Cdk5
A regulator of epithelial cell adhesion and migration

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Cell adhesion is a fundamental property of epithelial cells required for anchoring, migration and survival. During cell migration, the formation and disruption of adhesion sites is stringently regulated by integration of multiple, sequential signals acting in distinct regions of the cell. Recent findings implicate cyclin dependent kinase 5 (Cdk5) in the signaling pathways that regulate cell adhesion and migration of a variety of cell types. Experiments with epithelial cell lines indicate that Cdk5 activity exerts its effects by limiting Src activity in regions where Rho activity is required for stress fiber contraction and by phosphorylating the talin head to stabilize nascent focal adhesions. Both pathways regulate cell migration by increasing adhesive strength.

Anchoring of epithelial cells to their basement membrane is essential to maintain their morphology, normal physiological function and survival. Cells attach to extracellular matrix components by means of membrane-spanning integrins, which cluster and link to the actin cytoskeleton via components of focal adhesions. At focal adhesions, actin is bundled into stress fibers, multi-protein cellular contractile machines that strengthen attachment and provide traction during migration. Stress fiber contraction is generated by myosin II, a hexamer containing one pair of each non-muscle heavy chains (NMHCs), essential light chains, and myosin regulatory light chains (MRLC). Myosin motor activity is regulated by phosphorylation of MRLC at Thr18/Ser19 and is required to generate tension on actin filaments and to maintain stress fibers. Although a number of kinases have been identified which phosphorylate MRLC at Thr18/Ser19, the principal kinases in most cells are myosin light chain kinase (MLCK) and Rho-kinase (ROCK), a downstream effector of the small GTPases, RhoA.

Rho family small GTPases play a central role in regulating many aspects of cytoskeletal organization and contraction. These GTPases are subject to both positive regulation by guanine nucleotide exchange factors (GEFs), such as GEF-H1, and negative regulation by GTPase-activating proteins (GAPs), such as p190RhoGAP. As cells spread, the Rho-family GTPase, Cdc42, is activated at the cell periphery, leading to the formation of numerous filopodia. Focal adhesion formation is first seen at the tips of these filopodia as focal adhesion proteins such as talin and focal adhesion kinase (FAK) bind to the intracellular domains of localized integrins. Src is recruited to activated FAK at the nascent focal adhesion and generates binding sites for additional focal adhesion proteins by phosphorylating FAK and paxillin. Src activity is essential for the further maturation of the focal adhesion and for activating the Rho-family GTPase, Rac, leading to Arp2/3-dependent actin polymerization, formation of a lamellipodium and extension of the cell boundary. Simultaneously, Src inhibits RhoA by phosphorylating and activating its upstream inhibitor, p190RhoGAP. As the focal adhesion matures, Src is deactivated,
allowing the Rho activation necessary for mDia-dependent actin polymerization, myosin-dependent cytoskeletal contraction and tight adhesion to the extracellular matrix. Since new focal adhesions continually form at the distal boundary of the spreading cell, the most mature and highly contracted stress fibers are localized at the center of the cell.

Cell adhesion is an essential component of cell migration: if adhesion is too weak, cells cannot generate the traction necessary for migration; if it is too strong, they are unable to overcome the forces that anchor them in place. Thus, the relationship between adhesion force and migration rate is a bell-shaped curve. Migration rate increases as adhesive strength increases until an optimum value is reached. Thereafter, increases in adhesive strength decrease migration rate. Since the strength of adhesion depends on extracellular matrix composition as well as the types of integrin expressed in the cell, a decrease in adhesive strength may result in either faster or slower cell migration.

Several lines of evidence indicate that the proline-directed serine/threonine kinase cyclin dependent kinase 5 (Cdk5) plays an integral role in regulating cell adhesion and/or migration in epithelial cells. Cdk5 is an atypical member of cyclin dependent kinase family, which is activated by the non-cyclin proteins, p35 or p39. Cdk5 is most abundant in neuronal cells where it also regulates migration and cytoskeletal dynamics. In neurons, Cdk5 exerts its effects on migration at least in part by phosphorylating FAK, and the LIS1 associated protein, NDEL1. In contrast, recent findings have revealed two novel pathways involved in Cdk5-dependent regulation of migration in epithelial cells.

One of these newly discovered mechanisms links Cdk5 activation to control of stress fiber contraction. We have found that Cdk5 and its activator, p35, co-localize with phosphorylated myosin regulatory light chain (MRLC) on centrally located stress fibers in spreading cells. Moreover, Cdk5 is strongly activated in spreading cells as central stress fiber contraction becomes pronounced. Since contraction of these central stress fibers is primarily responsible for tight attachment between the cell and the extracellular matrix, the above findings suggested that Cdk5 might regulate cell adhesion by regulating MRLC phosphorylation. To test this possibility we inhibited Cdk5 activity by several independent means and found that MRLC phosphorylation was likewise inhibited. In addition, we found that inhibiting Cdk5 either prevented the formation of central stress fibers or led to their dissolution. The concave cell boundaries characteristic of contracting cells were also lost. Since MRLC lacks a favorable site for phosphorylation by Cdk5, we asked whether Cdk5 might affect the upstream signaling pathways that regulate MRLC phosphorylation. Experiments with specific pathway inhibitors indicated that the MRLC phosphorylation involved in stress fiber contraction in lens epithelial cells was regulated largely by Rho-kinase (ROCK). Inhibiting Cdk5 activity not only significantly reduced ROCK activity, but also blocked activation of its upstream regulator, Rho. To explore the mechanism behind the Cdk5-dependent regulation of Rho, we turned our attention to p190RhoGAP, which appears to play a major role in regulating Rho-dependent stress fiber contraction. This RhoGAP must be phosphorylated by Src to be active; as a result, Rho activity is low in the early stages of cell spreading, when Src activity is high. At later times, Src activity falls, p190RhoGAP activity is lost, and Rho-GTP is formed, enabling Rho-dependent myosin phosphorylation and stress fiber contraction. We have found that inhibiting Cdk5 activity during this later stage of cell spreading increases Src activity and Src-dependent phosphorylation of its substrate, p190RhoGAP. This in turn leads to decreased Rho activity accompanied by loss of Rho-dependent myosin phosphorylation, dissolution of central stress fibers, and loss of cell attachment.
phosphorylates the focal adhesion protein talin. The talin phosphorylation site has been identified as S425, near the FERM domain in the talin head region. Upon focal adhesion disassembly, this region is separated from the talin rod domain by calpain-dependent cleavage. Phosphorylation at S425 by Cdk5 blocks ubiquitylation and degradation of the talin head by inhibiting interaction with the E3 ligase, Smurf1. This leads, ultimately, to greater stability of lamellipodia and newly formed focal adhesions, thus strengthening adhesion to the substrate. Although the exact molecular events involved in this stabilization are not yet clear, it has been suggested that the Cdk5/p35 kinase, which regulates cell adhesion and migration in two distinct ways. Cdk5-dependent phosphorylation of the talin head domain at Ser425 prevents its ubiquitylation and degradation, allowing it to persist following calpain cleavage. The phosphorylated talin head may then bind to integrin at peripheral sites and recruit PIP- K, which converts P(4)P to P(4,5)P2. P(4,5)P2 may promote replacement of the talin head by full length talin. Full length talin recruits other focal adhesion proteins to form the mature focal adhesion. The talin tail provides the site for the actin binding and polymerization. Polymerized actin is subsequently bundled into stress fibers. Cdk5/p35 also regulates the Rho-dependent myosin phosphorylation necessary for stress fiber stability and cytoskeletal contraction by limiting Src activity. This in turn decreases Src-dependent phosphorylation of p190RhoGAP, favoring Rho-GTP formation, Rho-dependent stress fiber polymerization, stabilization and contraction. Both pathways modulate cell migration by increasing adhesive strength.
talin head may “prime” integrins to bind full length talin. One possible scenario describing how this might occur is shown in Figure 2. By permitting the isolated head region to escape degradation following calpain cleavage, Cdk5-dependent phosphorylation may stabilize a pool of talin head domains to bind focal contacts within the lamellipodium. It is known that the isolated talin head region can bind and activate integrins during cell protrusion. The resulting integrin activation would be expected to stabilize the lamellipodium by strengthening integrin-dependent adhesion. Since the head domain lacks sites for actin binding, which are located in the talin rod domain, the bound head domain would have to be replaced by full length talin to enable focal adhesion attachment to the cytoskeleton. The head domain might promote this replacement by recruiting the PIP-kinase needed to generate P(4,5)P₂, which facilitates binding of full length talin to integrin by exposing the auto-inhibited integrin binding site. The binding of full length talin and the resulting link between the integrins and the actin cytoskeleton would then further strengthen adhesion. This model predicts that full length talin would bind poorly in the absence of Cdk5 activity, due to degradation of the talin head and the resulting limited availability of P(4,5)P₂, and thus provides a possible explanation for the observed rapid turnover of peripheral focal adhesions. Clearly, other models may be proposed to explain the increase in adhesion produced by talin head phosphorylation, and deciding among them will be an active area for future investigation. Nonetheless, it is now certain that talin is a key substrate for Cdk5 at focal adhesions.

In summary, the presently available data indicate that Cdk5 has at least two distinct functions in cell adhesion (Fig. 2). On the one hand, it stabilizes peripheral focal adhesions and promotes their attachment to the cytoskeleton by phosphorylating the talin head. On the other hand, once the actin cytoskeleton has been organized into stress fibers, Cdk5 enhances the Rho activation essential for stability and contraction of central stress fibers by limiting Src activity. The discovery that Cdk5 is involved in two separate events required for efficient migration, suggests that it may coordinate multiple signaling pathways. The known involvement of Cdk5 and its activator, p35, in regulating microtubule stability suggests yet another mechanism by which Cdk5 activity may regulate cytoskeletal function. Microtubules are closely associated with stress fibers and their depolymerization has been shown to release the Rho activating protein, GEF-H1, leading to Rho activation and Rho-dependent myosin contraction. Since cell adhesion and migration play an important role in the progression of many pathological conditions, Cdk5, its substrates and its downstream effectors involved in cell adhesion may provide novel targets for therapeutic intervention.

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