Coupling membrane protrusion and cell adhesion

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Summary

The ability of cells to extend cell membranes is central to numerous biological processes, including cell migration, cadherin-mediated junction formation and phagocytosis. Much attention has been focused on understanding the signals that trigger membrane protrusion and the architecture of the resulting extension. Similarly, cell adhesion has been extensively studied, yielding a wealth of information about the proteins involved and how they signal to the cytoplasm. Although we have learned much about membrane protrusion and cell adhesion, we know less about how these two processes are coupled. Traditionally it has been thought that they are linked by the signaling pathways they employ – for example, those involving Rho family GTPases. However, there are also physical links between the cellular machineries that mediate cell adhesion and membrane protrusion, such as vinculin.

Key words: Adhesion, Protrusion, Actin polymerization, Phagocytosis

Introduction

Extension of the cell surface is an early step in several cellular processes, including cell migration, neurite outgrowth, phagocytosis and the formation of cell-cell junctions. Several types of membrane protrusion have been described. These include lamellipodia, pseudopodia, filopodia, microvilli, as well as various other structures (Bray, 1992). Lamellipodia are thin, sheet-like projections formed at the leading edge of many migrating cells. These can give rise to membrane ruffles when lamellipodia fail to adhere to a substrate and are propelled rearwards on the dorsal cell surface. Membrane ruffles can also arise on the dorsal surfaces of cells under some situations, although their function here is obscure. Pseudopodia are thicker protrusive structures, prominent in many amoeboid cells. Filopodia are finger-like projections that have an exploratory function, demonstrated by the behavior of filopodia on nerve growth cones. Microvilli are similar to filopodia in that they are finger-like projections, but they are generally stable extensions of the cell surface that are static and serve to increase the surface area of the cell. Consequently, they are abundant on the apical surfaces of epithelia involved in water or nutrient uptake. Here, we focus on dynamic membrane protrusions whose formation is driven by actin polymerization, such as lamellipodia and filopodia. Many growth factors or soluble agents stimulate the extension of these structures, and the signaling pathways involved are becoming well characterized. Here we discuss how adhesion and engagement of cell adhesion molecules can also stimulate membrane protrusion.

Protrusion driven by actin polymerization

The driving force for the formation and extension of membrane protrusions is the polymerization of actin. The leading edge of a lamellipodium is rich in actin filaments arranged in a highly branched and dendritic network with the barbed ends of the filaments concentrated close to the plasma membrane where they are primed for the addition of actin monomers (Svitkina and Borisy, 1999; Svitkina et al., 1997). A dendritic model for lamellipodial extension has been proposed. In this model, the entire actin filament network treadmills, growing at the filament barbed ends and later shrinking at the lamellipodial base (Svitkina and Borisy, 1999).

How these actin filaments assemble in this highly branched dendritic network in the seconds required for membrane protrusion to occur has been extensively studied. The Arp2/3 complex has been identified as a potent nucleator of actin polymerization, which initiates the formation of actin filaments in lamellipodia of moving cells (Machesky et al., 1994; Mullins et al., 1998; Svitkina and Borisy, 1999). The Arp2/3 complex comprises seven subunits and is assembled post-translationally (Welch and Mitchison, 1998). It is highly concentrated at the leading edge of migrating cells (Bailly et al., 2001; Machesky et al., 1997; Mullins et al., 1998; Svitkina and Borisy, 1999; Welch et al., 1997), where it undergoes a conformational change to provide a template for the polymerization of actin filaments (Robinson et al., 2001; Volkmann et al., 2001). Actin subunits then add to the new free barbed end, and the membrane is pushed outward.

Adhesion-stimulated protrusion

Conventional dogma is that protrusion of a leading edge is the first step in cell migration, followed by adhesion to the substratum, contraction and breaking of older adhesions at the cell rear (reviewed in Condeelis, 1998). But does adhesion precede extension in many situations and actually trigger extension? During cell spreading the cell first adheres to the substratum and then extends protrusions. The importance of adhesion for this event is underscored by the observation that perturbation of various adhesion molecules results in cells that are no longer able to spread onto an extracellular matrix

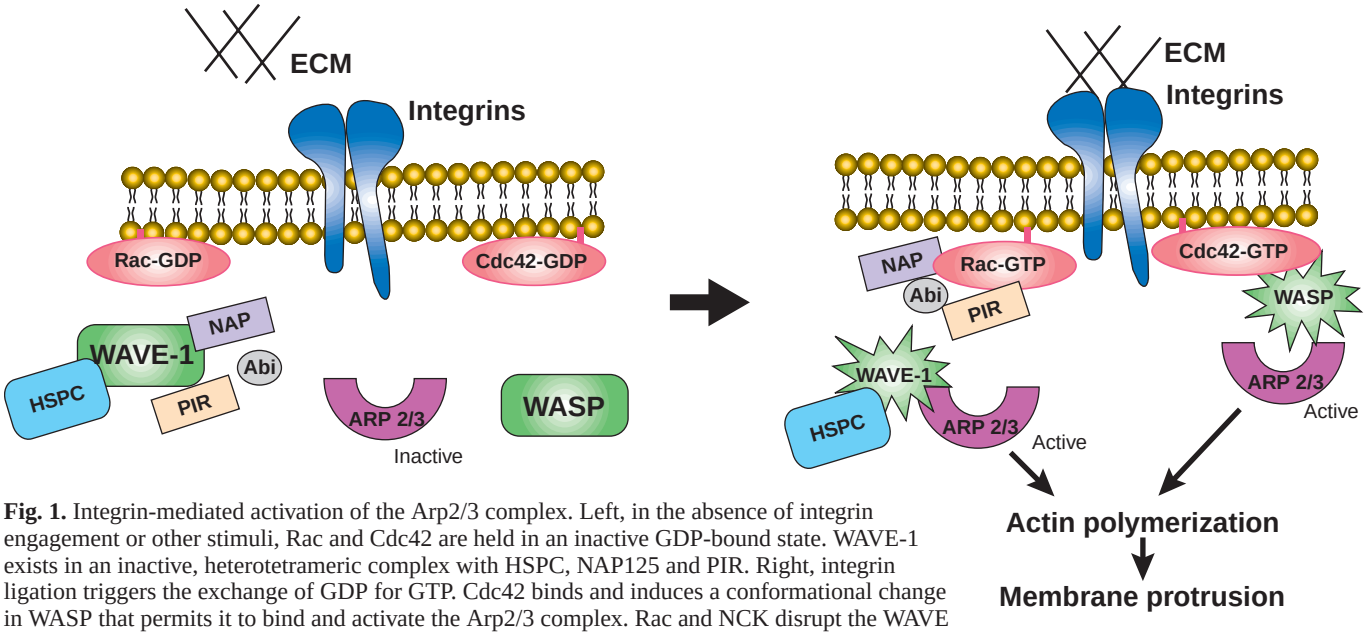

Fig. 1. Integrin-mediated activation of the Arp2/3 complex. Left, in the absence of integrin engagement or other stimuli, Rac and Cdc42 are held in an inactive GDP-bound state. WAVE-1 exists in an inactive, heterotetrameric complex with HSPC, NAP125 and PIR. Right, integrin ligation triggers the exchange of GDP for GTP. Cdc42 binds and induces a conformational change in WASP that permits it to bind and activate the Arp2/3 complex. Rac and NCK disrupt the WAVE complex, causing it to disassemble and release WAVE in association with HSPC300. WAVE binds and activates the Arp2/3 complex.

(Aznavoorian et al., 1996; LaFlamme et al., 1994; Ylanne et al., 1995). Similarly, perturbation of adhesion by blocking antibodies reduces leukocyte spreading on, and protrusion into, endothelial monolayers (Barry et al., 1998). Phagocytosis represents another situation during which contact-induced adhesion triggers membrane protrusion. Particles or pathogens adhere to the host cell and stimulate the formation of lamellipodia, which eventually engulf the foreign object. Perturbation of adhesion to the host cell renders it incapable of initiating the extension of membrane (Krukonis et al., 1998; Leong et al., 1990; Mengaud et al., 1996).

The Rho family of GTPases

Rac and Cdc42

How might adhesion stimulate protrusion? Adhesion to an extracellular matrix, engagement of integrins and cell-cell adhesion all activate members of the Rho family of GTPases (Clark et al., 1998; Cox et al., 2001; Del Pozo et al., 2000; Kim et al., 2000b; Nakagawa et al., 2001; Noren et al., 2001; Price et al., 1998). Two of these family members, Cdc42 and Rac, stimulate the formation of protrusions at the leading edge: Rac controls extension of lamellipodia and Cdc42 controls extension of filopodia. Members of the WASP family of proteins, including WASP and its ubiquitously expressed homolog N-WASP bind to active Cdc42 through its GBD/CRIB (GTPase-binding domain/Cdc42 and Rac interactive binding) motif. Binding of WASP/N-WASP to Cdc42 relieves an intramolecular interaction between the C-terminal VCA (verprolin and cofilin acidic) domain and the GBD/CRIB motif (Kim et al., 2000a). This unmasks the VCA domain, allowing it to bind and activate the Arp2/3 complex (Fig. 1). In contrast to Cdc42, Rac triggers activation of the Arp2/3 complex through a relative of WASP, WAVE/Scar (Machesky and Insall, 1998; Miki et al., 1998). WAVE does not have a GBD/CRIB motif, and direct binding of WAVE to Rac has not been detected (Miki et al., 1998). Two alternative mechanisms for how Rac activates WAVE have been described. Miki and colleagues provided evidence that IRSp53 acts as an adaptor protein that links WAVE-2 to active Rac (Miki et al., 2000). However, other work indicates that IRSp53 binds to Cdc42 rather than Rac (Krugmann et al., 2001). WAVE-1 exists in a heterotetrameric complex that includes orthologues of human p53-inducible messenger RNA (PIR121), a NCK-associated protein (Nap125) and HSPC300 (Fig. 1). Active Rac and NCK disrupt this WAVE complex, causing it to disassemble and release the active WAVE protein in association with HSPC300 (Eden et al., 2002). Active WAVE binds to and activates the Arp2/3 complex, providing the driving force for membrane protrusion at sites of integrin engagement.

Rho activity

In many cells, β1 integrin engagement initially results in a decrease in Rho activity (Arthur et al., 2000; O'Connor et al., 2000; Ren et al., 1999). This decrease involves Src, FAK and the activation of p190RhoGAP (Arthur et al., 2000; Ren et al., 2000). The transient dip in RhoA activity may contribute to membrane extension, because, in many situations, high RhoA activity appears to antagonize protrusion (Arthur and Burridge, 2001; Cox et al., 2001; Kozma et al., 1997; Nobes and Hall, 1999; Rottner et al., 1999). Inhibition of the Rho effector Rho kinase (ROCK) stimulates cell migration (Nobes and Hall, 1999) and promotes membrane protrusion (Rottner et al., 1999; Tsuji et al., 2002; Worthylake and Burridge, 2003), which lead to the idea that high Rho activity antagonizes membrane extension through ROCK. Because ROCK activity stimulates myosin contractility, there has been a presumption that the antagonism of membrane protrusion by Rho is due to excessive contractility. However, other pathways may also be important.

In addition to stimulating myosin activity, ROCK acts on and stimulates LIM kinase, which phosphorylates and inhibits cofilin (Maekawa et al., 1999). Active cofilin severs and depolymerizes actin filaments (reviewed in Bamburg, 1999). Inhibiting cofilin blocks growth-factor-induced actin polymerization and the extension of lamellipodia (Chan et al., 2000; Zebda et al., 2000). These results have been interpreted to be caused by active cofilin severing actin filaments, the resulting free barbed ends promoting polymerization, and the newly polymerized filaments recruiting the Arp2/3 complex (Condeelis, 2001). The Arp2/3 complex bound to the sides of the newly polymerized filaments will nucleate more filaments and give rise to the branched dendritic organization observed at the leading edge of cells. Consequently, the inhibition of cofilin by RhoA, via ROCK and LIM kinase, will tend to stabilize actin filaments and inhibit cofilin's role in promoting nucleation of actin polymerization. Expression of constitutively active cofilin results in inappropriate lamellipodial extensions (Worthylake and Burridge, 2003).

The conclusion that Rho universally antagonizes membrane extension, while appealing, is too simplistic. There are multiple examples, particularly in the case of epithelial cells, in which the converse has been observed. For example, Rho activity was shown to be critical in membrane ruffling induced by PMA (Nishiyama et al., 1994). Subsequent work confirmed the role of Rho and ROCK in PMA-induced membrane ruffling and identified adducin as a relevant target in the cell cortex (Fukata et al., 1999). Working with clone A colon carcinoma cells, O'Connor and colleagues provided evidence that RhoA promotes lamellipodial extension and noted that dominant-negative Rac constructs do not affect membrane extension in this system (O'Connor et al., 2000). A different type of apical membrane protrusion is induced by RhoA activation in NIH 3T3 fibroblasts (Shaw et al., 1998). This extension correlated with phosphorylation of ERM proteins that were recruited to these projections (Shaw et al., 1998). That Rho has been associated both with the generation of protrusive structures and with their inhibition indicates a complexity that is not yet understood. Some of this complexity may derive from different signaling pathways operating in different cell types, but other factors are also likely to be involved and merit further investigation.

Adhesion-mediated signaling

Numerous signaling pathways are initiated downstream of cell-matrix and cell-cell adhesion (reviewed in Juliano, 2002) (Yap and Kovacs, 2003; Zamir and Geiger, 2001a; Zamir and Geiger, 2001b). Some of these pathways affect the activity of Rho family proteins and cell migration. One of the best characterized is activation of focal adhesion kinase (FAK) (reviewed in Schaller, 2001). FAK activation initiates multiple other signaling cascades. At least two of the tyrosine phosphorylated proteins associated with FAK, p130cas (Cas) and paxillin, have been linked to activation of Rac and therefore to lamellipodial extension. Tyrosine phosphorylated Cas assembles a complex with the adaptor protein Crk (Cary et al., 1998; Klemke et al., 1998; Vuori et al., 1996), which associates with DOCK180 (Hasegawa et al., 1996; Matsuda et al., 1996). DOCK180 acts as a guanine nucleotide exchange factor (GEF) for Rac, even though it lacks the DH/PH domain signature motif of most Rho GEFs (Brugnera et al., 2002; Cote and Vuori, 2002). Paxillin also forms a multi-component complex with the proteins PKL and PIX, the latter being another Rac GEF (Bagrodia et al., 1998; Manser et al., 1998; Turner et al., 1999). Assembly and activation of these two signaling complexes depends on the tyrosine phosphorylation of FAK, Cas and paxillin. Not surprisingly, therefore, protein tyrosine phosphatases have key regulatory roles. Deletion of the phosphatase PTP-PEST results in increased spreading and membrane protrusion (Angers-Loustau et al., 1999), and both Cas and paxillin are substrates for PTP-PEST (Garton et al., 1996; Shen et al., 1998). Moreover, PTP-PEST regulates the level of active Rac (Sastry et al., 2002). Deletion of the phosphatase PTPα produces a decrease in cell migration and membrane protrusion (Zeng et al., 2003). This tyrosine phosphatase appears to be acting further upstream in the pathway, such that cells lacking PTPα have decreased Src family kinase activity and diminished FAK tyrosine phosphorylation and activation (Zeng et al., 2003). Decreasing the activation of FAK would be predicted to suppress the downstream activation of both Cas and paxillin and result in depressed Rac activity. Cell adhesion thus triggers numerous signaling pathways that may stimulate membrane protrusion.

Physical coupling between cell adhesion molecules and the protrusive machinery

In addition to the signaling links, new evidence indicates that there is direct physical coupling of the adhesion proteins to the actin assembly machinery. In response to EGF or cell spreading on fibronectin, the Arp2/3 complex is directly recruited to the hinge region of vinculin (Fig. 2) (DeMali et al., 2002). The interaction with vinculin does not itself activate the Arp2/3 complex. This interaction is regulated, requiring PI3K and Rac1 activation, and is sufficient to recruit the Arp2/3 complex to newly formed focal complexes. Cells that are unable to recruit the Arp2/3 complex to vinculin show diminished extension of lamellipodia (DeMali et al., 2002). Targeting of the activated Arp2/3 complex to vinculin thus represents one mechanism for directly coupling the actin polymerization machinery to sites of new cell-matrix adhesion.

In addition to vinculin, two proteins have been implicated in linking the actin polymerization machinery to integrins: N-WASP and cortactin. The β_1-integrin subunit can be co-immunoprecipitated with N-WASP (Sturge et al., 2002). It will be interesting to learn whether this association stimulates the nucleation activity of the Arp2/3 complex and the role this has in integrin-mediated events. Cortactin, a c-Src substrate that binds to the Arp2/3 complex and stimulates its ability to nucleate actin polymerization, represents another potential link between integrins and the Arp2/3 complex (Uruno et al., 2001; Weed et al., 2000). Cortactin becomes tyrosine phosphorylated in response to integrin-mediated adhesion, which suggests a close association with integrins, but it is not seen in focal adhesions and its mode of linkage to integrins remains to be determined (Vuori and Ruoslahti, 1995).

An intriguing link between adhesion and protrusion was suggested by recent work on myosin X. This unconventional myosin is found at the tips of filopodia and in phagocytic cups (Berg and Cheney, 2002; Cox et al., 2002). Overexpression of this myosin promotes formation of filopodia, whereas

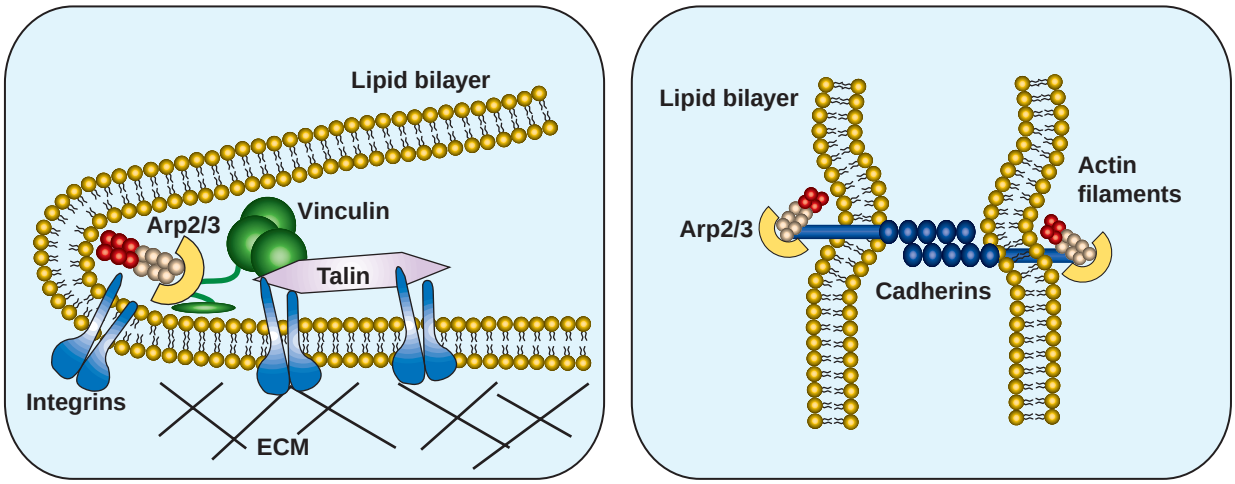

Fig. 2. Physical coupling of adhesion molecules to the actin polymerization machinery. Left, the Arp2/3 complex is directly recruited to sites of integrin engagement through an interaction with the linker region of vinculin, an integrin-associated protein. Right, the Arp2/3 complex is recruited to sites of cell-cell adhesion through an interaction with E-cadherin. Recruitment of the Arp2/3 complex to E-cadherin is thought to localize actin polymerization to sites of cadherin-engagement.

expression of truncated forms inhibits phagocytosis. Both results suggest a role for myosin X in generating membrane protrusions. Interestingly, this myosin possesses three PH domains, one of which binds to phosphatidylinositol 3,4,5-trisphosphate [Ptd(3,4,5)P_3], as well as a FERM domain. The latter have been implicated in binding to the cytoplasmic domains of membrane proteins. Little is known about the components of filopodial tips, but β1 integrins and Mena have been identified at this site (Grabham et al., 2000; Lanier et al., 1999), raising the possibility that myosin X may function together with these proteins, thereby coupling integrin-mediated adhesion to extension of the filopodial membrane.

Direct linkages between the actin polymerization machinery and adhesion molecules are also emerging in the context of cell-cell adhesion. E-cadherin colocalizes and co-immunoprecipitates with the Arp2/3 complex (Kovacs et al., 2002). This interaction localizes actin polymerization to sites of cadherin engagement. Although more work is needed to determine the effect of perturbing this interaction, these findings represent another example of a physical link between membrane protrusion and cell adhesion. Interestingly, vinculin, which acts as a link between integrins and the Arp2/3 complex, is also present at sites of cadherin-mediated adhesion and may represent another mechanism by which the Arp2/3 complex is localized to sites of cadherin engagement.

Lessons from phagocytosis

Phagocytosis provides a striking example of coupling actin polymerization to cell adhesion. In macrophages and neutrophils two distinct mechanisms have been described. One involves the Fcγ receptor (FcγR) that binds to immunoglobulins and the other involves the β_2 integrin complement receptor 3 (CR3). Morphologically, the two types of phagocytosis are different (reviewed in Aderem and Underhill, 1999). Phagocytosis via the FcγR involves the generation of prominent membrane ruffles that extend around the particle to be engulfed (a 'triggering' mechanism). In contrast, much less protrusive activity occurs when the CR3 receptor is engaged. In this latter situation, particles sink into the cell, and localized membrane protrusion surrounds the particle or bacterium by a 'zippering' mechanism (reviewed in Dramsi and Cossart, 1998). Associated with these different morphologies, the FcγR-mediated phagocytosis induces and requires activation of Cdc42 and Rac, whereas the CR3-mediated phagocytosis involves only RhoA (Caron and Hall, 1998). Both phagocytic pathways recruit actin and the Arp2/3 complex to the developing phagocytic cup. This recruitment is blocked by a dominant-negative construct of the Scar/WAVE protein (May et al., 2000). It will be interesting to determine whether the recruitment of the Arp2/3 complex to these specialized sites of adhesion is due only to the localized signaling occurring in these regions or whether it is also accompanied by a physical link to these receptors. The association of the Arp2/3 complex with vinculin is one way by which the Arp2/3 complex may be targeted to these sites and may link directly to the receptors.

Phagocytosis provides a means by which cells clear particulate material and pathogens from their immediate vicinity. However, it is also a route of entry into cells for many intracellular bacterial pathogens. The entry of these bacteria into host cells typically exploits cell adhesion molecules on the surface of the cells and is accompanied by phagocytosis either by the trigger or zippering mechanisms. *Listeria monocytogenes* adheres to host cells via the binding of a bacterial surface protein internalin (InlA) to E-cadherin (Fig. 3) (reviewed in Braun and Cossart, 2000) (Dramsi and Cossart, 1998). This induces phagocytosis via the zippering mechanism. Uptake involves actin polymerization as demonstrated by its inhibition with cytochalasin D (Gaillard et al., 1987). It also requires tyrosine phosphorylation and activation of PI3 kinase (Ireton et al., 1996). *Yersinia pseudotuberculosis* exploits a different adhesive interaction to enter cells. This bacterium expresses a protein, invasin, on its surface that binds with high affinity to β1 integrins (Cornelis, 2002; Isberg et al., 2000). *Yersinia* uptake requires Rac1 and

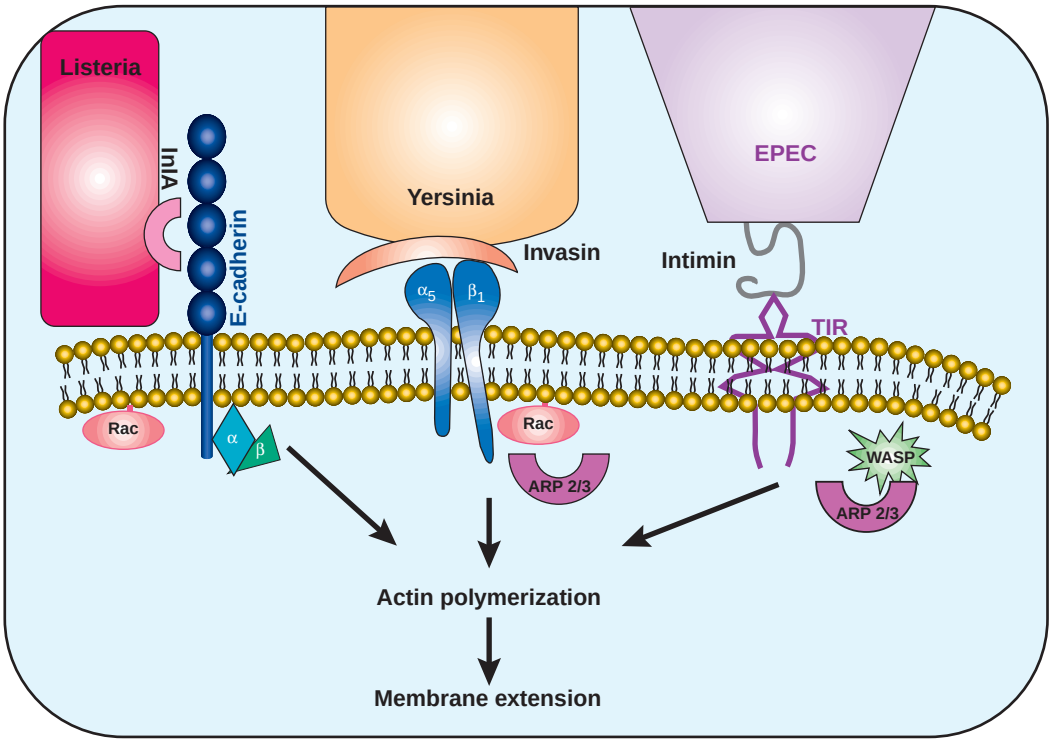

Fig. 3. Various bacterial pathogens and their mechanisms for stimulating membrane protrusion. *Listeria monocytogenes* adheres to host cells via the binding of a bacterial surface protein, internalin A (IlnA) to E-cadherin. E-cadherin, via Rac activation, can trigger dynamic events of actin polymerization and membrane extension, culminating in bacterial uptake. Yersinia expresses invasin on its surface, which binds with high affinity to α5β1 integrins. Yersinia uptake requires Rac1 and the Arp2/3 complex. EPEC attach to host cells through translocated intimin receptor (TIR), a receptor secreted by EPEC and inserted into the host cell plasma membrane where it acts as a receptor for intimin. WASP and the Arp2/3 complex are recruited to sites of attachment and stimulate actin polymerization required for pedestal formation.

the Arp2/3 complex, but some disagreement exists as to the role of N-WASP in this process (Alrutz et al., 2001; McGee et al., 2001; Wiedemann et al., 2001).

Association of enteropathogenic *Escherichia coli* (EPEC) with cells is a special example because it is not internalized but bound to a host cell protrusion known as a pedestal. Attachment to the pedestal is mediated via the translocated intimin receptor (TIR), a receptor secreted by EPEC, and inserted into the host cell plasma membrane, where it acts as a receptor for intimin expressed on the bacterial surface (Fig. 3) (Kenny et al., 1997). TIR shares a number of similarities with integrins in that it clusters, stimulates tyrosine phosphorylation of effector proteins and recruits several cytoskeletal proteins, including α-actinin, talin, cortactin, ezrin, VASP, villin and fimbrin to the site of EPEC attachment (Freeman et al., 2000; Huang et al., 2002) (reviewed in Goosney et al., 2001). Adhesion via TIR stimulates actin reorganization to form the pedestal structure that can extend up to 10 μm beneath the pathogen (Rosenshine et al., 1996). WASP and the Arp2/3 complex are recruited to the actin pedestal beneath the bacterium (Kalman et al., 1999; Lommel et al., 2001). Interference of WASP with dominant-negative constructs or cells lacking N-WASP prevents pedestal formation (Kalman et al., 1999; Lommel et al., 2001). Interestingly, cortactin, which binds and activates the Arp2/3 complex, is directly recruited to TIR, and dominant-negative mutants of cortactin block F-actin accumulation beneath the attached bacteria (Cantarelli et al., 2000; Cantarelli et al., 2002).

Adhesion and the inhibition of protrusion

In the preceding sections we have focused on how adhesion can trigger membrane extension, and yet there are many examples where adhesion is antagonistic to new membrane protrusion and cell migration. The concept of 'contact inhibition' of migration emerged from early observations of cells colliding in tissue culture (Abercrombie, 1967). This work revealed that the motile activity of a lamellipodium was often inhibited by contact with another cell (Abercrombie, 1967). Epithelial cells demonstrate contact inhibition particularly clearly. These cells in culture will form tight adhesions with their neighbors such that protrusive activity is confined to regions free of contact with other cells. The basis for contact inhibition is not fully understood. In some cases, the development of strong cell-cell adhesion may physically restrain cells, but signaling pathways downstream of the engaged cell adhesion molecules must also be involved. One might have anticipated that engagement of cadherins would have depressed Rac activity and thus have suppressed membrane ruffling, but, somewhat surprisingly, the opposite occurs. Initial cadherin engagement stimulates an increase in Rac and Cdc42 activity (Kim et al., 2000b; Nakagawa et al., 2001; Noren et al., 2001). Active Rac and Cdc42 promote assembly of cell-cell junctions in epithelia. Other signaling pathways must be activated that suppress membrane ruffling while allowing activation of Rac and Cdc42. Deciphering the pathways involved will be interesting and important for understanding the loss of contact inhibition that occurs with many tumor cells and for understanding the factors that contribute to tumor cells breaking away from the normal constraints of their neighbors.

In mature tissues, there is abundant adhesion either to other cells or to the matrix, and yet membrane protrusive activity is

usually suppressed. The stimulation of membrane extension by adhesion appears to be largely the result of new adhesions occurring. The time course of activation of Rac and Cdc42 in response to adhesion to the ECM (Cox et al., 2001; Del Pozo et al., 2000; Price et al., 1998) is consistent with this interpretation. An initial stimulation that occurs during the first few minutes declines to a baseline level over a period of hours. Similarly, the recruitment of the Arp2/3 complex to vinculin is a transient phenomenon. In this case, it lasts only for a few minutes following matrix adhesion and is then suppressed in mature adhesive structures such as focal adhesions (DeMali et al., 2002). Hence, the timing of interactions as well as the extracellular environment may dictate whether adhesion stimulates or inhibits protrusion.

Some of the best-characterized examples of adhesion molecules either inhibiting or stimulating membrane protrusive activity and cell migration occur in the nervous system. Several families of adhesion molecules have been identified that affect neurite outgrowth and growth cone guidance either in a positive or in a negative way (Dickson, 2002). Space limitations prevent discussion of this topic, but it is relevant that many of these receptors in nerve growth cones initiate signals that regulate the activities of Rho family proteins (reviewed in Giniger, 2002).

Just as lessons can be learned from bacteria that trigger phagocytosis through their adhesion to host cells, so too information can be acquired from bacteria that inhibit phagocytosis to promote their survival. Intriguing examples involve the enteropathic bacteria *Yersinia pseudotuberculosis* and *Y. enterocolitica.* Within the host intestine, these bacteria initially promote their own phagocytosis by M cells in the intestinal epithelium. Entry into these cells provides their passage out of the intestinal lumen and into the body. These bacteria then adhere to immune cells within Peyer's patches but inhibit phagocytosis that otherwise would lead to their destruction in phagolysosomes. As mentioned above, the *Yersinia* bacteria use adhesins (as well as other bacterial surface proteins) to adhere very strongly to β1 integrins on host cells (reviewed in Isberg and Barnes, 2001). The tightly adhering *Yersinia* use a protein delivery system to inject into the host cell a series of proteins that function to inhibit both phagocytosis and the development of the host's immune response. Three of the proteins (Yops) delivered by *Yersinia* affect, directly or indirectly, the Rho family GTPases. YopE is a RhoGAP that acts on RhoA, Rac and Cdc42 to promote their hydrolysis of GTP and consequent inactivation (Black and Bliska, 2000; Von Pawel-Rammingen et al., 2000). YopT is a protease that cleaves RhoA, Rac and Cdc42 close to their C-terminal prenylation site (Shao et al., 2003). Their prenyl groups link these small G-proteins to cell membranes and so this cleavage will release them from their sites of action. YopH is a potent tyrosine phosphatase that inhibits phagocytosis (Rosqvist et al., 1988; Zhang et al., 1992). Introduction of this phosphatase into the cells disrupts the organization of the actin cytoskeleton (Schneider et al., 1998), and this phosphatase targets many of the tyrosine phosphorylated proteins in focal adhesions such as FAK and p130cas (Black and Bliska, 1997; Persson et al., 1997). Given that FAK and p130cas signal downstream to Rac activation, this suggests at least one way by which this tyrosine phosphatase will depress Rac activity. An additional Yop protein, YopO (also known as YpkA, for *Yersinia* protein kinase A) is a serine-threonine protein kinase that binds RhoA, Rac and actin (Dukuzumuremyi et al., 2000; Galyov et al., 1993; Juris et al., 2000). This probably also contributes to the inhibition of phagocytosis, but the mechanism is not fully understood. The work on how *Yersinia* inhibits phagocytosis emphasizes once again the critical role Rho GTPases play in regulating membrane protrusion in response to adhesion.

Conclusions

In most models of cell migration, membrane protrusion precedes cell adhesion. Cell adhesion is generally considered to be important for providing traction to the underlying substratum on which the cell is moving. Undoubtedly, this is crucial, but here we have provided evidence that adhesion additionally contributes to membrane extension during cell migration, as well as in other situations. Engagement of adhesion molecules initiates signaling pathways that regulate the Rho family of GTPases in both negative and positive ways. In turn, these key regulatory proteins stimulate or inhibit membrane protrusive activity. Besides triggering signaling pathways that lead to extension of the plasma membrane, cell adhesion molecules such as integrins and cadherins can couple to the Arp2/3 complex that nucleates actin polymerization, thereby providing a direct link between adhesion and membrane protrusion.

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