also inhibited in cdh1 morpholino-injected embryos resulting in a shorter body length in surviving embryos, relative to controls. These results show that inhibiting E-cadherin expression during vertebrate embryogenesis disrupts early cleavage patterns and early morphogenetic cell movements.

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Visualization of N-Cadherin Protein Expression During the Emigration of Chicken Trunk Neural Crest Cells

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Emigration of trunk neural crest cells from the developing neural tube requires the breakage or rearrangement of cadherin-containing adherens junctions between the cells. I therefore examined the distribution of N-cadherin protein in chicken trunk neural crest cells as they emerged from the neural tube. To identify emerging neural crest cells, embryos were electroporated at the 12 to 16 somite stage of development with cDNA constructs encoding green or red fluorescent proteins and were allowed to develop for a further 12 to 24 hours before fixation. Sections or whole mounts then were stained with anti-cadherin antibodies and examined by conventional or confocal microscopy. As expected from previous work, fully-emigrated cells did not express detectable levels of surface N-cadherin. In some cases, cells that had not fully emerged from the neural tube appeared to retain surface-localized N-cadherin at areas of apposition to other neural tube cells. This suggests that modulation or breakage of N-cadherin-containing adherens junctions may be involved in control of neural crest emigration in the trunk. This work was supported by a Grant-in-Aid from the American Heart Association Western Regional Affiliates.

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E-cadherin is Internalized via a Dynamin-Dependent Pathway and is Trafficked to Sorting Endosomes

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E-cadherin is a fundamental molecular determinant of tissue patterning and epithelial cohesion. It is increasingly apparent that dynamic cadherin-based contacts play important roles in cellular recognition and epithelial motility. We recently reported that E-cadherin participates in a pathway of post-Golgi recycling and trafficking (Le et al., JCB 146,219). Such recycling may contribute to the remodelling of cadherin-based contacts predicted to be involved in dynamic cell contacts and intercellular movement. In order to elucidate the molecular mechanisms of E-cadherin recycling, we sought to first characterize the pathway and itinerary of E-cadherin internalization. We immuno-labelled E-cadherin on the surfaces of live MCF7 cells using mAb HECD-1 directed against human E-cadherin. By immunofluoresence, surface-labelled E-cadherin rapidly accumulated in peripheral vesicles that then redistributed to a perinuclear location reminiscent of recycling endosomes. Transferrin, a maker for receptor mediated endocytosis, showed a strong temporal co-localization with E-cadherin as it was trafficked through the cell, indicating E-cadherin may share the same trafficking pathway. E-cadherin internalization was also effectively inhibited by transient expression of dominant-negative dynamin (S45N), suggesting that this adhesion molecule was endocytosed by a clathrin-mediated mechanism. Comparison of E-cadherin internalization with other markers suggests that during post-Golgi trafficking surface E-cadherin accesses the endosomal network to be returned to the cell surface via a classical recycling compartment. Endocytosed E-cadherin localized with β-catenin but not α-catenin, raising the possibility that differential recruitment of actin-binding proteins may determine the fate of cell surface E-cadherin.

Supported by the National Health & Medical Research Council of Australia and the Wellcome Trust.

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A role for cholesterol-dependent membrane microdomains in E-cadherin adhesion

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E-cadherin is a major protein involved in determining epithelial integrity and morphogenesis. Although much is known about the role of E-cadherin in stable cell-cell adhesion, little is known about the mechanisms that determine E-cadherin activity in dynamic contacts. The dynamic nature of cholesterol-sphingolipid enriched plasma membrane rafts affords unique opportunities for the spatial and temporal coordination of discrete cellular events at the cell surface. Reports that E-cadherin associates with detergent resistant membranes prompted us to investigate the functional role of this association. Sucrose density gradients of 1% Triton X-100-resistant cellular material showed co-fractionation of E-cadherin and the raft protein, caveolin. Immunofluorescence studies showed that a pool of β-catenin (a key component of the cadherin adhesion complex) colocalized in a lipid-dependent manner with the known raft marker, GPI-PLAP, after GPI-PLAP was cross-linked at the cell surface. GPI-PLAP was also seen to accumulate with E-cadherin at the leading edges of lamellipodia where nascent E-cadherin based contacts are being established. The novel assay used to examine the formation of these nascent E-cadherin contacts was also used to investigate the possible functional dependence of E-cadherin on plasma membrane rafts. Methyl-β-cyclodextrin treatment or overexpression of a dominant negative caveolin mutant were employed to deplete plasma membrane cholesterol from cells expressing human E-cadherin (hE-CHOs). These manipulations, which disrupt raft integrity, inhibited the ability of cells to extend regions of E-cadherin specific contacts, a phenomenon which was rescued after repleting the membranes with cholesterol. This evidence indicates a role for plasma membrane rafts in regulating aspects of E-cadherin function, and suggests an interaction of the E-cadherin/catenin complex with cholesterol-dependent plasma membrane microdomains.

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E-cadherin activates PI3-kinase and Rac1 signaling to mediate adhesive contact formation

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E-cadherin, the prototypical epithelial cadherin, is responsible for mediating calcium dependent cell-cell interactions that are essential for tissue morphogenesis, embryogenesis and wound healing. Evidence suggests that upon initial cell-cell contact, the cytoplasmic tail of E-cadherin has the potential to influence cellular signaling pathways. Class IA PI3-kinases generate lipid second messengers that are involved in a multitude of signaling pathways and the p85α regulatory subunit of these PI3-kinases has been shown to bind the E-cadherin adhesive complex. We sought to investigate whether PI3-kinase regulates E-cadherin function through this association. We have developed a cadherin-specific assay in which E-cadherin expressing cells (hE-CHOs) adhere to and spread upon a synthetic cadherin ligand (hE/Fc) via lamellipodial protrusion. The leading edges of lamellipodia extensions where nascent cadherin contacts are being formed concentrate E-cadherin and are regions of dynamic actin assembly. Using our cadherin-specific assay, we first examined the importance of PI3-kinase activity in cadherin-based adhesion. We found PI3-kinase localized to the leading edges of cadherin-based lamellipodia, where PIP3 was identified using a GFP-PH probe. Biochemically, p85 was recruited to the E-cadherin/catenin complex specifically upon adhesive ligation. Wortmannin and LY294002 blocked both cadherin-based adhesion and lamellipodial extension. This was overcome by expression of a constitutively active Rac1 mutant. Furthermore we found that cadherin ligation specifically activated Rac1 signaling. Taken together we conclude that PI3-kinase and Rac1 participate in E-cadherin based signaling to the actin cytoskeleton.

Supported by National Health and Medical Research Council, Australian Research Council and Wellcome Trust.

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p120 Binding to E-cadherin Influences Cadherin Directed Actin Assembly

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E-cadherin, a prominent cell-cell adhesion molecule expressed in epithelial cells, is fundamental for initiating cell-cell contacts and forming adherens junctions. There is increasing evidence to show that the progression from nascent, dynamic cell contacts to stable contacts is subject to cellular regulation. While it is now clear that the cytoplasmic tail of E-cadherin and its associated catenins are involved with adhesive control, much remains to be understood about the effector mechanisms and the signalling pathways that control this process. The phosphoprotein $p120^{ctn}$ is a catenin that binds directly to the juxtamembrane region of the cadherin cytoplasmic tail to influence cadherin adhesion. In a cadherin-specific spreading assay, CHO cells expressing full-length E-cadherin (hE-CHO) formed extensive cadherin-based lamellipodia. Previous studies have shown that $p120^{ctn}$ associates with the cadherin complex in stable contacts. In our assays, we also observed the apparent accumulation of $p120^{ctn}$ at the leading edge of these cadherin-based lamellipodia, the sites of nascent cadherin contacts and dynamic actin activity. CHO cells expressing an E-cadherin mutant which does not bind $p120^{ctn}$ binding (hE-764 CHO) showed reduced adhesive strength compared to hE-CHO cells, as measured in laminar flow assays. In addition, hE-764 CHO cells were unable to support extensive cadherin-based lamellipodia, a phenotype that was rescued by transient expression of constitutively active Rac. Emerging evidence suggests a close relationship between cadherin adhesion and cytoskeletal re-organisation regulated by RhoGTPases such as Rac. We propose that $p120^{ctn}$ may participate in co-ordinating cadherin-based lamellipodial formation and Rac-directed actin assembly.

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Rho family GTPase Activity is Regulated by Cadherin Engagement

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Cadherins are calcium-dependent cell adhesion molecules responsible for strong cell-cell adhesions. In epithelial cells, cadherins are concentrated within adherens junctions where they bind homophilically to cadherins on adjacent cells. The