1118 Cell 133, June 13, 2008 ©2008 Elsevier Inc. DOI 10.1016/j.cell.2008.06.002

SnapShot: Tight and Adherens Junction Signaling

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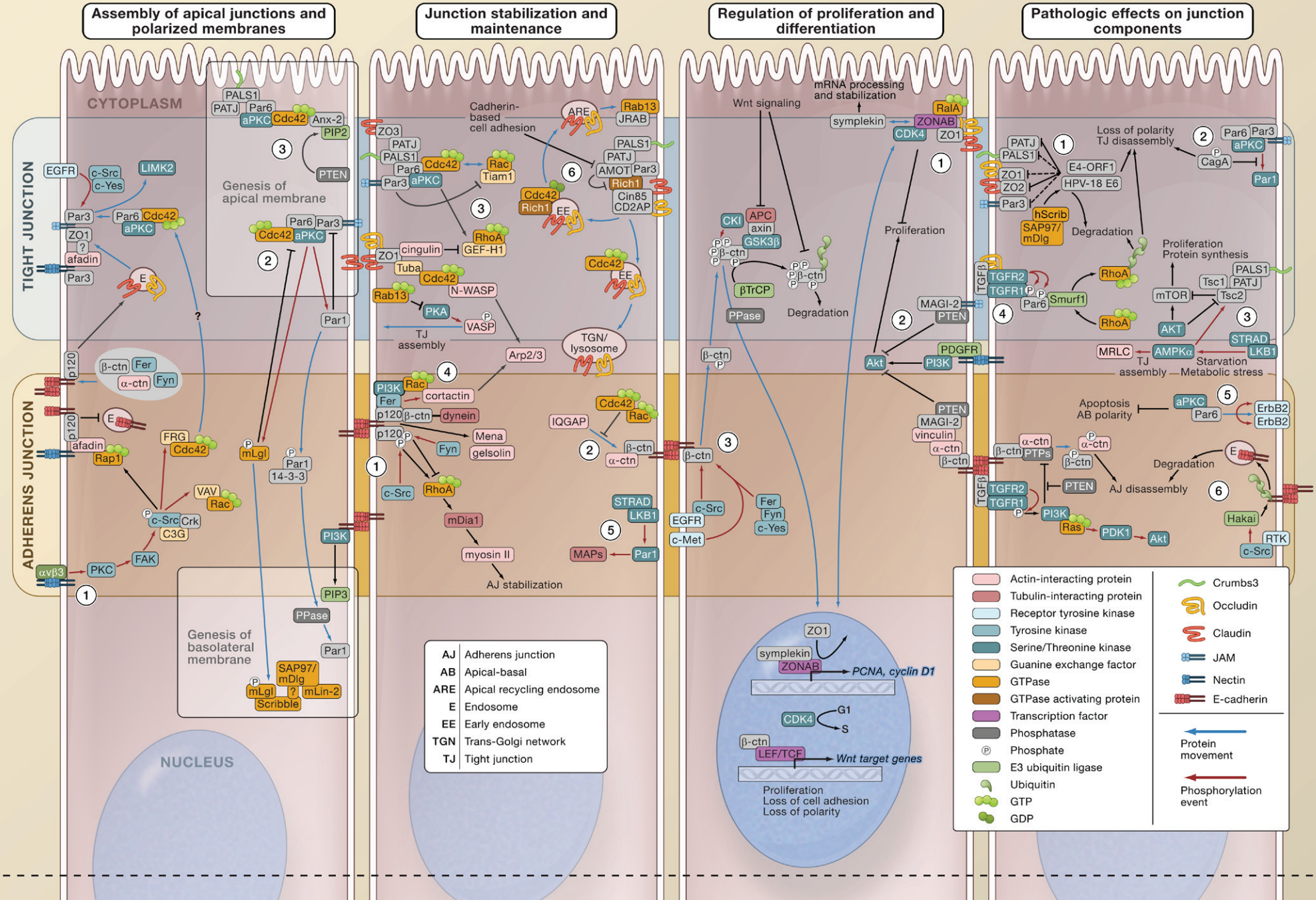
Cell



See online version for legend, abbreviations, and references.

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Signaling pathways converging upon and emanating from adherens junctions (AJs) and tight junctions (TJs) affect many cellular functions, including cytoskeletal dynamics, polarized vesicle trafficking, protein synthesis, and proliferation. Many pathologic processes target junctional proteins, resulting in the disassembly of junctions and breakdown of tissue integrity. As signaling pathways and protein components of junctions vary among organisms, this SnapShot highlights those pathways established in mammalian epithelia.

Assembly of Apical Junctions and Polarized Membranes

Transdimerization of heterophilic nectins between adjacent cells is a critical event leading to the development of AJs, TJs, and polarized membrane domains. (1) Upon association with dimerized nectin, integrin $\alpha_v\beta_3$ initiates a set of signaling events involving c-Src, which results in the activation of Rap1, Cdc42, and Rac. Subsequently, Rap1 complexes with afadin and p120^{ctn} resulting in stabilization of E-cadherin at the cell surface followed by recruitment of β- and α-catenin and Fer kinase. E-cadherin stabilization also triggers delivery of occludin and claudin to nascent TJs at the apical side of AJs. Concurrently, nectin accumulates JAM through its interaction with afadin and ZO1, developing a platform for Par3 localization. Par6 and aPKC join Par3 after LIMK is released, an event triggered by phosphorylation of Par3 downstream of EGFR activation. (2) aPKC at nascent TJs contributes to polarization of the apical membrane by targeting specific proteins for exclusion from this region. Phosphorylation of Par1 and mLgl by aPKC results in their recruitment to the nascent BLM. At the BLM, Par1 and mLgl inhibit incursion of the apical Par complex through sequestration and phosphorylation of complex components. (3) PTEN is recruited to junctional and apical surfaces where it generates PIP_2, which attracts Anx-2. The latter interacts with the Par complex which in turn associates with the Crumbs polarity complex consisting of Crumbs3, PALS1, and PATJ, thus firmly defining the apical-junctional membrane.

Junction Stabilization and Maintenance

Maturation and maintenance of both AJs and TJs requires the formation of an elaborate web of highly dynamic and specifically localized interactions with the cytoskeleton.

Competing Control of Actin Dynamics

(1) At AJs, tyrosine phosphorylation at one site on p120^{ctn} by c-Src activates RhoA and promotes activity of myosin II through mDia1 resulting in stabilization of AJs. These events are inhibited by Fyn-directed tyrosine phosphorylation of another site on p120^{ctn}. (2) IQGAP and α-catenin are rival binding partners for β-catenin at AJs. By preventing α-catenin's recruitment, IQGAP effectively blocks AJ anchorage to F-actin. Activated Rac/Cdc42 inhibit IQGAP's interaction with β-catenin, thus favoring α-catenin's association with proteins at the AJ. The presence of α-catenin triggers recruitment of other proteins that bind F-actin, culminating in maturation of AJ-actin cytoskeleton interaction. (3) At TJs, the Par complex is dependent on Rho GTPases to assemble and activate resident components. However, Par3 also has a negative feedback function; it inhibits activity of the Rac GEF Tiam1 thus preventing the propagation of locally activated Rac. Likewise, the RhoA GEF GEF-HI is activated by aPKC but inhibited by cingulin, which resides at the TJ in association with ZO1.

Regionalized Signaling at Junctions

Localized activation of actin effectors spatially regulates actin dynamics differentially at AJs and TJs. (4) At AJs, Rac activated by resident PI3K recruits cortactin, which is then activated by Fer kinase bound to p120^{ctn}. At TJs, ZO1 recruits a Cdc42 GEF, Tuba, resulting in activation of N-WASP. Localized activation of VASP, Mena, and gelsolin also occurs upon junction formation. Upstream signals also negatively regulate modulators of actin; at TJs, activated Rab13 inhibits PKA's activation of VASP, blocking its interaction with actin and preventing the assembly of TJ components.

Interactions with Microtubules

(5) Downstream of LKB1, activated Par1 phosphorylates MAPs, which results in their release from microtubules allowing for motors to move their cargos in an unhindered fashion. Additionally, interactions between p120^{ctn} and kinesin as well as β-catenin and dynein are described.

Directed Trafficking of Vesicles Carrying Membrane Components

(6) When Rich1, a Cdc42 GAP, is active at TJs, it assists in the redirection of endocytosed occludin and claudin back to the cell surface through the apical recycling pathway. When Rich1 is inactivated by AMOT, endocytosed proteins are directed for degradation instead. In turn, AMOT's activity is negatively regulated by the formation of AJs.

Regulation of Proliferation and Differentiation

The establishment of AJs and TJs in normal epithelia represses pathways for proliferation and promotes those for differentiation. (1) At high cell density, ZO1 sequesters ZONAB and CDK4 from the nucleus and shuttles them to TJs, preventing transcriptional control by ZONAB and entry into S phase triggered by CDK4. At low cell density, ZONAB localizes to the nucleus, complexes with symplekin, and regulates transcription. RalA also binds and regulates ZONAB in a manner similar to ZO1. (2) PTEN is recruited to both TJs and AJs through its interactions with MAGI proteins. At AJs, this interaction is stabilized by the interaction of MAGI with vinculin. At both junctions, PTEN's ability to suppress PI3K/Akt signaling is enhanced by binding to MAGI proteins, thereby repressing proliferation, preventing transcriptional repression of E-cadherin, and blocking events leading to loss of AB polarity. (3) When β-catenin is phosphorylated on specific tyrosine residues, it dissociates from E-cadherin and becomes part of a soluble cytoplasmic pool where it is targeted for degradation by the APC/Axin/GSK3β complex. Wnt signaling inhibits β-catenin degradation, which allows β-catenin to translocate to the nucleus where it binds TCF transcription factors and promotes the transcription of wnt target genes.

Pathologic Effects on Junction Components

Processes such as wound healing and tissue maintenance dictate that cells maintain dynamic control of junction assembly and disassembly. Many pathologic processes such as cancer and invasion of host cells by microbes affect these pathways. (1) Adenoviral E4-ORF1 directly binds and prevents TJ localization of PATJ and ZO2, stimulates the PI3K/Akt signaling pathway, and indirectly alters localization of Par3, PALS1, and ZO1, thus disrupting TJs and AB polarity. Similarly, the HPV-18 E6 oncogene sequesters PATJ and its paralog MPDZ/MUPP1 as well as hScrib/mDlg and targets them for degradation, contributing to loss of polarity. (2) The *Heliobacter pylori* protein cagA disrupts TJs and AB polarity. Upon entry into gastric epithelial cells, cagA is phosphorylated and activated by membrane-associated tyrosine kinases. It then interacts with Par1, preventing its phosphorylation by aPKC. Thus, Par1 remains pathologically associated with apical membranes where it interferes with proper membrane polarization. (3) Metabolic stressors such as starvation result in stimulation of AMPKα via LKB1. Activation of AMPKα preserves TJ assembly, possibly through MRLC, and prevents cell proliferation and protein synthesis through inhibition of the mTOR pathway. (4) Binding of TGFβ to TGFβR2 results in recruitment and activation of TGFβR1 at both TJs and AJs. At TJs, this interaction recruits Par6 and an E3 ubiquitin ligase, Smurf1, which ubiquitinates RhoA. RhoA's subsequent degradation results in disassembly of TJs and loss of AB polarity. At AJs, TGFR signaling recruits PI3K-Ras-GTPase complex, which results in the phosphorylation and disassembly of β- and α-catenin complexed with E-cadherin. Disassembly of AJs then ensues. (5) Activation of the oncogene ErbB2/HER2/Neu receptor tyrosine kinase by homodimerization at the basolateral membrane recruits Par6-aPKC without Par3, thereby disrupting AB polarity and preventing apoptosis. (6) The activation of various growth factor receptor tyrosine kinases results in recruitment and activation of an E3 ubiquitin ligase, Hakai, which ubiquitinates E-cadherin at the AJ and promotes its endocytosis and degradation, thus destabilizing AJs.

Abbreviations

α-ctn, α-catenin; AB polarity, apical-basal polarity; AJ, adherens junction; AMOT, angiomotin; AMPKα, AMP-activated protein kinase; Anx-2, annexin-2; APC, adenomatosis polyposis coli; aPKC, atypical protein kinase C; β-ctn, β-catenin; βTrCP, beta-transducin repeat-containing protein; CD2AP, CD2-associated protein; CDK4, cyclin-dependent kinase 4; CKI, casein kinase I; c-Met, hepatocyte growth factor receptor; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GSK3β, glycogen synthase kinase 3-beta; hScrib, human homolog of scribble; JAM, junctional adhesion molecule; JRAB, junctional Rab13-binding protein; LEF/TCF, lymphoid enhancer factor-1/T cell factor-1; LIMK2, LIM domain kinase; MAGI,

(*continued on next page*)

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membrane-associated guanylate kinase protein; MAPs, microtubule-associated proteins; mDia1, mammalian homolog of *Drosophila* diaphanous 1; mDlg/SAP97, mammalian homolog of discs large; mLgl, mammalian homolog of lethal giant larvae; MPDZ/MUPP1, multiple PDZ domain protein; MRLC, regulatory light chain of nonmuscle myosin II; mTOR, mammalian target of rapamycin; N-WASP, neuronal Wiskott-Aldrich syndrome protein; p120/p120^{ctn}, p120 catenin; mLin-2, mammalian homolog of Lin-2; PATJ, PALS1-associated tight junction protein; PDGFR, platelet-derived growth factor receptor; PDK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol (3,4) bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A; PKC, calcium-dependent protein kinase C; PPase, generic protein phosphatase; PTEN, phosphatase and tensin homolog; PTP, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatase 1B; RTK, generic receptor tyrosine kinase; TGFβ, transforming growth factor β; TGFR; TGFβ receptor; TJ, tight junction; Tsc, tuberous sclerosis protein; Ub, ubiquitin; VASP, vasodilator-stimulated phosphoprotein; ZONAB, ZO1–associated nucleic acid binding protein.

REFERENCES

Brembeck, F.H., Rosário, M., and Birchmeier, W. (2006). Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. Curr. Opin. Genet. Dev. *16*, 51–59.

Forcet, C., and Billaud, M. (2007). Dialogue between LKB1 and AMPK: A hot topic at the cellular pole. Sci. STKE *2007*, pe51.

Hartsock, A., and Nelson, W.J. (2008). Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim. Biophys. Acta *1778*, 660–669.

Köhler, K., and Zahraoui, A. (2005). Tight junction: A co-ordinator of cell signalling and membrane trafficking. Biol. Cell *97*, 659–665.

Martin-Belmonte, F., and Mostov, K. (2008). Regulation of cell polarity during epithelial morphogenesis. Curr. Opin. Cell Biol. *20*, 227–234.

Mège, R.M., Gavard, J., and Lambert, M. (2006). Regulation of cell-cell junctions by the cytoskeleton. Curr. Opin. Cell Biol. *18*, 541–548.

Perez-Moreno, M., and Fuchs, E. (2006). Catenins: Keeping cells from getting their signals crossed. Dev. Cell *11*, 601–612.

Sakisaka, T., Ikeda, W., Ogita, H., Fujita, N., and Takai, Y. (2007). The roles of nectins in cell adhesions: Cooperation with other cell adhesion molecules and growth factor receptors. Curr. Opin. Cell Biol. *19*, 593–602.

Shin, K., Fogg, V.C., and Margolis, B. (2006). Tight junctions and cell polarity. Annu. Rev. Cell Dev. Biol. *22*, 207–235.

Yap, A.S., Crampton, M.S., and Hardin, J. (2007). Making and breaking contacts: The cellular biology of cadherin regulation. Curr. Opin. Cell Biol. *19*, 508–514.

  DOI 10.1016/j.cell.2008.06.002