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J Biol Chem, Vol. 274, Issue 27, 19347-19351, July 2, 1999

 **Activation of the Protein Kinase Akt/PKB by the Formation of E-cadherin-mediated Cell-Cell Junctions
EVIDENCE FOR THE ASSOCIATION OF PHOSPHATIDYLINOSITOL 3-KINASE WITH THE E-CADHERIN ADHESION COMPLEX**[**\***](http://www.jbc.org/cgi/content/full/274/27/19347#FN150)

**Salvatore Pece, Mario Chiariello, Cristina Murga, and J. Silvio Gutkind**

From the Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, Bethesda, Maryland 20892-4330

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E-cadherins are surface adhesion molecules localized at the level of adherens junctions, which play a major role in cell adhesivenessby mediating calcium-dependent homophylic interactions at sitesof cell-cell contacts. Recently, E-cadherins have been also implicatedin a number of biological processes, including cell growth anddifferentiation, cell recognition, and sorting during developmentalmorphogenesis, as well as in aggregation-dependent cell survival.As phosphatidylinositol (PI) 3-kinase and Akt play a criticalrole in survival pathways in response to both growth factors andextracellular stimuli, these observations prompted us to explorewhether E-cadherins could affect intracellular molecules regulatingthe activity of the PI 3-kinase/Akt signaling cascade. Using Madin-Darbycanine kidney cells as a model system, we show here that engagementof E-cadherins in homophylic calcium-dependent cell-cell interactionsresults in a rapid PI 3-kinase-dependent activation of Akt andthe subsequent translocation of Akt to the nucleus. Moreover,we demonstrate that the activation of PI 3-kinase in responseto cell-cell contact formation involves the phosphorylation ofPI 3-kinase in tyrosine residues, and the concomitant recruitmentof PI 3-kinase to E-cadherin-containing protein complexes. Thesefindings indicate that E-cadherins can initiate outside-in signaltransducing pathways that regulate the activity of PI 3-kinaseand Akt, thus providing a novel molecular mechanism whereby theinteraction among neighboring cells and their adhesion statusmay ultimately control the fate of epithelialcells.

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The maintenance of structural and functional integrity of epithelia requires highly dynamic cell-to-cell and cell-to-matrixinteractions, which are mediated by adhesion mechanisms involvingdifferent types of cell-surface receptors. Among them, cadherinsand integrins play a major role, as they are able to recognizeand interact with other cell adhesion receptors on neighboringcells or with proteins of the extracellular matrix, respectively([1-3](http://www.jbc.org/cgi/content/full/274/27/19347#B1)). E-cadherins belong to the family of integral membraneglycoproteins promoting homophylic calcium-dependent cell-cellinteractions and are well characterized adhesion receptors foundwithin adherens-type junctions in epithelia. The extracellulardomain of E-cadherins is able to mediate *per se* calcium-dependenthomotypic interactions at sites of cell-cell contacts, while itshighly conserved intracytoplasmic tail is involved in the strengtheningof the homophylic adhesions by binding a set of related proteinscalled catenins which, in turn, link the complex to the actincytoskeleton and elicit certain nuclear responses ([4](http://www.jbc.org/cgi/content/full/274/27/19347#B4), [5](http://www.jbc.org/cgi/content/full/274/27/19347#B5)).Recently, the dynamic aspects of cell adhesion and its relationshipto physiological and pathophysiological events have been intensivelyinvestigated. They include cell growth and differentiation, cellrecognition and sorting during developmental morphogenesis (reviewedin Ref. [2](http://www.jbc.org/cgi/content/full/274/27/19347#B2)), and a role in certain pathological processes, includingthe correlation between loss of E-cadherins at the level of cellsurface and enhanced cell invasiveness *in vitro* ([6-9](http://www.jbc.org/cgi/content/full/274/27/19347#B6)) and tumorprogression *in vivo* ([10](http://www.jbc.org/cgi/content/full/274/27/19347#B10), [11](http://www.jbc.org/cgi/content/full/274/27/19347#B11)).

Several lines of evidence indicate that the E-cadherin-mediated adhesion system is subject to regulation from the cytoplasmicside in response to intracellular events ([9](http://www.jbc.org/cgi/content/full/274/27/19347#B9), [12-15](http://www.jbc.org/cgi/content/full/274/27/19347#B12)). In contrast,the generation of signals at the level of adherens junctions asa consequence of E-cadherin engagement has been thus far poorlyinvestigated, although newly available evidence suggest that E-cadherinsmay participate in transducing outside-in signals ([16](http://www.jbc.org/cgi/content/full/274/27/19347#B16)). Of interest,it has been reported recently that E-cadherins can mediate aggregation-dependentcell survival in a variety of experimental settings ([17-19](http://www.jbc.org/cgi/content/full/274/27/19347#B17)). Asthe Akt kinase is an integral component of survival pathways utilizedby both growth factors and extracellular stimuli ([20-23](http://www.jbc.org/cgi/content/full/274/27/19347#B20)), theseobservations prompted us to investigate whether E-cadherins couldaffect the activity of signaling molecules controlling Akt function.In this study, we used an *in vitro* model for the disruption andsubsequent re-formation of E-cadherin-dependent interactions inepithelial MDCK1 cells to explore the possibility that E-cadherin-mediated cellularaggregation could result in Akt activation. We provide evidencethat engagement of E-cadherins in homophylic adhesion with neighboringcells promotes a remarkable PI 3-kinase-dependent increase inthe state of activation of Akt and the rapid translocation ofAkt to the nucleus. We also demonstrate that engagement of E-cadherinsis necessary and sufficient for the induction of Akt activityupon adherens junction assembly, and co-immunoprecipitation experimentsdemonstrate a physical association between PI 3-kinase and E-cadherin-containingmultiprotein complexes in response to cell-cell contact formation,thus providing a likely mechanism for Akt activation. Overall,these findings indicate that E-cadherins may initiate outside-insignal transducing pathways, thus supporting an active role forE-cadherins in the control of key early post-aggregationevents.

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*Cell Culture and Expression Plasmids--* An expression vector for hemagglutinin-(HA) tagged Akt (pCEFL-HA-Akt) has been reported elsewhere ([22](http://www.jbc.org/cgi/content/full/274/27/19347#B22)). Early passage MDCKcells were maintained in Dulbecco's modified Eagle's medium supplementedwith 10% fetal bovine serum. For transfections, cells were grownat 70% confluence in 60-mm cell culture dishes and transfectedwith 4 µg of HA-tagged PKB/Akt cDNA by the calcium phosphate precipitationtechnique and selected with 500 µg/ml G418. Transfected cloneswere maintained in 500 µg/ml G418 to provide selectionpressure.

*Antibodies and Immunologic Detection Methods--* Antibodies specific for E-cadherins, purchased from Transduction Laboratories (Lexington, KY), were used for immunoprecipitationand Western blotting. Antibodies to the extracellular domain ofE-cadherin molecule (DECMA-1 clone, Sigma) were used for immunostainingand for antibody inhibition experiments. An anti-HA-specific monoclonalantibody (HA11, Babco, Richmond, CA) and a goat polyclonal anti-Aktantibody (C20, Santa Cruz Biotechnologies, Inc.) were used todetect ectopic and endogenously expressed Akt, respectively. Antibodiesagainst the p85 regulatory subunit of PI 3-kinase and anti-phosphotyrosine(anti-Tyr(P), G410 clone) antibodies were from Upstate BiotechnologyInc. (Lake Placid, NY). Anti-mouse and anti-rabbit secondary antibodiescoupled to horseradish peroxidase were from Cappel Laboratories(Durham, NC). Co-immunoprecipitation and Western blotting experimentswere performed as described previously ([22](http://www.jbc.org/cgi/content/full/274/27/19347#B22)).

*Akt and PI 3-Kinase Assays--* MDCK cells were serum-starved overnight in the presence of 10 mM Hepes, and E-cadherin-mediated cell-to-cell contacts weredisrupted by treatment with EGTA to a final concentration of 4mM for 30-40 min at 37 °C. Thereafter, intercellular interactionswere allowed to re-establish in the presence of fresh, calcium-containingmedium (final concentration CaCl2 ~1.8 mM) ([24](http://www.jbc.org/cgi/content/full/274/27/19347#B24)). At differenttime points after calcium restoration, cells were harvested, lysedon ice, and assayed for Akt activity in immunocomplex kinase reactions,using histone 2B as a substrate in the presence of [-32P]ATP ([22](http://www.jbc.org/cgi/content/full/274/27/19347#B22)). Comparable immunoprecipitation and loading of wild-typeand epitope-tagged Akt kinase were determined by immunoblottingmembranes with anti-Akt and anti-HA antibodies,respectively.

For measurement of PI 3-kinase activity, whole cell extracts were incubated with anti-Tyr(P) antibodies for 2 h at 4 °C underconstant agitation. The PI 3-kinase assay was subsequently performedas described previously ([25](http://www.jbc.org/cgi/content/full/274/27/19347#B25)) by evaluating the ability of theimmunoprecipitates to phosphorylate PI to yield phosphatidylinositol3-phosphate (PI3P). After thin layer chromatography, 32P-labeled phospholipids were detected by autoradiography. In someexperiments, the PI 3-kinase inhibitor wortmannin (50 nM, Sigma)was used. Autoradiograms were quantified on a PhosphorImager usingthe ImageQuantsoftware.

*Immunofluorescence Microscopy--* MDCK cells were grown on glass coverslips to confluence, washed with PBS, and fixed for 20 min at room temperature in 3.7%formaldehyde in PBS. Cells were then permeabilized with 0.5% TritonX-100 for 10 min. After washing with PBS, cells were blocked with1% bovine serum albumin in PBS and incubated with the appropriateprimary antibody for 1 h at room temperature. Rat anti-E-cadherin(1:1000) and mouse anti-HA (1:50) antibodies were used to detectE-cadherin and HA-tagged Akt, respectively. Following incubationwith the corresponding secondary antibodies (1:200) conjugatedwith tetramethylrhodamine B isothiocyanate or fluorescein isothiocyanate,respectively, the cells were washed, mounted in anti-fade medium(Molecular Probes, Eugene, OR), and examined under an UV microscope(Zeiss Axiophot) at a × 40 or × 63 magnification using the appropriatefilters. Images were processed using Adobephotoshop.

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*E-cadherin-mediated Adherens Junction Formation Elicits Akt/PKB Activation--* Using epithelial MDCK cells, we first evaluated whether adherens junction assembly could affect the state of activation ofendogenous Akt. As epithelial cells require Ca2+ to form homophylic cell-cell adhesions, a simple method to studythe adhesive properties of surface molecules involves the disruptionof Ca2+-dependent homotypic boundaries among cells by EGTA-treatmentand the re-establishment of cell-cell contacts by the subsequentrestoration of Ca2+ ions ([24](http://www.jbc.org/cgi/content/full/274/27/19347#B24)). Whereas control cells present a typical patternof E-cadherin immunostaining at the level of cell-cell contacts(Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*A*, *panel a*), in cells treated with the calcium chelator,EGTA, E-cadherins appear to be diffusely distributed (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*A*,*panel b*). In this case, the loss of E-cadherins at the level ofcell-cell contacts is most likely due to their redistributionthroughout the cell surface rather than to the internalizationof E-cadherins, as reported for other types of cadherins ([26](http://www.jbc.org/cgi/content/full/274/27/19347#B26)).After restoration of calcium, *de novo* formation of adherens junctionscould be observed as early as 5-10 min (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*A*, *panel c*), andthe process appeared to be almost complete after 30-40 min (Fig.[1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*A*, *panel d*).

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| http://www.jbc.org/content/vol274/issue27/images/small/bc2791415001.gif**View larger version** (66K): [[in this window]](http://www.jbc.org/cgi/content/full/274/27/19347/F1) [[in a new window]](http://www.jbc.org/cgi/content-nw/full/274/27/19347/F1)   | **Fig. 1.** **E-cadherin-mediated cellular aggregation induces Akt activation.** *A*, MDCK cell monolayers were left untreated (*panel a*) or treated with 4 mM EGTA (*panel b*). The EGTA-containing medium was then replaced with serum-free, calcium-containing medium for 5 (*panel c*) and 30 min (*panel d*). Cells were fixed and stained with anti-E-cadherin-specific antibodies. *B*, MDCK cells (*panel a*) and MDCK cells expressing an epitope-tagged Akt (*panel b*) were serum-starved and treated with 4 mM EGTA for 30-40 min and then calcium was restored for 5-60 min, as indicated. As controls, starved cells were left untreated (*control*) or stimulated with 100 nM EGF for 5 min. After calcium restoration, lysates were immunoprecipitated with anti-Akt or anti-HA antibodies and used for Akt kinase reaction as described under "Experimental Procedures." 32P-Labeled substrate (histone 2B (*H2B*)) is indicated. Autoradiograms showing the time course of Akt activity correspond to representative experiments. Similar results were obtained in three to five independent experiments. Data represent the average (mean ± S.D.) of three to five independent experiments, expressed as fold increase with respect to untreated cells (*control*). *C*, MDCK cells were stably transfected with HA-Akt, as described above. Following starvation, cells were left untreated (*panel a*), treated with 4 mM EGTA (*panel b*), or restored in calcium-containing medium for 30 min after EGTA treatment (*panel c*). Cells exposed to 100 nM EGF for 5 min were used as positive control (*panel d*). After stimulation, cells were fixed, permeabilized, stained with anti-HA monoclonal antibodies followed by a fluorescein isothiocyanate-conjugated secondary antibodies and prepared for microscope analysis at a × 63 magnification.  |

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To explore the possibility that E-cadherin engagement leads to Akt activation, MDCK cells were grown as long confluent culturesby maintaining cells in complete medium for at least 24-48 h afterreaching confluence, to optimize cell-cell contacts as well asto minimize the influence of integrin-extracellular matrix interactions([27](http://www.jbc.org/cgi/content/full/274/27/19347#B27)). Cells were then serum-starved for 18-24 h and cell-cellcontacts disrupted by the treatment with EGTA (4 mM) for 30-40min. Subsequently, adherens junctions were allowed to re-formin the presence of fresh calcium-containing medium, and, at theindicated time points, cells were lysed and assayed for Akt kinaseactivity. As shown in Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*B*, under these experimental conditions,the reassembly of adherens junctions induced a remarkable elevationof Akt kinase activity. Kinetic studies demonstrated a rapid increasein Akt activity as early as 15 min after calcium restoration,with a peak at 30-40 min followed by a decrease over a 1-h timecourse. Stable expression of a HA-tagged Akt in transfected MDCKcells revealed to be a useful tool to enhance the detectabilityof Akt activation upon E-cadherin engagement. Kinetics of activationof ectopically expressed Akt (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*B*, *panel b*) was similar tothat observed for the endogenous enzymatic activity (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*B*,*panel a*), thus suggesting that endogenous levels of E-cadherinsand Akt are sufficient to sustain a potent Akt activity followingadherens junction assembly under physiologicalconditions.

*Activation of Akt following E-cadherin Engagement Leads to Akt Translocation to the Nucleus--* Following activation by a PI 3-kinase-mediated pathway, Akt has been shown previously to translocate to the nucleus ([28](http://www.jbc.org/cgi/content/full/274/27/19347#B28)),where it participates in the regulation gene expression ([29](http://www.jbc.org/cgi/content/full/274/27/19347#B29)).On the basis of these observations, we examined whether E-cadherinengagement could lead to a change in the subcellular distributionof an ectopically expressed Akt. Serum-starved cells stimulatedwith EGF (100 nM) for 5-10 min were used as a positive control(Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*C*, *panel d*). Whereas Akt appeared to be diffusely distributedin the cytoplasm of untreated and EGTA-treated cells (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*C*,*panels a* and b), respectively, calcium restoration caused a markedtranslocation of Akt to the nucleus (Fig. [1](http://www.jbc.org/cgi/content/full/274/27/19347#F1)*C*, *panel c*).

*Akt Activation in Response to Adherens Junction Assembly Requires E-cadherin Engagement--* To confirm that Akt activation is not due to the manipulation of calcium levels but to the ability of calcium restorationto mimic the physiological engagement of E-cadherins in cell-cellcontacts, we took advantage of the availability of function-perturbinganti-E-cadherin antibodies, DECMA-1 clone, which have been describedpreviously to be effective in blocking E-cadherin mediated adherensjunction formation ([30](http://www.jbc.org/cgi/content/full/274/27/19347#B30)). As illustrated in Fig. [2](http://www.jbc.org/cgi/content/full/274/27/19347#F2) (*A* and B,*sixth lanes*), pretreatment with anti-E-cadherin antibodies, thathinders the formation of adherens junctions, led to a dramaticsuppression of Akt activity upon calcium restoration. This suggeststhat E-cadherin-dependent homophylic interactions among cellsare strictly required for induction of Akt activity. In contrast,the presence of anti-E-cadherin antibodies did not affect EGF-inducedAkt activation (Fig. [2](http://www.jbc.org/cgi/content/full/274/27/19347#F2), *A* and *B*, *third lanes*), thus establishingthe specificity of the experimental approach and suggesting thatE-cadherin-mediated Akt activation occurs through a growth factor-independentmechanism. Unexpectedly, during the course of dose-response experimentswith blocking antibodies, we observed that high antibody dilutionscaused a dramatic increase in the activity of Akt (Fig. [2](http://www.jbc.org/cgi/content/full/274/27/19347#F2), *A* and*B*, *eighth lanes*). Antibody immobilization experiments, using MDCKcells suspended in 4 mM EDTA and plated on cell culture dishesprecoated with different antibody dilutions, confirmed these observations(data not shown). The most straightforward explanation for theseseemingly conflicting results relies on the fact that in the presenceof a vast excess of DECMA-1 antibodies the majority of the antibody-boundE-cadherins would be expected to remain in a monomeric, inactiveform. However, at high antibody dilutions each molecule of antibodywould be expected to bind two molecules of E-cadherin, thus causinglateral dimerization and clustering of E-cadherins, which canmimic E-cadherin activation by calcium-dependent homophylic interactions([31](http://www.jbc.org/cgi/content/full/274/27/19347#B31), [32](http://www.jbc.org/cgi/content/full/274/27/19347#B32)). Together, these observations suggest that E-cadherinengagement is necessary and sufficient for Akt activation in responseto adherens junction assembly.

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| http://www.jbc.org/content/vol274/issue27/images/small/bc2791415002.gif**View larger version** (26K): [[in this window]](http://www.jbc.org/cgi/content/full/274/27/19347/F2) [[in a new window]](http://www.jbc.org/cgi/content-nw/full/274/27/19347/F2)   | **Fig. 2.** **E-cadherin-mediated adhesion is necessary for enhanced Akt activity.** Serum-starved MDCK cells untransfected (*A*) or stably expressing HA-Akt (*B*) were treated with 4 mM EGTA to disrupt E-cadherin-mediated cell-cell contacts. Cells were pretreated for 30 min with the indicated dilutions of anti-E-cadherin antibodies (DECMA-1 clone) and lysed after 30 min of calcium restoration. As controls, serum-starved cells were left untreated, treated with 100 nM EGF for 5 min, or treated with EGF and anti-E-cadherin antibodies, as indicated. Cellular lysates were assayed for Akt activity, as described under "Experimental Procedures." 32P-Labeled substrate (histone 2B (*H2B*)) is indicated. Values represent the average ± S.D. of triplicate samples from a typical experiment expressed as fold induction relative to controls. Nearly identical results were obtained in three additional experiments.  |

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*Role of PI 3-Kinase in the Activation of Akt in Response to E-cadherin-mediated Cellular Aggregation--* The Akt/PKB kinase represents a downstream target of PI 3-kinase in a pathway critical for signaling cell survival in responseto several stresses or growth factor deprivation ([20-22](http://www.jbc.org/cgi/content/full/274/27/19347#B20)). We thereforeused a PI 3-kinase inhibitor, wortmannin, to address the possibleinvolvement of PI 3-kinase in the induction of Akt kinase activityby adherens junction assembly. As shown in Fig. [3](http://www.jbc.org/cgi/content/full/274/27/19347#F3), preincubationof cells with wortmannin (50 nM) for 30 min before addition ofcalcium completely abolished Akt activation, with Akt kinase levelsfalling even below the unstimulated background in wild-type aswell as in stably transfected MDCK cells. These observations suggestedthat the functional activity of PI 3-kinase is required for theactivation of Akt in response to E-cadherin engagement.

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| http://www.jbc.org/content/vol274/issue27/images/small/bc2791415003.gif**View larger version** (25K): [[in this window]](http://www.jbc.org/cgi/content/full/274/27/19347/F3) [[in a new window]](http://www.jbc.org/cgi/content-nw/full/274/27/19347/F3)   | **Fig. 3.** **Akt activation by E-cadherin-dependent aggregation is wortmannin-sensitive.** MDCK cells were serum-starved overnight. After EGTA treatment for 30-40 min and subsequent calcium restoration for 30 min, kinase activity of endogenous (*A*) and ectopically expressed (*B*) Akt was evaluated in the presence or absence of 50 nM wortmannin, which was added 20 min prior to calcium stimulation. As controls, cells were left untreated or treated with 100 nM EGF for 5 min, as indicated. After immunoprecipitation with anti-Akt or anti-HA antibodies, Akt kinase assays were performed. Autoradiograms correspond to representative experiments. Data represent the mean ± S.D. from three to five separate experiments.  |

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*Formation of Adherens Junctions Causes Association of PI 3-Kinase with E-cadherin-containing Protein Complexes--* Agonist activation of PI 3-kinase frequently involves the translocation of this enzyme to the plasma membrane where it cangain access to its lipid substrates (reviewed in Ref. [33](http://www.jbc.org/cgi/content/full/274/27/19347#B33)). Thus,it was tempting to speculate that a similar mechanism may underliethe activation of Akt upon organization of E-cadherin-mediatedcell-cell contacts. In preliminary experiments we found that thep85 regulatory subunit of PI 3-kinase was tyrosine-phosphorylatedas a function of time after calcium restoration, overlapping withthe kinetics of Akt activation (data not shown). Therefore, wedecided to evaluate directly the pattern of activation of PI 3-kinaseafter immunoprecipitation of MDCK cell lysates with anti-Tyr(P)antibodies. Of interest, the profile of PI 3-kinase activity aftercalcium restoration mirrored that of Akt, as illustrated in Fig.[4](http://www.jbc.org/cgi/content/full/274/27/19347#F4)*A*. We next tested the possibility that Akt activation in responseto E-cadherin engagement could involve the docking of PI 3-kinaseto the adherens junction complex. As shown in Fig. [4](http://www.jbc.org/cgi/content/full/274/27/19347#F4)*B*, upon calciumrestoration, PI 3-kinase was found to be associated to the E-cadherinimmunoprecipitates in a time-dependent manner, as judged by Westernblotting with specific anti-p85/PI 3-kinase antibodies.

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| http://www.jbc.org/content/vol274/issue27/images/small/bc2791415004.gif**View larger version** (40K): [[in this window]](http://www.jbc.org/cgi/content/full/274/27/19347/F4) [[in a new window]](http://www.jbc.org/cgi/content-nw/full/274/27/19347/F4)   | **Fig. 4.** **Adherens junction assembly induces PI 3-kinase activation and association of the p85/PI 3-kinase subunit to the E-cadherin multiprotein complex.** *A*, MDCK cells were serum-starved overnight, treated with 4 mM EGTA for 30-40 min, and then calcium was restored in serum-free medium, as indicated. Serum-starved cells were left untreated (*control*) or exposed to 100 nM EGF for 5 min. After lysis, PI 3-kinase activity was assayed in anti-Tyr(P) immunoprecipitates, as detailed under "Experimental Procedures." The chromatographic mobility of 32P-labeled PI3P is indicated. Autoradiogram corresponds to a representative experiment that was repeated three times. *B*, MDCK cells were treated as described above. E-cadherins were immunoprecipitated from total cell lysates with anti-E-cadherin-specific monoclonal antibodies. The presence of the p85/PI 3-kinase regulatory subunit in anti-E-cadherin immunoprecipitates was detected with specific anti-p85 polyclonal antibodies. As controls, untreated (*control*) or EGF-treated cells were also included. *Top*, the immunoblot depicts the amount of p85 recovered in anti-E-cadherin immunoprecipitates. *Bottom*, equal amounts of E-cadherins were observed in each immunoprecipitated sample. Similar results were obtained in three independent experiments.  |

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Taken together, overall results of this study indicate that one of the molecular events resulting from the E-cadherin-mediatedcellular aggregation is the rapid activation of a PI 3-kinase/Aktcascade. Although the mechanism whereby E-cadherin engagementstimulates this biochemical route is not fully understood, itmost likely involves a yet to be identified tyrosine kinase, which,when activated in response to E-cadherin mediated-cellular aggregation,might facilitate the recruitment of PI 3-kinase to E-cadherin-containingcomplexes at the level of the plasma membrane. Indeed, the recoveryof the p85 regulatory subunit of PI 3-kinase in co-immunoprecipitationexperiments with anti-E-cadherin antibodies, along with the kineticpattern of PI 3-kinase activity detected in anti-Tyr(P) immunoprecipitates,strongly argue in favor of thishypothesis.

As PI 3-kinases are known to play a central role in a number of cellular processes, including mitogenic signaling and cellsurvival, cytoskeletal remodeling, as well as metabolic controland vesicular trafficking (reviewed in Ref. [33](http://www.jbc.org/cgi/content/full/274/27/19347#B33)), our presentfindings may have broad implications to the understanding of epithelialcell biology. In this scenario, E-cadherins might function as"relationship molecules" between the extracellular and the intracellularenvironment, initiating the transduction of intracellular signalingpathways stimulating the PI 3-kinase/Akt cascade. This might providean interesting mechanism whereby the adhesion status of the cellsmay control the cell fate, including cell survival or death byapoptosis, as well as other critical events occurring during thestepwise organization of theepithelium.