

MAGI-1 Is Required for Rap1 Activation upon Cell-Cell Contact and for Enhancement of Vascular Endothelial Cadherin-mediated Cell Adhesion

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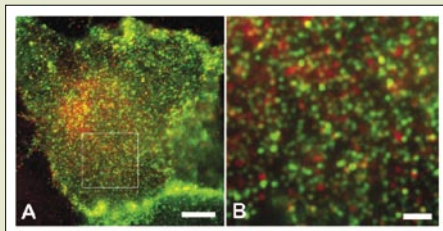
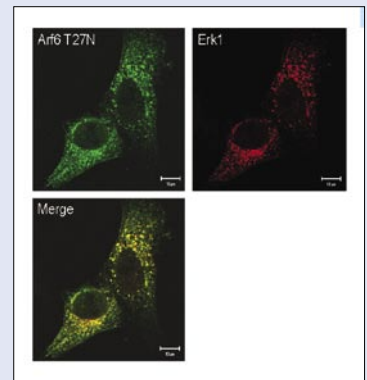
Intercellular adhesion between vascular endothelial cells must be sufficiently strong to serve as a barrier separating blood from tissue, yet dynamically regulated to allow for leukocyte transepithelial migration. Here it is established that the Ras-family GTPase Rap1 functions in a positive feedback loop to tighten VE-cadherin-mediated cell adhesion. Raichu-Rap1, a chimera consisting of yellow fluorescent protein (YFP)-Rap1 and the Ras-binding domain of Raf fused to cyan fluorescent protein (CFP), was used as a reporter molecule to localize Rap1 activation. In its GTP-bound, activated state Raf binds intramolecularly to Rap1, inducing fluorescence resonance energy transfer between the N- and C-terminal YFP and CFP moieties. Rap1 activation was shown to occur at sites of cell-cell

contact in a Ca^{2+} - and VE-cadherin-dependent manner. Recruitment and activation of Rap1 required the scaffolding molecule MAGI-1, which binds to both VE-cadherin through β -catenin and to a Rap1-guanine nucleotide exchange factor, PDZ-GEF1. Activated Rap1 induces

Erk Signaling Regulates Clathrin-independent Endosomal Trafficking

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The extracellular signal-regulated kinases, Erk 1 and 2, are among the most abundant kinases. Erk is recruited to and activated at diverse subcellular locations, including the Golgi, late endosomes, focal adhesions, and the leading edge, by a growing number of distinct scaffold molecules. In addition to its well-established roles in cell proliferation and survival, when activated at these distinct subcellular locations Erk functions in cell adhesion, migration, Golgi fragmentation, and phagocytosis. This paper demonstrates a role for Erk at the Arf6 tubular endosome. Internalization and recycling through the clathrin-independent, Arf6-dependent endocytic pathway has also been implicated in a wide variety of cellular functions, including adhesion, migration, phagocytosis, and immune surveillance. Here it is shown that the scaffold molecule KSR1 targets Erk and MEK to Arf6 tubular endosomes and that Arf6 activity is required for Erk activation by EGF. These data suggest a possible link between trafficking through this still enigmatic pathway, the regulation of Erk activation, and their function in diverse cellular processes.



Syntaxins 3 and 4 Are Concentrated in Separate Clusters on the Plasma Membrane Prior to the Establishment of Cell Polarity

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SNARE proteins are critical components of the vesicular trafficking machinery because they function both in establishing the specificity of vesicle targeting and in mediating membrane fusion. Here it is shown that the t-SNARE proteins syntaxin 3 and 4, which direct vesicular targeting and fusion to the apical and basolateral surfaces of polarized epithelial cells, respectively, exist in mutually exclusive, uniform clusters on the plasma membrane even in

non-polarized cells. Interestingly, the mechanisms of clustering are also distinct: The formation and/or maintenance of syntaxin 3 clusters require an of t-SNAREs may be necessary for their function, perhaps to ensure correct and specific localization of fusion events or to enhance the efficiency of membrane fusion after docking.

Unusual Kinetic and Structural Properties Control Rapid Assembly and Turnover of Actin in the Parasite *Toxoplasma gondii*

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Toxoplasma gondii and other obligate intracellular parasites of the phylum Apicomplexa exhibit an unusual form of motility, called gliding, which they use to actively penetrate and invade host cells. Actin filament polymerization mediates the movement and coordinates its directionality, yet little is known about how actin assembly/disassembly is regulated in these parasites, and paradoxically, actin filaments are rarely detected. This paradox is partly explained by the in vitro assembly properties of purified *Toxoplasma* actin (TgACT1). The authors show that TgACT1 is adapted for rapid cycles of assembly and disassembly. It assembles at concentrations 3- to 4-fold lower than conventional actin but forms only short, unstable filaments that are, on average, 10–20 times shorter than filaments formed from conventional actin. Structural modeling of TgACT1, which is 83% identical to vertebrate actin, reveals conserved sequence changes in residues that stabilize actin filaments. The unique biochemical properties of parasite actins may render them useful targets of therapeutic intervention. ■

