected as fixed modification with oxidised methionine and protein N-acetylation set as variable modifications. The search was conducted with an initial mass tolerances of 6 ppm for the precursor ion and 0.5 Da for MS/MS spectra. The false discovery rate was fixed at 1% at the peptide and protein level.

Quantitative analysis of mass-spectrometric data of co-immunoprecipitations in Perseus

Downstream statistical analysis was carried out using the Perseus interface of MaxQuant (https://maxquant.net/perseus/). Prior to statistical analysis, peptides mapped to known contaminants, e.g. human keratin, reverse sequence hits and protein groups only identified by site, were removed. Only protein groups identified with at least two peptides, of which one had to be unique, and two quantitation events were considered for data analysis with Perseus. Three experiments were analysed for Zasp52-GFP and Armadillo-YFP each and two for wild type. To compare samples of interest with control, the triplicate or duplicate datasets were transformed to Log2(x), missing values replaced by imputation, and the volcano plot tool used to compare two datasets in which the x-axis represents a difference score and the y-axis visualises the negative, logarithmic P-value generated by an unpaired two-tailed Student’s t-test. The significance threshold was set to a P-value of 0.75, so that known interaction partners in the Armadillo-YFP control sample were identified.

Computational modelling

We represent an epithelial tissue by a 2D vertex model of 127 hexagonal cells initially arranged in a hexagonal orientation, essentially following previous reports (Durney and Feng, 2021; Durney et al., 2018). In brief, an individual cell has six peripheral nodes and one central node connected by passively elastic edges. To represent a supracellular actomyosin cable that is enriched by myosin across several junctional boundaries, we have taken select edges (indicated in bold black, Fig. 7E,F) and endowed them with two different properties: an increased elastic modulus, μ, and a resistance to bending. The latter is implemented by a penalty against deviation in the angle of adjacent edges resulting in a restoring force, Fb. Select edges (indicated in green, Fig. 7E) are prescribed to contract and generate active pulling forces. This contraction pulls on the tissue exterior of the boundary, compressing the interior region.

The total force, \( F_c \), on node \( i \) is given by,

\[
F_i = \mu (l_{ij} - l_0) \frac{\mathbf{x}_j - \mathbf{x}_i}{|\mathbf{x}_j - \mathbf{x}_i|} + F_b + F_{active},
\]

where \( l_{ij} \) is the current edge length between nodes \( i \) and \( j \), \( l_0 \) is the edge rest length, \( F_b \) is the force due to bending (only on edges associated with the supracellular actomyosin cable) and \( F_{active} \) is the active force that causes contraction of select edges. Nodal motion is governed by over-damped dynamics with a viscous friction factor \( \eta \).

\[
\eta \frac{d\mathbf{x}_i}{dt} = F_i.
\]

The force, \( F_{active}(\theta - \pi) \), tends to align the adjacent edges. As the tissue is in an initial hexagonal configuration, this term circularises the supracellular cable and reduces its circumference. To maintain a zero-energy ground state, we set the bending modulus, \( k \), and then allow the tissue to relax to equilibrium. For edges of the supracellular cable, we adopt the equilibrium length to be its new rest length. This procedure is repeated until there are no more changes in \( l_{ij}/\forall i,j \). This guarantees a consistent ground state as \( \mu \) is increased. Tissue deformation is initiated by \( F_{active} \) contracting specific edges.

The main output of the model is the average radial movement of the \( n \) peripheral nodes of the epithelial tissue,

\[
r_{avg} = \frac{1}{n} \sum_{n} |x_n^t - x_n^0|,
\]

where the superscript denotes simulation time.

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Competing interests

The authors declare no competing or financial interests.

Author contributions


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