Frontiers in Bioscience 14, 510-522, January 1, 2009]

Phosphatidylinositol 3-kinase: a key regulator in adherens junction formation and function

Nathalie Rivard

Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, University of Sherbrooke, 3001, 12th north avenue, Sherbrooke,(Quebec), Canada, JIH 5N4

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1. ABSTRACT

The activity of E-cadherin-adhesion complexes is under stringent control of signaling pathways. Conversely, these adhesion complexes are preferential sites for signal transduction. One class of signaling molecules reported to regulate adherens junction and to be activated by adherens junction assembly are phosphatidylinositol 3-kinases. While the exact molecular mechanisms involved are not clear, present data indicate that one of the earliest events likely involves c-Src which is rapidly activated by E-cadherin-mediated cellular aggregation and may facilitate the recruitment and activation of PI3K to E-cadherin-containing complexes. Beta-catenin, gamma-catenin and hDlg which are present at cell-cell adhesions can act as docking proteins for PI3K. Hence, cell-cell interaction leads to PtdIns(3,4,5)P_3 production in nascent cadherin contacts triggering the recruitment of proteins containing pleckstrin homology domains including the kinase PKB/Akt and the exchange factors for Rac, Tiam and Vav. PKB/Akt may be involved in the regulation of survival and proliferation while Tiam and Vav may activate Rac, resulting in reorganization of actin cytoskeleton which ultimately serves to mediate adhesive cell-cell recognition as well as epithelial cell differentiation and polarity.

2. INTRODUCTION

In epithelial tissues, the cells are tightly bound together into sheets called epithelia. These epithelia form a boundary between discrete compartments within an organism whilst also constituting the interface between the organism and the environment. The development and maintenance of epithelia require selective cell-cell and cell-extracellular matrix adhesion (reviewed in 1) which must be strong enough to maintain tissue architecture yet be sufficiently dynamic to allow essential processes such as morphogenesis or cell division to occur (reviewed in 2).

Cell adhesion is achieved by a superfamily of transmembrane glycoproteins collectively known as cell adhesion molecules and found in a diverse range of cellular junctions (reviewed in 2). A subfamily known as cadherins mediate homotypic Ca^{2+}-dependent intercellular adhesion in the majority of cell types (reviewed in 3-5) and are structurally characterized by conserved 110-amino-acid repeat sequences (cadherin repeats) present within the extracellular domain (Figure 1) (3, 4). Cadherins can be divided into four subclasses: classical cadherins, responsible for cell-cell adhesion at the zonula adherens; desmosomal cadherins, responsible for cell-cell adhesion at

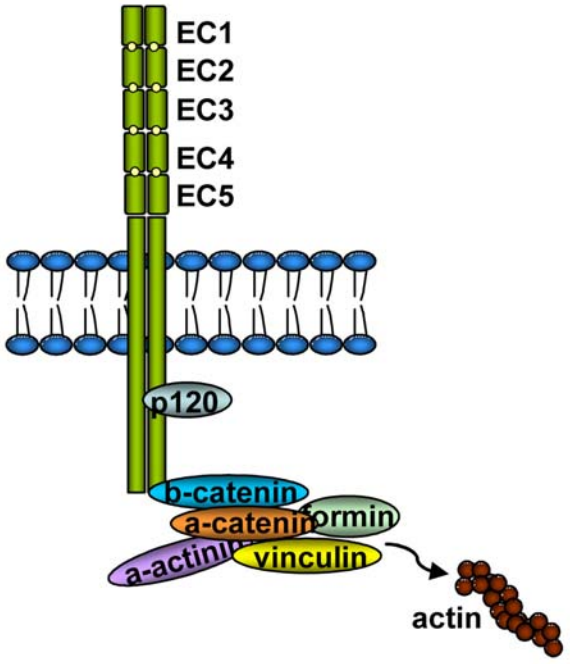

Figure 1. The classic cadherin-catenin complex. The cadherin is a parallel or cis homodimer. The extracellular region of E-cadherin consists of five cadherin-type repeats (EC domains; extracellular cadherin domains) that are bound together by Ca^{2+} ions. The core-universal catenin complex consists of p120 catenin and beta-catenin which in turns binds alpha-catenin. In a less understood way, alpha-catenin binds to actin and actin-binding proteins, such as vinculin, alpha-actinin and formin-1 (32).

desmosomal junctions; and protocadherins and cadherin-related proteins whose functions are not fully elucidated (3). Individual cadherins are differentially expressed during embryonic development and mediate intercellular interactions in distinct cell types. E-cadherin is the prototypic member of the subfamily of classical cadherins and is expressed in most epithelial tissues (6). Without diminishing the importance of other cellular junctions, E-cadherin-mediated adherens junctions are particularly important in controlling the specificity, formation and maintenance of intercellular adhesion in epithelial tissues (7-11).

3. ADHERENS JUNCTIONS

Conceptually, the life of a cadherin-mediated adherens junction can be separated into three stages: formation, maintenance and disassembly (12). Adherens junctions are assembled at cell-cell contacts. During the early stages of intercellular adhesion, cells extend filopodial/lamellipodial extensions which enhance cell-cell contacts (13, 14). Such membrane protrusions involve the actin cytoskeleton and Rho GTPases including Rho, Rac and Cdc42 (15-17). Specifically, Rac has been shown to be required for efficient recruitment of F-actin to the adherens junctions in epithelial cells (18-20). Cell surface proteins such as nectins and JAMs may be involved in initial contacts by facilitating early transient cadherin interaction and bringing membranes into apposition and/or forming signaling complexes (21-23). Cadherin-associated proteins are subsequently recruited to these nascent contacts. E-cadherin binds directly to several cytoplasmic proteins including beta-catenin and p120 (24). p120 may indirectly regulate the cadherin-actin cytoskeleton nexus by locally controlling the activity of the Rho inhibitor p190RhoGAP, hence the activation of Rho and Rac (25), as well as the rate of cadherin endocytosis (26). Beta-catenin plays a more direct role by binding to alpha-catenin (27), an actin filament binding/bundling protein that also binds other actin-binding proteins (Figure 1) (28). Clustering of adhesion molecules and recruitment of these intracellular components connecting the junctional complex to the actin cytoskeleton induce further maturation of the junction and subsequent formation of tight junctions (12, 29). However, the current view that actin filaments bind directly to alpha-catenin and therefore to E-cadherin-catenin complex was recently re-questioned (30-31). Indeed, it has been shown that alpha-catenin assembled into the cadherin-catenin complex does not bind to actin (30-31). However, when alpha-catenin dimerizes, alpha-catenin homodimers are released from the cadherin-catenin complexes and bind to actin and antagonize Arp2/3 function, inhibiting actin branching and facilitating formation of the belt of unbranched actin filaments. Alpha-catenin then acts as a molecular switch to regulate actin dynamics at adherens junctions. Alternative models could also explain the association between actin and adherens junctions. One possibility is that other molecules mediate a direct connection. One attractive candidate is the nectin-afadin system; another possibility is that the link is mediated by many weak and transient interactions (which cannot be detected biochemically) between actin binding proteins (such as zonula occludens-1, spectrin, vinculin, afadin and alpha-actinin) and adherens junction components (reviewed in 32).

The activity of E-cadherin-adhesion complexes is under stringent control of signaling pathways, many of which are dysregulated during neoplastic progression. Conversely, it is now well accepted that these adhesion complexes are also preferential sites for signal transduction. Hence, not only may signaling events regulate E-cadherin adhesive function, but cadherins themselves also participate in transducing extracellular signals to the interior. One class of signaling molecules reported to regulate adherens junction formation and to be activated by adherens junction assembly are phosphatidylinositol 3-kinases.

4. PHOSPHATIDYLINOSITOL 3-KINASES

There are three classes of phosphatidylinositol 3-kinases (PI3Ks) in mammals based on primary structure, substrate specificity and mode of regulation. Class I PI3Ks include four distinct p110 catalytic isoforms, further divided into Class IA (-alpha, -beta, -delta) and IB (-gamma); among these, p110alpha and p110beta are the isoforms mostly expressed in epithelia (reviewed in 33). *In vitro*, they are capable to convert phosphatidylinositides (PtdIns) to PtdIns-3-*P*, PtdIns-4-*P* to PtdIns(3,4)P_2, and

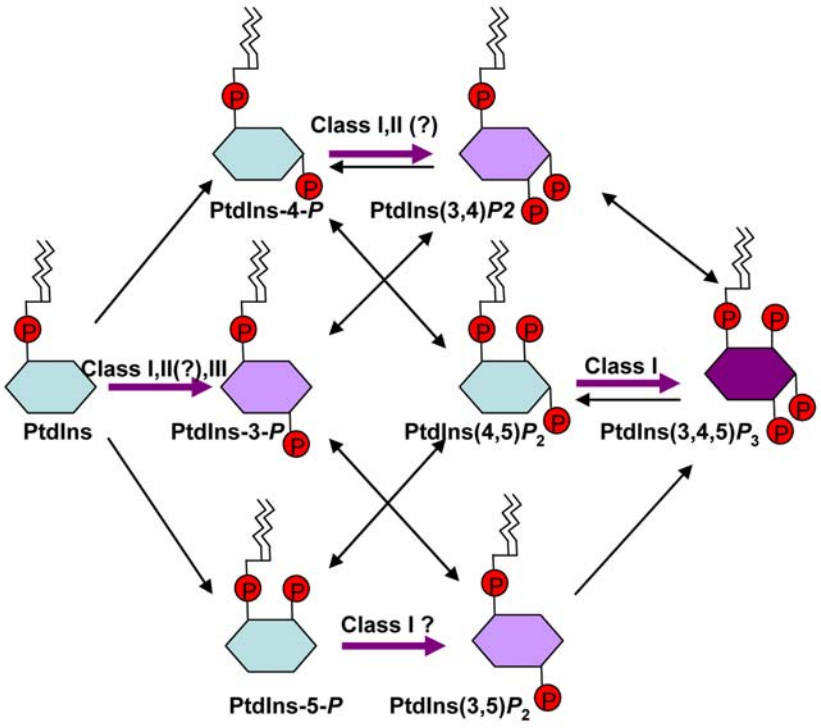

Figure 2. Biochemical activities of PI3K. The substrates (in blue) and catalytic products of class I, II and III (in purple) are indicated. The most important phosphorylation (indicated in dark purple) is of PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3 which can serve as a docking site for signaling proteins with PH domains. Other inositol phospholipid kinases (and phosphatases) catalyze the reactions indicated by the black arrows.

PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3, but the *in vivo* substrate is PtdIns(4,5)P_2 (Figure 2). Class I PI3Ks primarily generate PtdIns(3,4,5)P_3, which binds to pleckstrin homology (PH) domains and activates a variety of proteins including serine/threonine kinase PKB/Akt and exchange factors for small GTPases such as Tiam and Vav. Class IA PI3Ks are comprised of an 85kDa regulatory subunit (p85) and a 110kDa catalytic subunit (p110) (34, 35) which is activated in a phosphotyrosine-dependent manner via Src homology domain 2 (SH2 domain)-mediated recruitment to tyrosine kinase receptors or docking proteins. SH2 domains mapped within the p85 subunit specifically recognize the phosphotyrosine binding motif pY*XX*M (36). Class II PI3Ks have the potential to generate PtdIns(3,4,5)P_3, although the type of PtdInsPs that they produce *in vivo* has not been fully characterized. *In vitro*, they are capable to phosphorylate PtdIns and PtdIns-4-*P* (Figure 2). The sole class III PI3K, Vps34, produces PtdIns-3-*P* (Figure 2). Class I PI3Ks as well as 3-phosphosphorylated PtdIns have been implicated in a number of signaling pathways in response to extracellular stimuli which in turn regulate diverse cellular programs such as cell survival, proliferation/migration, phagocytosis and glucose homeostasis (37). Class I PI3Ks have also been implicated in cancer: for example PTEN phosphatase removes the 3-phosphate from 3-phosphorylated PtdIns generated by PI3Ks and has been identified as a tumor suppressor gene mutated in a variety of human cancers (38). In addition, mutation, amplification and/or overexpression of some PI3K Class I catalytic subunits have been observed in some carcinomas (37, 39). Furthermore, Class I PI3Ks are activated by a plethora of stimuli (36), one of which is adherens junction formation itself. Although the Class I PI3K family has been implicated in multiple cellular processes (40-42), much less is known as to the function of Class II and Class III PI3K enzymes (41, 42). In the ensuing text, PI3K will refer specifically to Class IA PI3K.

5. REGULATION OF E-CADHERIN-MEDIATED CELL-CELL CONTACTS BY PI3K

The first evidence suggesting the involvement of PI3K in adherens junction regulation in mammalian cells was reported by Hordijk and colleagues (43) who demonstrated that Tiam1 is localized at sites of cell-cell contacts in MDCK renal epithelial cells. Tiam1 is an exchange factor for Rac that requires membrane localization via its NH2-terminal PH domain interacting with PtdIns(3,4,5)P_3 in order to activate endogenous Rac (44, 45). Overexpression of Tiam1 inhibits hepatocyte growth factor-induced cell scattering by increasing E-cadherin-mediated cell-cell adhesion. Furthermore, Tiam1/Rac signaling inhibits motile and invasive behavior of Ras-transformed MDCK cells due to restoration of E-cadherin-mediated adhesion (43). Sander and colleagues (46) subsequently confirmed that Tiam1-mediated Rac1 activation and E-cadherin-mediated cell-cell adhesion are both dependent on PI3K activity, by using pharmacological

inhibitors of PI3Ks, namely LY294002 and wortmannin, and the dominant-negative mutant deltap85 which carries a deletion in the binding site for the p110 catalytic subunit. Other studies further demonstrated that Rac1 is recruited at intercellular junctions during establishment of cadherin-mediated adhesion and is activated by E-cadherin-mediated homophilic interactions through PI3K (20, 47). Specifically, upon formation of nascent contacts, Rac1 becomes activated and is recruited to these sites in a PI3K-dependent manner (44-49). This in turn leads to the recruitment and activation of Arp2/3 at cadherin-mediated contacts, promoting the formation of actin branched networks (47). On the other hand, Rac1 contributes to the stable formation of nascent contacts through its effector IQGAP. By inhibiting IQGAP, Rac1 facilitates the interaction of alpha-catenin with beta-catenin (17). In Xenopus, IQGAP localizes at adherens junctions in regions undergoing active morphogenetic movements (50). Thus, Rac1 may regulate the balance between a dynamic to a more static state. In intestinal epithelial cells, inhibition of PI3K was also found to alter adherens junction integrity by reducing the amount of cytoskeletal-associated E-cadherin and beta-catenin at the site of cell-cell contact (51). Therefore, there appears to be a signaling hierarchy leading from PI3K to Tiam1 to Rac to the actin cytoskeleton resulting in adherens junction formation. However, the role of Tiam1 in the activation of Rac1 by E-cadherin-mediated cell-cell adhesion was recently re-questioned (52, 53). Vav2, another Rac-GDP/GTP exchange factor that localizes to the membrane via its NH2-terminal PH domain interacting with PtdIns(3,4,5)P_3, has recently been shown to be necessary for PI3K-dependent activation of Rac1 during adherens junction formation (53). Interestingly, Vav2 is also recruited to E-cadherin-based cell-cell adhesion sites where it is tyrosine phosphorylated and activated by c-Src (53).

Recent evidences suggest that the recruitment and activation of PI3K and Rac1 upon E-cadherin engagement are, however, transient. Indeed, it has been reported that Rac1 tends to localize at the dynamic, expanding edges of a forming contact, but not when contacts had already formed and the membranes had become more quiescent (54). Moreover, direct analysis of subcellular sites of Rac1 activation between contacting MDCK using the Raichu FRET-based biosensors (55, 56) revealed that Rac1 activity is high in a restricted zone at the edges of expanding cell-cell contacts but is very low within the established cell-cell contact where E-cadherin has accumulated. More recently, a study from Nelson's laboratory describes the spatiotemporal immediate-early signaling induced by E-cadherin engagement and adhesion (57). By using laser tweezers to place beads coated with functional E-cadherin extracellular domain on cells, they quantified the kinetics of E-cadherin, PI3K and Rac1 distributions immediately following E-cadherin engagement. They found that cellular E-cadherin accumulates rapidly around beads and phosphoinositides and Rac1 co-accumulate with E-cadherin, reach peak levels with E-cadherin but then rapidly disperse. E-cadherin accumulation was dependent on membrane dynamics and actin polymerization, but actin did not stably co-accumulate with E-cadherin. Thus, the recruitment of PI3K and Rac1 upon E-cadherin engagement appears to be transient. They propose a model in which initial E-cadherin accumulation requires active membrane dynamics and involves diffusion-mediated trapping at contact sites; to propagate further contacts, PI3K and Rac1 are transiently activated by E-cadherin engagement and initiate a new round of membrane dynamics, but they are subsequently suppressed at that site to allow maintenance and strengthening of weak E-cadherin-mediated adhesion (57).

6. RECRUITMENT AND ACTIVATION OF PI3K SIGNALING BY E-CADHERIN-MEDIATED CELL-CELL CONTACTS

Pece and colleagues (58) were the first to assess whether adherens junction assembly can affect the state of activation of PI3K signaling. As epithelial cells require Ca^{2+} in order to form homophilic cell-cell adhesions, a simple method to study the adhesive properties of surface molecules involves the disruption of intercellular Ca^{2+}-dependent homotypic boundaries by EGTA-treatment and the re-establishment of cell-cell contacts by the subsequent restoration of Ca^{2+} ions (so-called Ca^{2+}-switch) (59). By using Ca^{2+}-switch experiments, Pece and colleagues demonstrated that engagement of E-cadherins in homophilic adhesion with neighboring cells promotes a substantial activation of PI3K-dependent PKB/Akt and the rapid translocation of PKB/Akt to the nucleus (58). Such stimulation of PI3K by E-cadherin-mediated cell-cell contact has also been reported in other epithelial cell types (51, 60). Furthermore, a physical association between PI3K and E-cadherin-containing multiprotein complexes is observed in response to cell-cell contact formation, thus providing a likely mechanism for PKB/Akt activation (51, 61, 62). However, the interaction between E-cadherin and p85/PI3K is indirect and may involve intermediate proteins (61, 62).

The molecular mechanisms by which PI3K is recruited and activated to E-cadherin-mediated cell-cell contacts have been studied to some extent, although the exact sequence of molecular events involved in this process is still not clear. As in many other models of PI3K signaling, recruitment of PI3K to the plasma membrane is a key step in the cadherin-activated signaling pathway. In many instances of receptor-activated PI3K signaling, binding of p85 is in itself a response to upstream tyrosine kinase (reviewed in 63). Recruitment commonly entails high affinity interactions between SH2 domains and tyrosine-phosphorylated sequences in components of the receptor complex (64, 65). Many tyrosine kinases are known to concentrate at cadherin-adhesive contacts (66-68) while c-src has been shown to co-recruit to cadherin homophilic contacts and to be critical for the recruitment and activation of PI3K to E-cadherin (69, 70).

Three cadherin-associated proteins have been reported to bind the p85 subunit of PI3K at E-cadherin-mediated cell-cell contacts (Figure 3), namely beta-catenin (61, 71, 72), gamma-catenin (73) and hDlg (62). Beta-catenin has been shown to directly interact with the N-

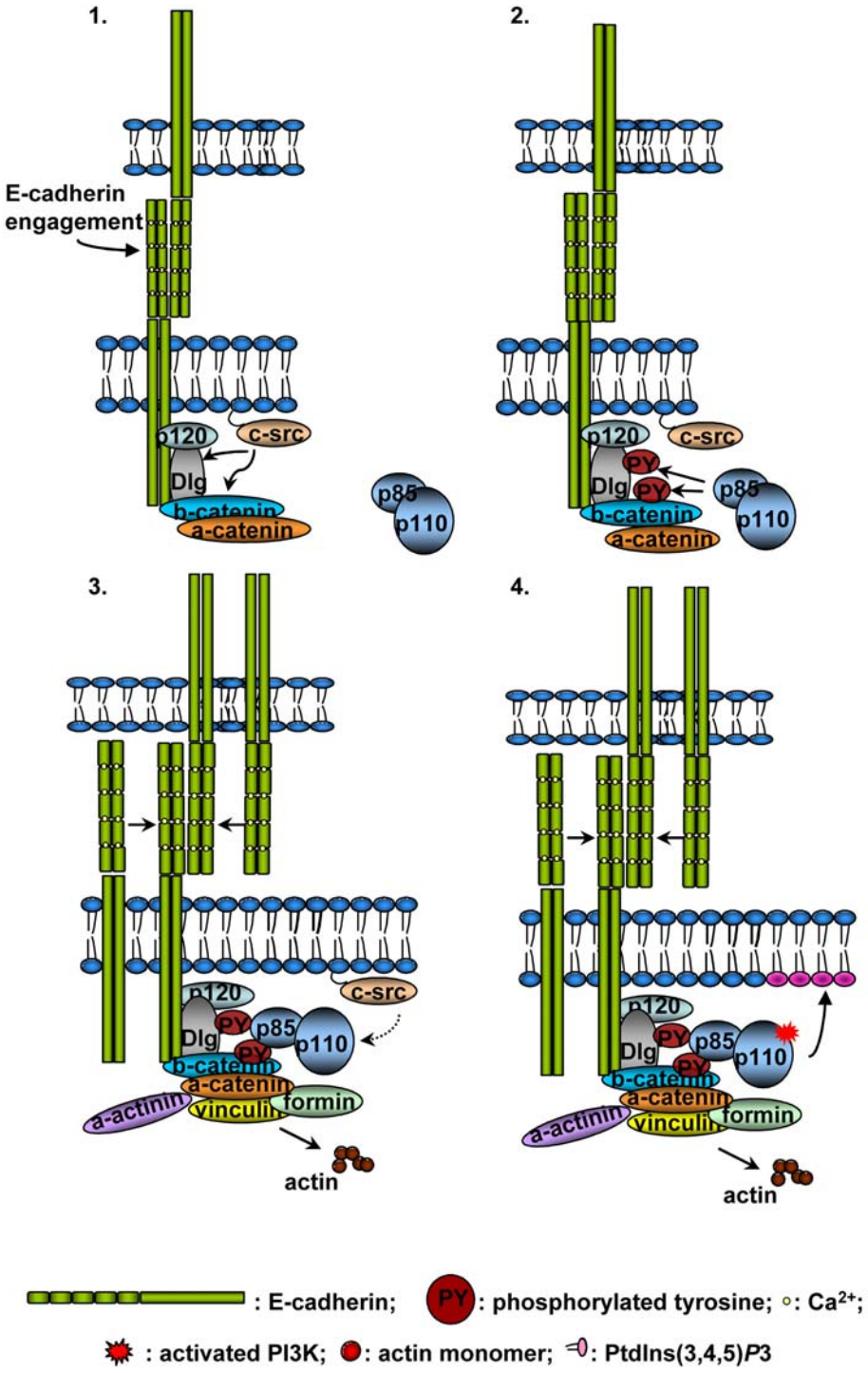

Figure 3. Model of how PI3K is activated by E-cadherin adhesion complex. 1. One of the earliest events likely involves c-src which is rapidly activated by E-cadherin-mediated cellular aggregation and may facilitate the recruitment of PI3K to E-cadherin-containing complexes. 2. Whether this reflects a role of c-src in regulating binding of PI3K to the cadherin-catenin complex and/or catalytic activation of PI3K by c-src remains to be determined. PI3K may not be the only target for cadherin-activated c-src signaling as other potential c-src substrates such as beta-catenin, gamma-catenin (not shown) and hDlg are found at cell-cell adhesions. 3. Once tyrosine phosphorylated, some of these proteins might provide binding sites for PI3K. 4. Once recruited, PI3K becomes activated and leads to PtdIns(3,4,5)P_3 production at the lateral membrane. Phosphoinositides and E-cadherin co-accumulate rapidly at the site of cell-cell contact (57).

terminal SH2 domain of p85 in the cadherin-based adhesion complex of human keratinocytes and MDCK epithelial cells. However, this interaction does not require tyrosine phosphorylation (61) and thus the mechanism by which beta-catenin interacts with p85 needs to be clarified.

Gamma-catenin may also represent an additional docking protein for PI3K within E-cadherin complexes. This protein is known to co-precipitate with the p110alpha catalytic subunit in keratinocytes and demonstrates prominent reactivity to an antibody recognizing phosphorylated Y*XX*M motifs (73). In parallel, tyrosine kinase inhibition, in addition to suppressing the activation of PI3K pathway, also blocks the association of PI3K with E-cadherin-mediated complexes and the reactivity of gamma-catenin to the PI3K docking site antibody (73).

hDlg, a member of the membrane-associated guanylate kinase (MAGUK)-like protein family, has been shown to be involved in PI3K recruitment and binding to E-cadherin adhesion complex (62). Interestingly, members of the MAGUK-like protein family contribute to proper junction and cell polarity (74-76). DLG-1, a *C. elegans* homologue of the Drosophila disc-large (Dlg), is required for the proper assembly and function of adherens junctions (77-79). Indeed, Firestein and Rongo (79) have shown that DLG-1 is predominantly localized at the adherens junctions of the epidermis, intestine and pharynx of embryonic and adult nematodes. Conversely, DLG-1-deficient embryos exhibit abnormal adherens junction formation and disorganization of the actin cytoskeleton (79). Human Dlg (hDlg) contains many protein-protein interaction domains including three PDZ (PSD-95/Dlg/ZO-1) domains, e.g. a SH3 domain, a GUK domain and alternatively spliced insertions (80-82). Consistent with the cellular localization of Dlg to lateral junctions (77), hDlg localizes to regions of cell-cell contact in human epithelial cells (62, 83, 84). We have recently reported that hDlg is required for the recruitment and maintenance of p85/PI3K to the E-cadherin adhesion complex (62). Indeed, epithelial cells containing low hDlg levels fail to recruit PI3K to E-cadherin-mediated cell-cell contacts and are unable to organize their cortical actin cytoskeleton. PI3K recruitment together with an organized cortical actin cytoskeleton are hence indispensable for proper adherens junction formation and stabilization as well as for morphological and functional epithelial differentiation (51). In addition to demonstrating a role for hDlg in the regulation of adherens junction assembly and function, our recent data also provide insights into how hDlg recruits PI3K signaling to the E-cadherin adhesion complex (62). Firstly, the direct interaction between p85/PI3K and hDlg is mediated by both SH2 domains of p85/PI3K. Accordingly, tyrosine phosphorylation of hDlg is necessary for p85/hDlg association. Since the binding of both SH2 domains of p85 is required for full activation of PI3K (63), this suggests that hDlg/p85 interaction may contribute to PI3K activation. Preliminary experiments demonstrate that hDlg is indeed tyrosine phosphorylated in intestinal epithelial cells (N. Rivard, unpublished data). However, the tyrosine kinase(s) which phosphorylate(s) hDlg in epithelial cells is(are) unknown, although c-Src may constitute a plausible candidate (69, 70). Secondly, Far-Western analysis demonstrates that the regulatory p85 subunit of PI3K directly interacts with specific variants of hDlg and more specifically with hypo-phosphorylated forms (fast migrating bands) present in differentiated polarized intestinal and renal epithelial cells. Interestingly, the dephosphorylation of hDlg on serine and threonine residues by PP1 phosphatase clearly reveals an interaction of the SH2 domains of p85 with the fast migrating forms of hDlg, suggesting that in undifferentiated subconfluent non polarized cells, hDlg is already tyrosine phosphorylated but its serine/threonine phosphorylation prevents its association with p85/PI3K (62). Therefore, it appears that the p85 binding site on hDlg protein is only unmasked upon confluency and differentiation. The change in hDlg phosphorylation state on serine and threonine during differentiation, together with the association of p85 with hypo-phosphorylated forms of hDlg, are consistent with the hypothesis that serine/threonine phosphorylation negatively regulates the association of p85/PI3K with hDlg to the E-cadherin adhesion complex and hence its scaffolding and clustering activity. In this respect, several phosphorylation consensus sequences for various serine/threonine kinases are distributed throughout the hDlg protein. However, further studies are required at this point to identify the actual phosphorylated serine and/or threonine on hDlg and their role in hDlg function in epithelial cells as well as to clarify the mechanism by which hDlg phosphorylation specifically inhibits p85/PI3K association.

7. PI3K IS INVOLVED IN E-CADHERIN-DEPENDENT REGULATION OF EPITHELIAL CELL DIFFERENTIATION AND POLARITY

In epithelial cells in culture, the localization and the cellular effect of PI3K activation clearly differ depending on the conditions in which cells are maintained, and in particular on the degree of confluence. For example, in intestinal epithelial cells, the p85 regulatory subunit of PI3K exhibits a cytoplasmic distribution in subconfluent growing cells, probably associated with Focal Adhesion kinase (FAK) (85) or other proteins, while a localized distribution pattern to cell-cell interfaces has been observed in confluent differentiating cells (51). Co-immunoprecipitation experiments confirmed the significant association of E-cadherin with p85/PI3K only in confluent differentiating intestinal epithelial cells (51). Accordingly, Tiam1 is localized in lamellipodia and membrane ruffles of migrating fibroblastoid cells while it is found in the adherens junctions of non-motile epithelial monolayers (46). Hence, when localized into the cytoplasm, PI3K is involved in cell migration (86-91) and proliferation (reviewed in 37, 38) probably by its association with FAK or other partners whereas PI3K may control not only adherens junction assembly but also differentiation and polarity when it is translocated to E-cadherin-mediated cell-cell contacts at confluency. Indeed, E-cadherin-mediated cell-cell attachment plays an important role in the morphological and functional differentiation of many epithelia (92-98). One of the key questions in epithelial

development is what triggers the differentiation process. In this regard, it has been demonstrated that cell-cell junction systems, particularly adherens junctions, play a critical role in the control of cell differentiation during ontogeny as well as during the continuous renewal of epithelial cells in the mature organ. For instance, studies with E-cadherin knockout mice have revealed that E-cadherin-mediated cell adhesion is essential for the compaction of mesenchymal cells and their transition to a polarized epithelium (99, 100). In a chimeric-transgenic animal model, expression of a dominant-negative N/E-cadherin mutant in villous enterocytes resulted in perturbation of cell-cell adhesion associated with an increased enterocyte migration rate along the crypt-villus axis, the loss of the differentiated, polarized phenotype and increased apoptosis (101). The tissue-specific ablation of E-cadherin impairs the differentiation of both epidermal keratinocytes and mammary epithelial cells (102-104). However, signaling components that relay the signal from E-cadherin to nuclear targets for the control of epithelial tissue-specific gene expression remain elusive. The fact that PI3K is recruited to and activated by E-cadherin-mediated cell-cell contacts in epithelial cells suggests that it may constitute one of these signaling components. For instance, inhibition of PI3K activity has been shown to repress the expression of intestine-specific genes such as sucrase-isomaltase and to delay functional and morphological differentiation of enterocytes (51). In addition, blockade of PI3K inhibits the expression of late differentiation markers such as loricrin and filaggrin in epidermal keratinocytes (73). PI3K is also involved in 3-dimensional morphogenesis and tissue-specific differentiation in the mammary gland (104). Hence, PI3K plays a primary role in regulating the integrity of adherens junctions, which in turn appears crucial for the efficient functional and morphological differentiation of epithelial cells. Conversely, PI3K may act as an intermediate in the formation of adherens junctions and differentiation, suggesting a bi-directional regulation between PI3K activity and adherens junction assembly such as previously observed for Rac and Cdc42 (20, 105). The manner in which PI3K relays the signal from E-cadherin to nuclear targets for the control of epithelial tissue-specific gene expression remains to be further clarified however. In intestinal epithelial cells, one of the downstream molecular events resulting from E-cadherin-mediated cellular aggregation is the activation of the mitogen-activated protein kinase p38alpha (Figure 4) (51), which controls differentiation of enterocytes (106); indeed, the stimulation of intestine-specific gene expression by p38alpha is mediated by the Cdx2 transcription factor, a p38 substrate known to be essential in enterocyte differentiation (106). In addition to regulating epithelial differentiation, E-cadherin-dependent PI3K activation may have other unexpected roles in the biology of epithelial cells. Indeed, we have demonstrated that E-cadherin-mediated cell-cell adhesion triggers PKB/Akt-induced inhibition of Raf-1/MEK/ERK cascade in a PI3K-dependent manner (Figure 4) (107). Strong evidences exist for the critical involvement of the Raf-1/MEK/ERK signaling cascade in the regulation of intestinal epithelial cell proliferation (108, 109). Hence, PI3K may play a role in the transition between proliferation and differentiation in the intestinal epithelium, a role shared by E-cadherin (10, 11).

Importantly, PtdIns(3,4,5)P_3 has recently emerged as a key determinant of epithelial polarity. For example, in chemotaxing cells, PtdIns(3,4,5)P_3 accumulates at the leading edge of migrating cells (110). This results in the recruitment of many PH domain-containing proteins which in turn produce the polarized phenotype and motility of chemotaxing cells. In polarized epithelial cells, PtdIns(3,4,5)P_3 is stably localized at the basolateral membrane and is excluded from the apical plasma membrane (111, 112). Recently, Mostov and colleagues reported that PtdIns(3,4,5)P_3 is a key signal for the formation and expansion of the basolateral surface (112). In their experiments, exogenous PtdIns(3,4,5)P_3 ectopically implanted into the apical surface constituted a sufficient signal to transform the composition of the apical surface to a basolateral type. Interestingly, inhibition of endogenous PtdIns(3,4,5)P_3 production by LY294002 causes a decrease in the amount of lateral surface, resulting in shorter cells (51, 112). Hence, E-cadherin-mediated cell-cell interaction (and perhaps integrin-mediated cell-matrix interaction), by activating PI3K, maintains PtdIns(3,4,5)P_3 production at the basolateral membrane (Figure 4). The mechanism by which a gradient of this freely diffusible lipid is maintained may also involve PTEN which strongly localizes to the apical plasma membrane. In fact, PTEN segregates PtdIns(4,5)P_2 to the apical surface, recruiting annexin 2 and Cdc42 which spatially regulate actin assembly. In turn, Cdc42 goes on to recruit the Par6/aPKC complex, which further stabilizes axial polarity (113).

8. CONCLUSION

The studies outlined above illustrate the significant progress achieved to date in understanding the importance of the PI3K signaling network in adherens junction formation and signaling function (Figure 4). However, numerous questions still remain unanswered. The upstream mechanisms that allow cadherin adhesion to modulate PI3K activity remain to be clarified. Obviously, one of the earliest events likely involves c-Src which is rapidly activated by E-cadherin-mediated cellular aggregation and may facilitate the recruitment of PI3K to E-cadherin-containing complexes. Whether this reflects a role of c-Src in regulating the binding of PI3K to the cadherin-catenin complex and/or catalytic activation of PI3K by c-Src remains to be determined. PI3K may not be the only target for cadherin-activated c-Src signaling as other potential c-Src substrates such as p120, beta-catenin, gamma-catenin, cortactin and hDlg are also present at cell-cell adhesions. As they become tyrosine-phosphorylated, some of these proteins may provide binding sites for PI3K. Whether the upstream docking protein for PI3K varies with differences in cellular background or context also needs to be addressed. It will be important in the future to understand in detail, how phosphorylation of the cadherin/catenin complex and of other substrates in its

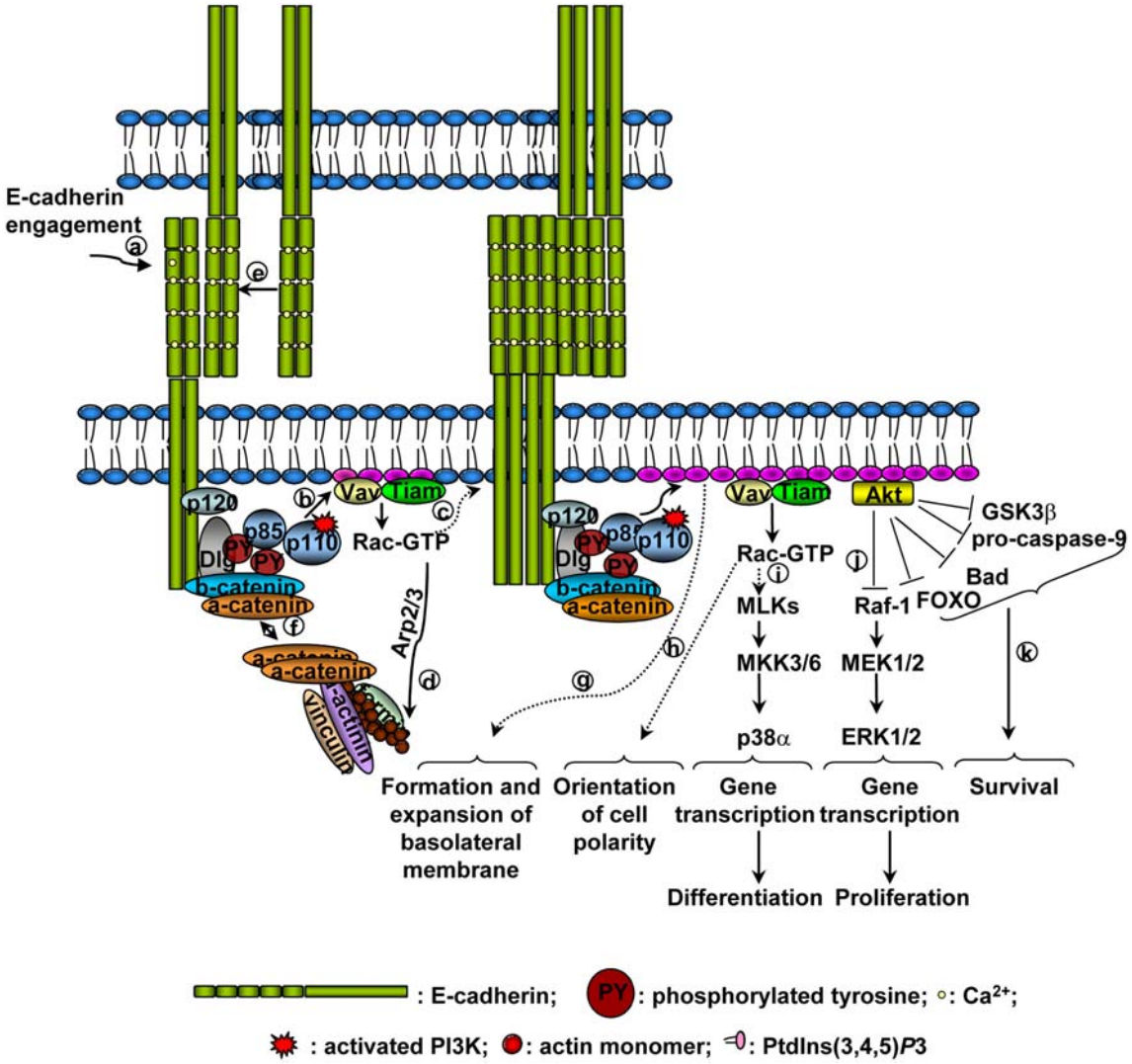

Figure 4. Outside-in signaling, receptor-like function of E-cadherin involving PI3K. After primary adhesion (a), PI3K is recruited to E-cadherin and activated. PtdIns(3,4,5)P_3 may participate to the recruitment of PH-containing proteins including the Rac exchange factors, Tiam and Vav2 (b), and the protein kinases PKB/Akt and PDK1 (not shown). Vav2 and Tiam promote Rac1 activation which, as recently reported (54, 57), stimulates membrane (c) and actin dynamics (d) adjacent to the initial site of contact, increasing the probability of additional, new E-cadherin engagements (e) (57). Alpha-catenin dimerizes, alpha-catenin homodimers are released from the cadherin-catenin complexes, bind to actin (f) and antagonize Arp2/3 function, inhibiting actin branching and facilitating formation of the belt of unbranched actin filaments. Alternatively, other actin-binding proteins such as vinculin, afadin (not shown) and alpha-actinin may provide a link with actin cytoskeleton. Meanwhile, accumulation of PtdIns(3,4,5)P_3 in the membrane signals for the formation and expansion of the basolateral surface (g) (112) and Rac1 promotes orientation of polarity and lumen formation (h) (114). In addition, in specific cellular contexts, Rac1 triggers the activation of downstream signaling effectors such as the mitogen-activated protein kinase p38alpha (i) while PKB/Akt leads to the inhibition of ERK signaling cascade (j), hence promoting cell cycle arrest of confluent epithelial cells. PtdIns(3,4,5)P_3-dependent activation of PKB/Akt may also promote survival of polarized epithelial cells (k).

close vicinity translates into changes in adhesive strength and downstream signaling. Finally, more studies are needed to understand how the spatial and temporal expression of PI3K activity intersects with cellular context, polarity determinants and the signaling of diverse receptors, including cell-cell adhesive receptors such as E-cadherin.

9. ACKNOWLEDGEMENTS

I thank Pierre Pothier for the critical reading of the manuscript. Some of the work reported in this manuscript was supported by a grant from Canadian Institutes of Health Research (MT-14405). Nathalie Rivard

is a recipient of a Canadian Research Chair in Signaling and Digestive Physiopathology.

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Abbreviations: PI3K: phosphatidylinositol 3-pinase; PtdIns: phosphoinositides; PH: pleckstrin homology; PKB: protein kinase B; MDCK: Madin-Darby Canine Kidney; SH domain: src homology domain; Dlg: disc large; PDZ: PSD-95/Dlg/ZO-1; MAGUK: membrane-associated guanylate cyclase; ERK: extracellular signal-regulated kinase; FAK: focal adhesion kinase

Key Words: Adherens junctions, phosphatidylinositol 3-kinase, PI3K, Phosphatidylinositol-3,4,5-trisphosphate, Phosphoinositides, Cell Adhesion, Epithelium, E-Cadherin, Actin Cytoskeleton, Catenin, Dlg, Review

Send correspondence to: Nathalie Rivard, Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, University of Sherbrooke, 3001 12th north avenue, Sherbrooke, Quebec, Canada, J1H5N4, Tel: 819-564-5271, Fax: 819-564-5320, E-mail: Nathalie.Rivard@USherbrooke.ca

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