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Dynamin as a mover and pincher during cell migration and invasion

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Summary

The large GTPase dynamin, long known for its role in endocytosis, has most recently been implicated as a facilitator of cell migration and invasion. Recent observations link dynamin to the cycle of membrane expansion and retraction essential for cell motility. Its role in actin polymerization, membrane deformation and **vesiculation, and focal adhesion dynamics are all important for this process, and the new findings provide exciting directions for studies of this ubiquitous and diverse protein family.**

Key words: Dynamin, Cell Motility, Migration, Invasion

Introduction

Cell migration is an essential function that is used for normal cell processes, such as wound healing (Lambrechts et al., 2004), differentiation (Weston, 1971), and neuronal development (Kanatani et al., 2005), as well as aberrant processes, such as tumor cell invasion and metastasis (Titus et al., 2005). Migrating cells exhibit pronounced membrane extension at the leading edge, which is promoted largely by actin polymerization, concomitant with cycles of retraction mediated by the assembly and disassembly of adhesions (Vicente-Manzanares et al., 2005). While the leading edge of a migrating cell is expanding and establishing new connections to the extracellular matrix, the rear disassembles its focal adhesions, allowing the cell to retract. In addition, migration involves the rearrangement of specific cellular structures, such as the Golgi complex (Bershadsky and Futerman, 1994), the centrosome (Xie and Tsai, 2004) and focal adhesions (Tamura et al., 1998). The discovery that the large GTPase dynamin resides at these diverse cellular locations suggests that, along with its well-documented role in endocytosis, dynamin plays a significant role in cell migration. Here, we review recent findings indicating that this is indeed the case and provide some insights into how the dynamin family might contribute to the complex process of pushing and pulling a cell forward.

Dynamins as polymeric contractile scaffolds: attributes helpful for cell motility

Since the isolation of the first dynamin protein in 1989 (Shpetner and Vallee, 1989), a larger superfamily of dynamins has been described, including three conventional dynamins (Dyn1, Dyn2 and Dyn3), several related forms (dynamin-like proteins, DLP/DRP) that have been implicated in mitochondrial dynamics (Labrousse et al., 1999), and the previously identified Mx proteins (MxA and MxB), which have antiviral capacity (Haller and Kochs, 2002). Additional diversity is provided by considerable alternative splicing, leading to scores of different variants in mammalian cells (Cao

et al., 1998). Not all of these forms are expressed in a single cell type: Dyn1 is restricted to neuronal cells; Dyn2 is ubiquitously expressed; and Dyn3 may be limited to brain, lung and testis. Because dynamin has been implicated largely in the migration of epithelial cells, we focus here predominantly on Dyn2.

All of the conventional dynamins are large molecular weight GTPases ~96 kDa in size, which differentiates them from the class of small GTPases. In addition, the dynamins share significant homology in several different domains that appear important for function (Fig. 1). These include a highly conserved N-terminal GTPase domain, a 'middle domain' that is also well conserved but has unknown function, a pleckstrin-homology (PH) domain that interacts with phosphoinositides such as phosphatidylinositol 4,5-bisphosphate (PIP2) (Barylko et al., 1998), and a GTPase effector domain (GED) that is thought to function as an internal GTPase-activating protein (GAP) domain and thus participates in self-regulation (Muhlberg et al., 1997). At the C-terminus resides a proline-rich domain (PRD) whose sequence varies significantly between the dynamin isoforms. This domain interacts with a wide variety of SH3-domain-containing endocytic adaptor proteins, such as Grb2 (Gout et al., 1993) and intersectin (Zamanian and Kelly, 2003), BAR-domain-containing proteins such as amphiphysin and endophilin (Ringstad et al., 1997), and actin-regulatory proteins (see below) such as cortactin (McNiven et al., 2000) and Abp1 (Kessels et al., 2001) (see Table 1).

A remarkable property of the dynamins is their ability to self-assemble into complex polymers of defined dimensions. This trait distinguishes dynamin from small GTPases that act as switches. Furthermore, unlike tubulin, a GTPase that also forms large polymers, dynamin polymers constrict when GTP is hydrolyzed (Fig. 1). Indeed, a variety of different structural approaches have demonstrated that upon nucleotide hydrolysis the internal diameter of the dynamin polymer is reduced (Danino et al., 2004). This hydrolysis-constriction cycle can be
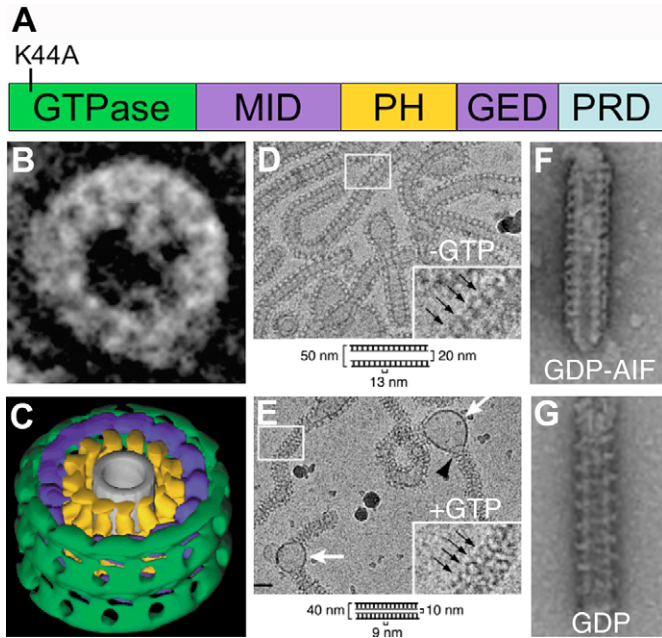
Fig. 1. Dynamin self-assembles and tubulates lipids. (A) Scheme of dynamin protein showing the GTPase domain, the middle domain (MID), the pleckstrin-homology domain (PH), the GED domain, and the proline-rich domain (PRD). (B) Negative stain showing that dynamin protein self-assembles into rings in the presence of GTPγS; adapted from Hinshaw (Hinshaw, 2000), reprinted with permission. (C) The surface rendering depicts dynamin's ability to constrict around tubules in an ordered manner. The dynamin head domain is shown in green, the stalk is blue, the leg is gold, and the inner lipid leaflet is grey; adapted from Zhang and Hinshaw (Zhang and Hinshaw, 2001), reprinted with permission. Cryo-EM of ΔPRD tubules before (D) and 5 seconds after (E) addition of GTP. The diagrams below each panel depict the constriction of the tubules in the presence of GTP. White arrows indicate undecorated lipid bulges and black arrowheads indicate transitions between constricted, decorated tubules and lipid bulges; adapted from Danino et al. (Danino et al., 2004), reprinted with permission. Bar, 100 nm. Negative stain of GDP-AlF (F) and GDP (G) dynamin on tubules depicting the change in pitch of dynamin rings after hydrolysis of GTP occurs; adapted from Stowell et al. (Stowell et al., 1999), reprinted with permission.

activated by interaction of the PH domain with membrane lipids such as PIP2 or by binding of the PRD to some of the effectors mentioned above (Lin et al., 1997). Through this constriction process, dynamin can deform membranes and, in association with other proteins, such as BAR-domain-containing proteins and the actin cytoskeleton (Itoh et al., 2005), serves as a pinchase, releasing vesicles from donor membrane compartments. This pinchase activity is essential for the role of dynamin in endocytosis. In fact, McMahon and colleagues have shown that such a constriction not only coincides with membrane scission but is also required for this event to proceed (Marks et al., 2001).

Perhaps the most dramatic demonstration to date of the combined actions of dynamin and actin in membrane tubulation and vesiculation comes from a recent study by Itoh et al. (Itoh et al., 2005). They identified a novel subset of related proteins that contain F-BAR domains, which are similar to the previously identified BAR-domain proteins, some of

which regulate the actin cytoskeleton (Peter et al., 2004). F-BAR proteins are cytosolic proteins that tubulate and deform membranes, probably by inducing membrane curvature. They interact with dynamin and work in synergy with it to tubulate and vesiculate cellular membranes. Interestingly, expression of F-BAR proteins leads to a dramatic tubulation of the plasma membrane; however, when dynamin is co-expressed, the cells cannot form pronounced tubules (Fig. 2). Instead, these tubules are vesiculated, presumably because the pinchase activity of dynamin vesiculates the F-BAR-induced long tubules. Vesiculation of these tubules can be attenuated either by expression of a K44A mutant Dyn2 protein or, interestingly, treatment with the actin antagonist Latrunculin, which indicates that the pinchase function of Dyn2 on the F-BAR-formed tubules depends on an intact actin cytoskeleton.

This capacity for rapid assembly and constriction while interacting with adaptor, signaling and cytoskeletal molecules that leads to the deformation and scission of cellular membranes makes dynamin unique. We can thus view it as a contractile polymeric scaffold (Thompson and McNiven, 2001) that could also have a regulatory role (Song and Schmid, 2003) at multiple cellular sites by engaging in spatial and temporal interactions with many binding partners. Interestingly, although dynamin localizes to the leading edge of migrating cells, little caveolae- or clathrin-based endocytic activity occurs at the lamellipodium of a cell.

One might imagine that a dynamic dynamin polymer that can pinch, pull, push, or sever membranes in concert with an actin framework could make a substantial contribution to lamellipodium extension, cell adhesion, and uropodial retraction. Indeed, recent studies on the interaction of dynamin with lipids, actin and several new binding partners are providing some insight into its lamellipodial localization and support this notion.

Dynamin and actin dynamics

Several seminal studies provided the initial impetus for pursuing a relationship between dynamin and the actin cytoskeleton. Initially, De Camilli and colleagues (Ochoa et al., 2000) found Dyn2 to be situated at podosomes of transformed cells and osteoclasts. These matrix-degrading structures at the base of cells are formed from tubular invaginations of the plasma membrane and appear to require both actin and Dyn2, because expression of Dyn2 mutants in these cells disrupts podosome formation. Although the precise mechanisms by which Dyn2 might participate in podosome formation or function are still unknown, this study provided the first functional link between components of the actin cytoskeleton and the previously categorized endocytic protein Dyn2.

Three studies subsequently showed that actin-binding proteins interact with dynamin. We and others observed that at the plasma membrane Dyn2 strongly interacts with cortactin (McNiven et al., 2000), an SH3-domain-containing protein that demonstrates actin-binding and -remodeling activity in response to Erk phosphorylation (Martinez-Quiles et al., 2004). Overexpression of a dominant-negative cortactin mutant that lacks the SH3 domain and cannot interact with Dyn2 significantly reduces the recruitment of dynamin to membrane ruffles and increases the number of actin stress fibers. This cortactin-Dyn2 interaction is essential for vesicle formation both at the plasma membrane (Cao et al., 2003) and at the
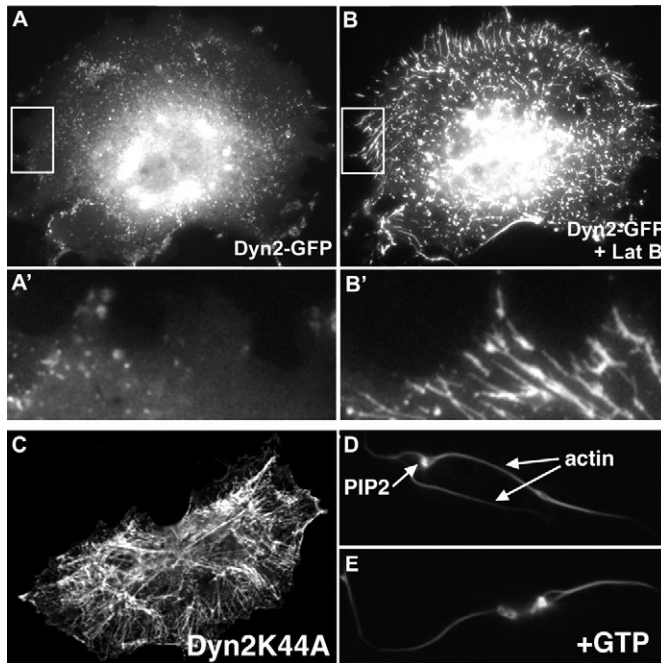
Fig. 2. Dynamin tubulates lipids cooperatively with the actin cytoskeleton. (A,B) Co-expression of RFP-FBP17, an F-BAR-domain-containing protein, and GFP-Dyn2 results in antagonization of formation of lipid tubules, which is relieved by disruption of the actin cytoskeleton by Latrunculin B treatment (B). (A') and (B') provide a higher magnification of the vesiculation (A') or tubulation (B') of lipids at the plasma membrane. These data demonstrate the importance of the cooperation of Dyn2 and actin in vesiculation of the membrane; adapted from Itoh et al. (Itoh et al., 2005), reprinted with permission. (C) Fluorescence image of a rat fibroblast expressing a GFP-Dyn2abK44A mutant, which results in formation of dynamin-coated lipid tubules extending inwards from the plasma membrane (Cao, H. and M.A.M., unpublished data). (D,E) Two examples of PIP2-containing membranes developing long actin cables in the presence of GTP. Long actin filament bundles form, demonstrating dynamin's ability to alter the organization of actin filaments; adapted from Schafer et al. (Schafer et al., 2002), reprinted with permission.

trans-Golgi network (Cao et al., 2005). At the same time, Qualmann and Kelly (Qualmann and Kelly, 2000) observed that isoforms of the scaffolding protein syndapin bind to Dyn1 and appear to participate in a variety of dynamic cellular functions, such as extension of filopodia and endocytosis. Finally, Kessels and co-workers demonstrated a link between dynamin and the actin-binding protein Abp1 (Kessels et al., 2001); overexpression of dominant-negative Abp1 constructs that disrupt interaction of the wild-type protein with dynamin resulted in a significant reduction in transferrin endocytosis. These three studies were among the first to show a direct link between the endocytic machinery and the actin cytoskeleton, implicating a role for dynamin in the process. They also suggested that, in addition to pinching off membranes during endocytosis, dynamin also plays a wider role in regulation of actin dynamics.

Specific forms of dynamin appear to preferentially interact with cortactin. For example, in the dendritic spines of hippocampal neurons, which contain actin, cortactin and Dyn3

(Gray et al., 2003), one splice variant of Dyn3 (Dyn3baa) induces the formation of long, immature, filopodium-like spines that can extend several micrometers in length. By contrast, a splice variant (Dyn3aaa) that is also expressed in neurons but has an eight-residue insert just upstream of the PH domain does not induce this marked morphological change. In vitro binding experiments revealed that the Dyn3baa form exhibits a 200% higher affinity for cortactin than does Dyn3aaa.

Further evidence that Dyn2 regulates actin dynamics comes from studies (Lee and De Camilli, 2002; Orth et al., 2002) showing that it associates with actin-propelled vesicle comets formed as a consequence of overexpression of type I phosphatidylinositol phosphate 5-kinase (PIP5KIα). Expression of the GTPase-deficient DynK44A mutant or a DynΔPRD mutant inhibits the formation, growth and speed of these comets. Interestingly, Dyn2 is concentrated at the actin–vesicle-membrane interface but also extends along the length of the comet tails. This suggests a direct structure-function relationship with the actin filament network, implicating dynamin in the regulation of actin polymerization. At the same time, an in vitro study by Schafer and colleagues (Schafer et al., 2002) provides insights into the actin-nucleating and -organizing capacity of Dyn2. In vitro actin polymerization assays demonstrate that Dyn2 has a biphasic effect on actin polymerization. In the presence of cortactin, low concentrations of dynamin enhance actin polymerization whereas higher concentrations inhibit this. Association of dynamin with lipid vesicles also increases rates of actin polymerization. The Dyn2K44A GTPase mutant inhibits the effect, which suggests that both its enzymatic activity and its binding to actin-binding proteins are important.

Forming and extending lamellipodia
Recent work indicates that dynamin might promote efficient cell migration by participating in lamellipodial extension. Recruitment of Dyn2 to lamellipodia depends upon a pre-established cortactin-rich cortical network, because expression of a cortactin protein lacking the SH3 domain greatly reduces localization of Dyn2 to the leading edge. Expression of a Dyn2ΔPRD protein prevents its recruitment to the lamellipodium but has no effect on cortactin distribution (McNiven et al., 2000). Dynamin may thus link the cortical actin cytoskeleton with other processes, including lamellipodial extension.

Krueger and colleagues (Krueger et al., 2003) have viewed GFP-tagged Dyn2 in living cells upon treatment with motogenic growth factors, such as PDGF or EGF. They observe a dramatic recruitment of both Dyn2 and cortactin to circular dorsal ruffles or 'waves' (Fig. 3). These dynamic and ephemeral structures form at the leading edge of cells and appear to function, in part, as sites of active actin remodeling that aid lamellipodial protrusion. When dorsal waves form, there is a dramatic reorganization of actin; large, rigid stress fibers disassemble and a finer, more pliable actin meshwork is formed within the lamellipodium. This occurs almost exclusively at the site of lamellipodial extension, and preventing wave formation greatly attenuates extension of the leading edge of an activated cell along with subsequent motility (Krueger et al., 2003). The recruitment of a Dyn2-cortactin complex to dorsal waves is essential and concomitant with that
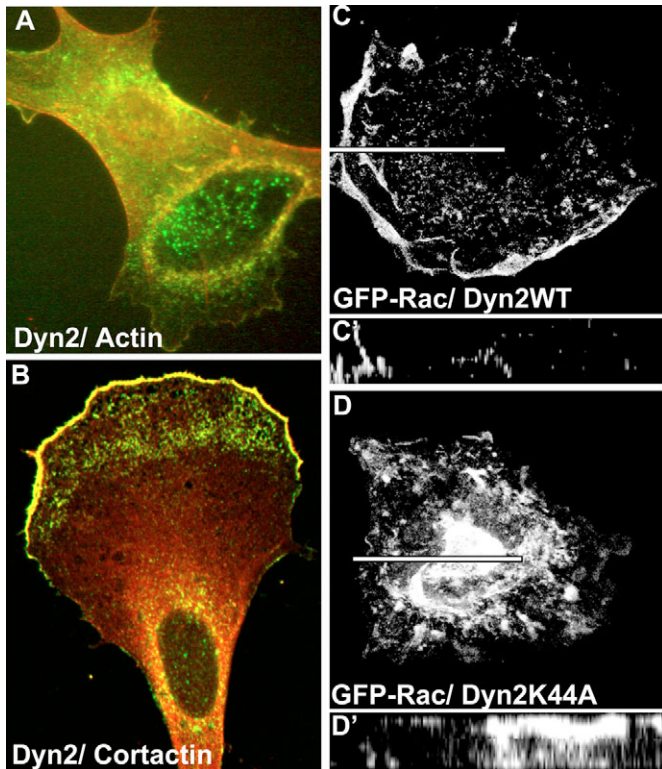
Fig. 3. Dynamin participates in dorsal wave and lamellipodia formation. (A) Fluorescence image of a dorsal wave in a rat fibroblast stimulated for 5 minutes with PDGF that accumulates both Dyn2 (green) and actin (red) at the progressing wave structure (Orth, J. D. and M.A.M., unpublished data). (B) Dyn2 and cortactin accumulate at the leading edge of a rat fibroblast stimulated with EGF (Orth, J. D. and M.A.M., unpublished data). (C) Wild-type dynamin and Rac accumulate in the lamellipodium of an NIH3T3 fibroblast co-expressing constitutively active Rac, whereas dominant-negative Dyn2K44A (D) disrupts lamellipodial extension as well as accumulation of Rac at the lamellipodium. Confocal imaging was used to create Z-series reconstructions, indicated by the lines in (C) and (D) and shown in (C') and (D'); adapted from Schlunck et al. (Schlunck et al., 2004), reprinted with permission.

of multiple components of the Arp2/3–N-WASp network, which is central to actin reorganization during cell migration.

The small GTPase Rac, a protein known to mediate lamellipodium extension (Nobes and Hall, 1995), associates with wave complexes. Although there is no direct physical interaction between dynamin and Rac, several functional interdependencies between the two GTPases are observed (Schlunck et al., 2004). Expression of mutant Dyn2 or knocking down Dyn2 by RNAi reduces lamellipodial extension while preventing recruitment of Rac (Fig. 3). Surprisingly, despite these disruptions, there is an increase in total Rac activity. In mutant-Dyn2-expressing cells, Rac appears to be localized to long tubules emanating from the plasma membrane. Thus, there seems to be an intimate relationship between these two GTPases that plays an essential role in migration. It will be important to define this interaction further. Insights have been provided by Gomez and colleagues (Gomez et al., 2005), who have shown that these proteins

participate in the formation of the immunological synapse in activated T-cells. Synapse formation in these cells is regarded as a modified form of lamellipodial activity that is dependent upon dynamic actin reorganization. Interestingly, Dyn2 appears to regulate this process through direct interaction with the SH3 domain of the Rac guanine nucleotide exchange factor (GEF) Vav1. Dyn2 does not appear to affect the GEF activity of Vav1. Instead it is recruited to the synapse by this GEF and appears to recruit other cytoskeletal proteins and affect downstream signaling.

Related observations in the amoeba proteus support findings in mammalian cells. Injection of anti-dynamin antibodies into this organism results in a loss of directional migration (Dominik et al., 2005) as well as a reduction in the rate of uroidal translocation. Amoeba Dyn2 appears to precipitate with actin pellets in an ATP-independent manner, which emphasizes its role in actin dynamics. Inactivation of amoeba orthologs of Dyn2 disrupts the polarity of the cell and results in the extension of pseudopodia in opposite directions. Although little is known about focal adhesions in amoeba, blocking Dyn2 might affect the formation of focal adhesion structures, leading to defective uroidal retraction and a loss of directed cell movement.

Dynamics at the cell base

The physical interaction between the cell and the substratum is tightly regulated and an essential factor in cell migration. Dyn2 plays an important role in several structures that mediate cell-matrix interactions, including focal adhesions, podosomes and invadopodia.

Focal adhesions are complex structures composed of many proteins assembled around integrins, which provide a transmembrane link between the cytoplasm and the extracellular matrix. Regulated assembly and disassembly of these structures is essential for cells to move forward at the leading edge while retracting at the rear. The number of cytoskeletal and signaling components found at adhesion sites now exceeds 20, and dynamin is a newly confirmed addition to this complex, exhibiting several interactions with focal adhesion components. Recent findings now extend a seminal observation by Cypher and Letourneau (Cypher and Letourneau, 1991), who observed that dynamin is enriched at adhesion sites in growth cones from embryonic chick brain.

Prominent components of focal adhesions are the syndecans, a family of membrane glycoproteins known to promote the formation of stress fibers and focal adhesions. Using a yeast two-hybrid approach, Yoo et al. showed that the PH domain of Dyn2 interacts with syndecan-4 (Yoo et al., 2005). Importantly, Dyn2 redistributes from a diffuse cytoplasmic distribution to colocalize with syndecan-4 and paxillin at focal adhesion sites after stimulation of cells with lysophosphatidic acid (LPA) (Yoo et al., 2005). At the same time, Ezratty et al. (Ezratty et al., 2005) observed that Dyn2 interacts and colocalizes with focal adhesion kinase (FAK) at focal adhesions. Dyn2 appears to play a functional role in cell adhesion, because a direct interaction between these two proteins is important for cell migration. Furthermore, a dominant-negative Dyn2 mutant inhibits the disassembly of focal adhesions in translocating cells. Indeed, cells expressing the Dyn2 mutant have reduced rates of migration into a wound and exhibit a characteristic drag of the uropod, which suggests an inappropriate

Table 1. Dynamin binding partners

|	Dynamin binding partner	Function	Reference
Scaffolding proteins	ArgBP2/nArgBP2	Adaptor protein colocalizing with actin at stress fibers and adhesion sites	Cestra et al., 2005
		Substrates of Abl/Arg tyrosine kinases and Cbl ubiquitin ligase	
	Intersectin 1L	Scaffold protein in endocytosis	Zamanian and Kelly, 2003
		Guanine nucleotide exchange factor for CDC42	
	Tuba	Scaffold protein that binds actin regulatory proteins	Salazar et al., 2003
	Cbl	Adaptor protein and E3 ubiquitin ligase	Bruzzaniti et al., 2005
	Grb-2	Adaptor protein linking tryosine phosphorylated receptors to downstream effector proteins	Seedorf et al., 1994
	Nck	Adaptor protein mediating downstream signals	Wunderlich et al., 1999
Cytoskeletal proteins	Abp1	Actin binding protein involved in receptor mediated endocytosis	Kessels et al., 2001
	Caveolin	Major component of caveolae and a stuctural/scaffolding protein	Yao et al., 2005
	Cortactin	Actin binding protein and prominent substrate of Src kinase	McNiven et al., 2000
	Profilin	Regulator of actin assembly by binding monomeric actin	Witke et al.,1998
	Focal Adhesion Kinase (FAK)	Mediator of integrin signaling and component of focal adhesions	Ezratty et al., 2005
	Src	Non-receptor tyrosine kinase involved in secretion and upregulated in several cancers	Foster-Barber, 1998
	Syndecan-4	Membrane proteoglycan that mediates focal adhesion and stress fiber formation	Yoo et al., 2005
	Phospholipase C (PLC-γ)	GEF for dynamin-1 and substrate for receptor tyrosine kinases	Seedorf et al., 1994
	Vav-1	Rho family GEF involved in formation of the immunological synapse	Gomez et al., 2005
	Formin-binding protein 17	Involved in endocytosis and deforming plasma membrane	Kamioka et al., 2004
	γ-Tubulin	Centrosomal protein involved in microtubule nucleation	Thompson et al., 2004
	Microtubules	Structural support and cell locomotion	Shpetner and Vallee, 1989
Endocytic regulators	Endophilin-1	Synaptic vesicle endocytosis	Ringstad et al., 1997
	Amphiphysin-II	Synaptic vesicle endocytosis	David et al., 1996
	Syndapin/PASCIN	Coupling membrane traffic to the actin cytoskeleton	Qualmann and Kelly, 2000
	Phosphatidylinositides	Lipid components of membranes	Zheng et al., 1996
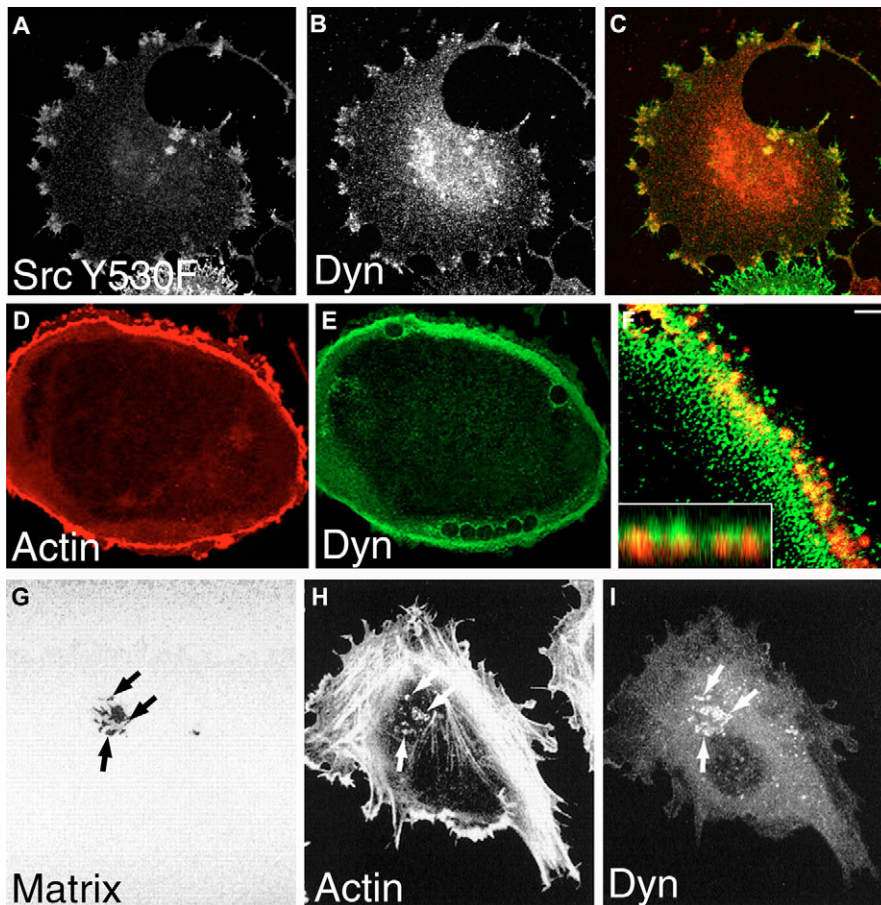
Fig. 4. Dynamin localizes to focal adhesions, podosomes and invadopodia. Dynamin (A) colocalizes with constitutively active SrcY530F (B) in HeLa cells at the sites of focal adhesions (C, merge) (Cao, H. and M.A.M., unpublished data). Osteoclasts form a podosome ring, which accumulates both actin (D) and Dyn2 (E). (F) Confocal imaging shows the overlap of Dyn2 (green) and actin (red) at high resolution. A375MM melanoma cells demonstrate an association of Dyn2 (I) with actin (H) at invadopodia where the degradation of extracellular matrix occurs (G, arrows); adapted from Baldassarre et al. (Baldassarre et al., 2003), reprinted with permission.

persistence of focal adhesion attachment. These findings highlight the idea that focal adhesion disassembly is not simply the reversal of assembly, because Dyn2 is not required for focal adhesion assembly. Rather, Dyn2 appears to regulate a specific process of focal adhesion disassembly through its interaction with FAK and other members of the cytoskeleton. Note that, along with the actin cytoskeleton, focal adhesions also associate with microtubules, which may have a role in focal adhesion turnover and retraction of the rear of the cell during cell migration.

In addition to focal adhesions at the cell base, normal and neoplastic cells form membranous ventral invaginations such as podosomes and invadopodia that protrude into the extracellular matrix. Dyn2 appears to associate with actin-coated tubules at the site of podosome assembly in BHK and 3T3 cells, and a temperature-sensitive dominant-negative dynamin mutant results in a marked loss of podosomes. FRAP studies demonstrate that mutant Dyn2 significantly reduces the turnover of actin at the podosome site, which suggests that it may participate in actin dynamics during podosome formation. The precise role of podosomes in cell migration is not well defined, because it is unclear whether these structures are actually invadopodia, which are nearly identical in form and content. Like podosomes, invadopodia appear as actin-rich tubules of the ventral plasma membrane that both protrude into the extracellular matrix and invaginate into the cell interior. Providing a secretory and endocytic function, invadopodia are sites of protease release and subsequent endocytosis, facilitating matrix degradation and thereby promoting cell translocation and invasion.

Dynamin is now known to have an essential role in focal matrix degradation by invadopodia/podosomes in a variety of neoplastic and primary cells. Reduction of Dyn2 levels in a human melanoma cell line by RNAi or expression of K44A Dyn2 reduces the cellular capacity to form invadopodia and degrade the extracellular matrix (Baldassarre et al., 2003). Electron microscopy reveals the invadopodia to be reduced in number and in the size of the extension. Dyn2 might therefore be involved in both the structural aspects of invadopodia formation as well as the functional aspects of extracellular matrix degradation.

Matrix degradation by osteoclasts is perhaps the best studied of several cell models of matrix degradation, and Dyn2 is a key player in this process. A prominent function of osteoclasts is the degradation and resorption of matrix during bone remodeling, allowing free movement through the osteo-lacunae. Dynamin and cortactin are significantly enriched at sites of matrix degradation and resorption in osteoclasts and, furthermore, overexpression of dynamin in these cells leads to increased matrix resorption and migration. Accordingly, inhibition of dynamin function via the expression of the dominant-negative K44A mutant inhibits both migration and resorption (Fig. 4) (Bruzzaniti et al., 2005). How Dyn2 actually participates in osteoclast function is unclear, although it has been shown that it is part of a complex of Pyk2, Src and Cbl. This interaction is negatively regulated by the tyrosine kinase Src. Thus, the Dyn2-Cbl-Src connection may regulate the turnover of podosomes by Src signaling. Because podosome turnover is a key step in the attachment, matrix resorption and migratory processes of osteoclasts, the existence of a regulatory complex involving dynamin demonstrates that Dyn2

has a significant role in regulating cell motility and other cellular events that can lead to metastases.

Dynamin function: substantially more complicated than previously thought

As is almost always the case, additional information and insights into dynamin function generates more questions than answers. Indeed, since 1989 when dynamin was first identified, the scope of dynamin distribution and function has been shown to extend well beyond the focused localization of a budding clathrin-coated basket. Although the dynamins are a complex protein family that participates in many seemingly unrelated cell processes, this family does possess some general features that can help bring some order and clarity to this confusing functional diversity. Lipid-based membrane binding and vesiculation involve its PH and GTPase domains, allowing dynamin to support membrane dynamics during both endocytosis and membrane extension. The PRD mediates interactions with various binding partners, linking dynamin to sites of high actin turnover, such as endocytic vesicles, lamellipodial and filopodial protrusions, focal adhesions, podosomes and invadopodia. While illuminating its role in multiple membrane trafficking events in the endocytic and secretory pathways, which in retrospect might have been somewhat predictable, investigation of the dynamins has expanded into a new, exciting phase. This new direction includes less-conventional cellular structures and organelles where a role for a membrane pinchase or scaffold may, at first, seem less obvious (Fig. 5). Yet, upon closer examination, a central role for the dynamins in dynamic processes such as lamellipodial extension and cell adhesion may become more apparent. Indeed, it should be no surprise that a protein that exhibits contractile and polymeric properties and contains a PH domain as well as a PRD might act as an actin regulator, membrane tubulator and constrictor, or a dynamic scaffold that supports the docking and integration of a host of other cytoskeletal and signaling components (see Table 1). Our understanding of dynamin's role in cell motility is still in the most nascent of stages. What precisely is dynamin doing at
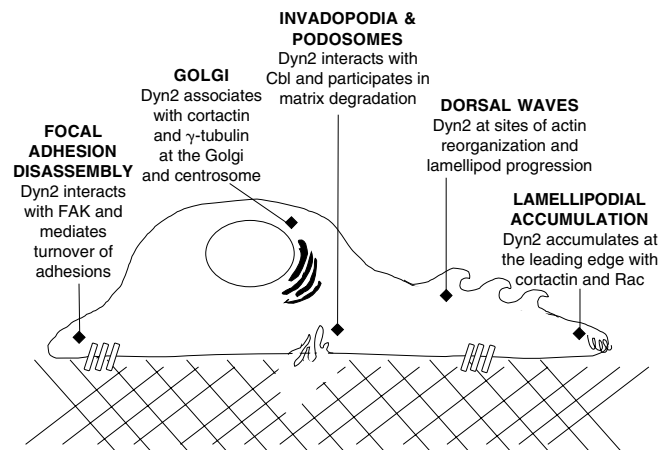

INVADOPODIA & PODOSOMES
Dyn2 interacts with Cbl and participates in matrix degradation

GOLGI
Dyn2 associates with cortactin and γ-tubulin at the Golgi and centrosome

DORSAL WAVES
Dyn2 at sites of actin reorganization and lamellipod progression

FOCAL ADHESION DISASSEMBLY
Dyn2 interacts with FAK and mediates turnover of adhesions

LAMELLIPODIAL ACCUMULATION
Dyn2 accumulates at the leading edge with cortactin and Rac

Fig. 5. Dynamin regulates multiple aspects of cell migration. Through its action as a polymeric scaffold protein and a GTPase, Dyn2 localizes to focal adhesions, invadopodia, podosomes, dorsal waves, and lamellipodia to promote cell migration.

lamellipodia and invadopodia to support cell motility? What are the mechanistic implications for dynamin in actin dynamics, membrane ruffling, and cytoskeletal interactions? Even more enigmatic is the recently identified structure-function relationship with non-membrane-based cell structures, such as focal adhesions (Ezratty et al., 2005) and the centrosome (Thompson et al., 2004). Because these organelles are generally thought to have limited interactions with membranes, a role for dynamins at these sites is difficult to model; however, these structures do contribute to directed cell migration. Thus, the new studies bring dynamin into yet another cellular theater: one with implications for cell polarity and migration. As if the field did not have enough to do defining the role of dynamin in severing the neck of a clathrin-coated tubule.

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