Chapter 16. Intracellular mechanical coupling, signalling receptors and PCP functions at the cortical membrane interface.

The cortical actin cytoskeleton is coupled between adjacent cells via multiple trans-membrane linkers, including cadherins, integrins, catenins, laminins, nectins and dystroglycans. These anchoring functions may transduce mechanical strain across epithelial interfaces and provide structural integrity to the cytoplasmic boundary. Additional specialised signalling receptors include N, Delta, Serrate (Ser), Dscam, Tkv and Tl. By implication, trans-membrane coupling may generate entangled interactions between (otherwise) discrete signalling pathways.

Cadherin-mediated interactions are predominantly transduced via the *adherens* junctions (AJs), while the N and Ser receptors signal through focal adhesions (FAs). In particular, E-cadherin (E-cad, aka *shg*) is a structural component of AJs, with functions during embryonic segmentation and in the germline stem-cells [1]. The Ft and Ds cadherins regulate growth; while the Stan cadherin (aka, Flamingo, Clsr) functions during terminal differentiation. Similarly, the integrins form structural components of FAs, connecting the apical actin cytoskeleton to the extracellular matrix. Mammalian integrins anchor the N-WASP (Wiscott-Aldrich Syndrome protein) and Arp2/3 (Actin related protein-2/3) to the cortical actin cytoskeleton, with a stress-sensing function. This complex remains in a closed configuration unless bound to Cdc42 (and/or PIP_2) and may nucleate microfilament assembly at the cortical membrane interface [2]. An additional stress-sensitive component is provided by the Paxilin (Pax) scaffold, which has 4 Zn-finger LIM domains and is a target of Focal Adhesion Kinase (FAK) [3] [4]. Pax can also localise to perinuclear microtubule organising centres (MTOC), where it binds α and γ tubulins [5], consistent with microtubule nucleating and anchoring functions.

The Pk family proteins have 3 LIM domains, closely resembling those of Pax, and an adjacent PET domain. In addition, Pk contains several low complexity peptide segments and multiple short linear motifs (SLiMs) including the C-terminal CAAX prenylation motif that allows reversible membrane localisation [6] [7] [8]. The alternatively-spliced 5' exons of *pk*, *PB* and *sple* encode N-terminal peptides of 13, 79 and 349 AAs, respectively; with additional low sequence-complexity segments in PB and Sple [8]. Putative post-translational SLiM targets include FAK, AJ, microtubule-associated and cyclin-dependent kinases, but only the CAAX motif and a Mink1 (Mitotic spindle and nuclear kinase1) phosphorylation site have been validated [9]. In addition, the *Drosophila* and vertebrate Pk proteins all carry (unrelated) putative nucleolar localisation motifs (NoLS) [10] outside their conserved peptide segments.

The Pk^{pk} and Pk^{sple} protein isoforms co-localise with Arm (β-catenin) at AJs in imaginal wing discs. F-actin localisation at lateral cell boundaries is reduced in somatic clones of pk^{pk}, which lose their regular hexagonal packing. By contrast, pk^{sple} mutant cells remain hexagonal, with Pk^{pk} localised to apico-lateral vertices [11]. Thus, the Pk^{pk} isoform may favour PCP signalling through lateral cell contacts; while the Pk^{sple} may function though the Ap epithelial surface, consistent with Notch pathway signalling [12] [13] [14] [15] [16]. Notably, the bald patch in the anterior notum of $pk^{pk\text{-}sple}$ mutants is consistent with reduced delivery of cytoskeletal components to Ap cell surfaces (Fig. 24), see Chapter **24**. The antagonistic interactions of the Pk^{pk} and Pk^{sple} isoforms may result from their competitive binding to Dsh, through their common C-terminal domain, with preferential localisation to proximal cell vertices. Meanwhile, Dsh binding is also required at distal PCP complexes, in combination with Diego [17]. It follows that lowering Pk activity may favour formation of the Dsh/Diego complexes. Such a "bipolar" Dsh requirement would be consistent with the long-range domineering polarity reversals associated with Pk overexpression within the wing blade. In particular, expression of LIM-deleted Pk^{pk} and Pk^{sple} constructs that lack essential scaffold-binding functions, still cause domineering PCP reversals in surrounding wild-type cells,

consistent with competitive inhibition of Diego/Dsh assembly. Meanwhile C-terminal deletions that remove the Dsh binding site of Pk^{pk} and Pk^{sple} do not reverse domineering PCP signalling [11]. By implication, PCP signal-transduction is dependent on the predominant Pk isoform and to what extent the activity of Diego (Dgo) is limited by lack of Dsh [17 18]. Both Dsh and Pk with carry putative NEK2 and GSK3 kinase SLiM target sites, consistent with scaffold functions that regulate microtubule anchoring and assembly, http://elm.eu.org/search.html [8]. In this context, Dsh may alter mitotic spindle orientation via Discs large1, Dlg1, with linkage to the Kinesin-II (Klp 64D) motor protein through Arm [20].

At the molecular level, the best characterised Pk-family protein may be mammalian Tes [21 22]. Deletions of the individual LIM domains of Tes alter its partitioning between FAs, stress fibres and the nucleus [23 24 21 22]. When localised to FAs, the Tes-LIM1 domain binds Zyxin, while the Tes-LIM3 domain binds Mena (Mammalian enabled), Talin and Actin related protein 7A (Arp7A) [23 24 25]. Arp7A forms a component of the vertebrate N-WASP-Arp2/3 scaffold complex, which includes Mena, Vasp and Evl [25]. In migrating cells, the WASP-Arp2/3 complex regulates filopodial and lamellipodial assembly by blocking microfilament capping and recruiting actin to FAs [26 27 25 28]. By implication, Tes may modify the stress-sensitivity of the WASP-Arp2/3 complex [2]. In particular, the PET domain of Tes may cap its LIM domains and/or modify membrane anchoring, unless displaced by mechanical stress, [24] and J. Sutherland, personal communication. The PET and LIM domains of *Drosophila* Pk and Esn are strongly conserved with respect to vertebrate Tes, and the orthologous regions of the fly Ena and Arp7A proteins fit the LIM2-3 domains of Pk and Esn (Figs. 26 & 27).

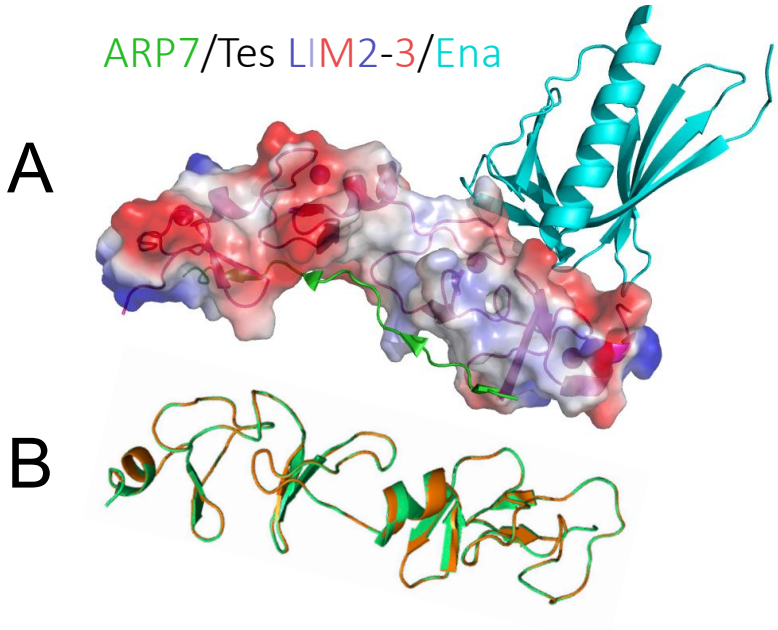

Fig. 26. ARP7/Tes/Ena scaffold assembly. A. Molecular model of mouse ARP7/Tes/Ena scaffold assembly. ARP7 and Ena are shown as ribbons, while the LIM2-3 domains of Tes are represented as solid; with red positively charged surfaces, blue negatively charged surfaces and grey apolar surfaces. Note that the scaffold assembly has only one-fold rotational symmetry around each of the Cartesian axes, as do the individual peptide components. **B.** Superposition of *Mus* TES LIM1-2 (green) and *Drosophila* Pk LIM1-2 (orange). A. Rojas and D. Gubb, unpublished.

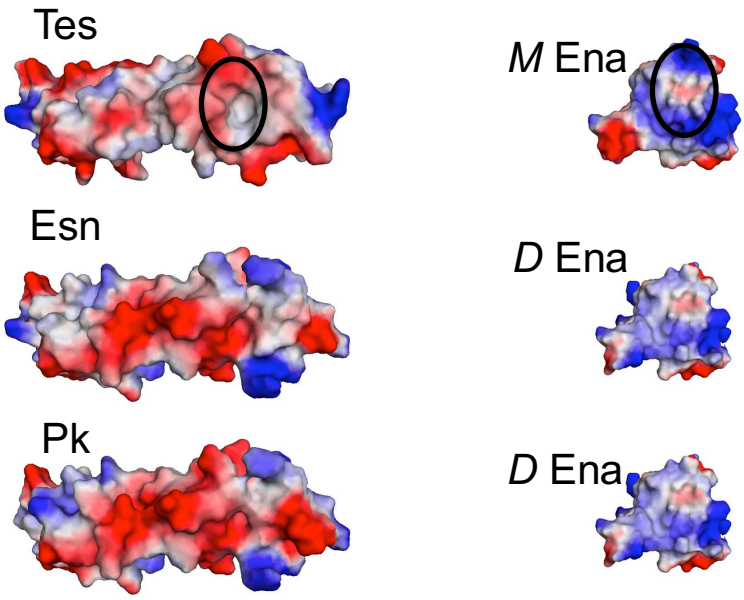

Fig. 27. Ena/Lim2-3 scaffold assembly models. A. *Mus* Mena/Tes Lim2-3, black oval indicates matching molecular interfaces; **B.** *Drosophila* Esn Lim2-3/Ena (E299 to S408), **C.** *D.* Pk Lim2-3/En (E299 to S408). A. Rojas and D. Gubb, unpublished.

Ena functions as a processive actin polymerase, adding G-actin monomers to the barbed end of F-actin filaments [29]. Notably, the Ap localisation of Ena and F-actin is suppressed by the Ableson kinase in the presumptive mesodermal cells to either side of the ventral furrow [30]. Ena localises to tension-dependent junctional complexes near cellular vertices in the embryo and imaginal wing disc [31]. Taken together, these results indicate that Pk family proteins provide stress-sensitive scaffold functions at cytoplasmic membrane interfaces.

Summary:

Cell adhesion molecules mediate the transduction of mechanical strain between cells, with AJs and FAs acting as foci for coupling to the extracellular matrix and signal transduction. Thus, scaffold protein assemblies at the cytoplasmic membrane interface may transduce mechanical stress and regulate cell shape, including Pk, Tes and Pax. Remodelling of the cortical actin cytoskeleton redistributes anchor molecules and signal receptors. In particular, the Pk/Dgo balance may mediate Dsh-dependent signal transduction, and mitotic spindle orientation, during terminal PCP signalling.

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