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THE ROLE OF DYNAMIN IN THE ASSEMBLY AND FUNCTION OF PODOSOMES AND INVADOPODIA

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TABLE OF CONTENTS

1. ABSTRACT

Cells make contact with the extracellular matrix (ECM) through extensions of the plasma membrane; these range from irregular dynamic structures, e.g. lamellipodia, ruffles and pseudopodia, to more localized and highly defined protrusions, e.g. podosomes and invadopodia. Both might be instruments through which cells sample the immediate extracellular environment and maintain polarized activities such as chemotaxis and focal degradation of the matrix. Podosomes are expressed in cells of the monocytic lineage, and most studies point to a role for podosomes in adhesion/motility. Invadopodia are prominent in certain aggressive cancer cells (or transformed cells) and appear to be directly responsible for focal ECM degradation. Recent studies have revived interest in these structures in terms of the actin regulation machinery. Within this framework, the atypical GTP-binding protein dynamin, a central modulator of protrusive events, has been associated to podosome and invadopodia structure and function. Here, we specifically discuss the role played by dynamin in controlling the activities and function of these structures.

2. INTRODUCTION

Cells make contact with the extracellular matrix (ECM) through extensions of the plasma membrane that are referred to as protrusive contacts. These display diverse morphological features, ranging from irregular dynamic structures, e.g. lamellipodia, ruffles and pseudopodia, to more localized and highly defined protrusions, e.g. podosomes and invadopodia. Generally speaking, these plasma membrane protrusions might be the instrument through which cells sample the immediate extracellular environment and maintain polarized activities such as cell locomotion and focal degradation of the matrix (reviewed in *1, 2*).

Podosomes are expressed in cells of the monocytic lineage (reviewed in *1, 3*), with few exceptions. They consist of highly dynamic, dot-shaped, F-actin-rich, close contacts that at the ultrastructural level appear as small plasma-membrane protrusions that assemble early during adhesion to the substratum (*4*). These structures are enriched in a full complement of actin cytoskeleton regulatory components and integrins. To date, most studies point to a role for podosomes in adhesion/motility, although this interpretation is far from conclusive as it is based solely on correlative evidence. It should be pointed out that only very recently has the possibility been discussed that podosomes might be associated with degradation of the ECM (*3*).

Cancer cells (or transformed cells), which have a generally increased tendency to form lamellipodia and spikes, express invadopodia instead (*5, 6*). At the ultrastructural level, these structures have been shown to be long membrane protrusions extending into the ECM, and which originate from a large invagination of the ventral plasma

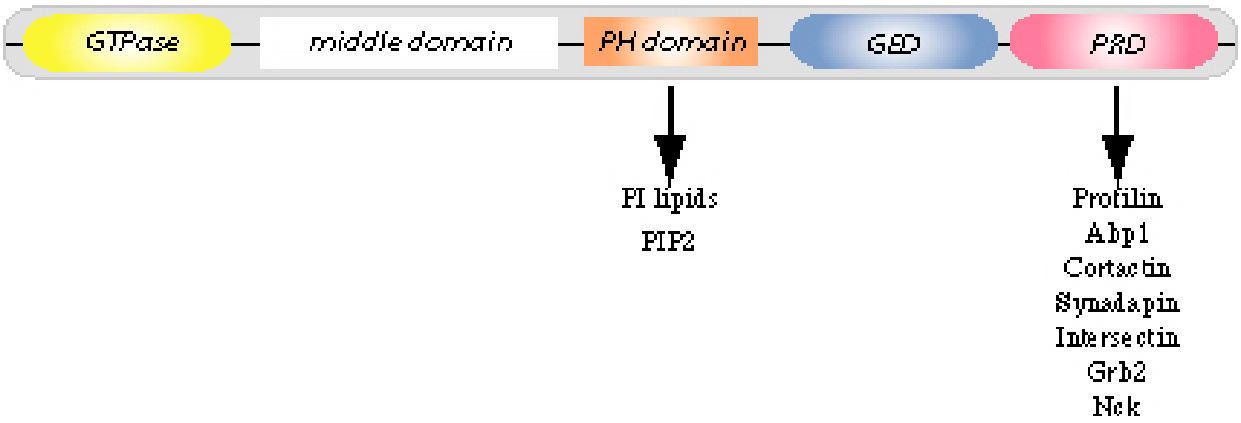

Figure 1. Domain structure of rat dynamin 2. GTPase; GTP hydrolysis domain. Middle: variable domain of unknown function; PH: pleckstrin homology domain; GED: GTPase effector domain and PRD: proline rich domain. Also shown are the main binding partners

membrane surface (*7*). Invadopodial protrusions are enriched in integrins and their associated tyrosine kinase signaling machinery, metalloproteases, and quite prominently, in actin and actin-associated proteins (*8-12*). These invadopodia are directly responsible for focal ECM degradation and invasion (*13*).

From the structural and functional point of view, an intermediate class of membrane protrusions might be represented by the podosomes expressed by osteoclasts, which are specialized cells engaged in mineralized bone reabsorption. Osteoclast podosomes essentially participate by ensuring the tight seal needed to maintain a contained subosteoclastic environment, where the pH and the concentration of proteases remains at optimal levels for bone matrix degradation (*14, 15*). Further to this, membrane-type metalloprotease 1 has been localized to osteoclast podosomes (*16*), supporting the view that osteoclast podosomes might produce focal degradation and hence increase "grip" with the calcified matrix, with a mechanism similar to invadopodia.

Most recently, a number of studies have rekindled interest in these structures in terms of the actin regulation machinery. Within this framework, the atypical GTP-binding protein dynamin appears to be a central modulator of protrusive events at the actin-membrane interface (*17*) and has been associated to podosome and invadopodia structure and function (*7, 18*). Here, we discuss the main features of dynamin, describe podosomes and invadopodia in detail, and consequently, analyze the role played by dynamin itself in controlling their structure and function.

3. DYNAMIN

The dynamins are a small family of 100-kDa proteins expressed by at least three distinct genes in mammals, with the most-studied isoforms being neuronal dynamin 1 (Dyn1) and the ubiquitously-expressed dynamin 2 (Dyn2). Dynamin has been demonstrated to be required in endocytic membrane fission (reviewed in *19*), caveolae internalization (*20*) and protein trafficking from the Golgi apparatus (*21*). Recent evidence has also shown Dyn2 to be active in the regulation of a number of actin-driven processes (for a review, see *17*), such as the internalization of particles during phagocytosis (*22*), the regulation of actin reorganization, and subsequently of cell shape (*23, 24*), the formation of podosomes (*18*) and the focal ECM degradation mediated by invadopodia (*7*). These findings demonstrate that Dyn2 is a key regulatory element in many types of actin-driven cytoskeletal machineries, and link the cytoskeleton to both membrane trafficking/remodeling and signaling events.

The dynamins are multidomain proteins with four conserved regions (Figure 1): a highly conserved N-terminal GTPase domain, a pleckstrin homology (Dyn-PH) domain of about 100 amino acids, a coiled-coil GTPase effector domain, and a moderately conserved arginine-proline-rich domain (Dyn-PRD) that contains several SH3 binding sites (reviewed in *25, 26, 27*). Dynamin self-assembles in solution into higher-order structures resembling rings, and helical stacks of rings composed of tetramers. Dynamin self-assembly stimulates its GTPase activity (*28*).

The coiled-coil domain interposed between Dyn-PH and Dyn-PRD was proposed to interact with the N-terminal GTPase domain, to stimulate GTP hydrolysis, by functioning as an assembly-dependent GTPase-activating protein. As a consequence, this region has been termed the GTPase effector domain (GED) (*29*). Indeed, experiments employing the GED mutants Dyn^{K694A} and Dyn^{R725A}, which feature reduced assembly-stimulated GTPase activity, accelerated early events in receptor-mediated endocytosis (*30*); it must be noted, however, that a later study failed to detect impaired GTPase activity of the same GED mutants (*31*).

Several *in vitro* and *in vivo* studies have convincingly demonstrated that dynamin binds to the phosphoinositides via its PH domain, thus mediating its interactions with membranes. The highest affinity of Dyn-PH is for phosphatidylinositol 4, 5-bisphosphate [PtdIns (4, 5) P_2], PtdIns(3, 4, 5) P_2 and PtdIns (3, 4)P_2. Of note, the

PH domains of Dyn1 and Dyn2 have similar phosphoinositide-binding specificities and require oligomerization for high affinity phosphoinositide binding (*32*). Finally, this Dyn-PH interaction with the phosphoinositides is essential for dynamin activity, at least in endocytosis (*33*).

Dyn-PRD has been shown to bind a variety of SH3-domain-containing proteins (Figure 1) that have been proposed to mediate and/or regulate dynamin-regulated activities. Among these, there are signaling proteins (*34, 35*), endocytosis-related proteins (*36-38*) and cytoskeleton related proteins (*39-41*) such as cortactin (*23, 24*).

3.1. The actin-binding partners of dynamin

We will here emphasize in particular the cytoskeleton-related dynamin partners that are relevant to the biology of podosomes and invadopodia.

3.1.1. Profilin

Historically, the first actin-binding protein found to bind directly to Dyn-PRD was profilin (*39*); profilin also binds actin monomers, PtdIns(4, 5)P_2, N-WASp and the Arp2/3 complex (*42*). This is consistent with a role for profilin in actin polymerization during cell motility since the Arp2/3 complex is the main actin-nucleating machine (for a review see *43*). Furthermore, N-WASP is a Wiskott-Aldrich syndrome protein (WASp) family member (together with WASp, Scar, Wave and others), a group of prominent actin-nucleation-promoting factors that act at the level of Arp2/3 (reviewed in *44*).

More recently, profilin has also been shown to act with formins to regulate Arp2/3-complex-independent actin nucleation (*45, 46*). The effect of dynamin on profilin activities remains, however, unclear.

3.1.2. Cortactin

Cortactin is an F-actin binding protein that was first characterized as a major substrate of non-receptor tyrosine kinases, such as Src, Syk and Fer, and that is overexpressed in several cancers (*47*). It binds Dyn-PRD through its SH3 domain and represents a partner of specific interest because it co-localizes with both dynamin and actin in all the actin-driven events that have been recently proposed to be regulated by dynamin, namely cortical membrane ruffles (*24*), podosomes (*18*), actin cometing activity (*48, 49*) and ECM degradation at invadopodia (*7*). Furthermore, cortactin is directly involved in tumor metastasis (*50*). Cortactin itself is a multidomain protein featuring an N-terminal acidic Arp2/3-binding domain, an F-actin-binding series of tandem repeats, and both proline-rich and SH3 domains at the C-terminus (*51*); interestingly, when cortactin is phosphorylated on tyrosine by Src, it exhibits reduced actin filament cross-linking ability (*52*). Also relevant in this context, cortactin can stimulate actin nucleation via Arp2/3 through its acidic domain (*53*), in synergy with WASp; this activity is regulated by its interaction with dynamin (*54*).

3.1.3. Abp1

The F-actin-binding protein mAbp1 is the mammalian homolog of yeast Abp1 and represents yet another potential functional link between the actin cytoskeleton and dynamin. Indeed, mAbp1 localizes to dynamin-rich growth-factor-induced ruffles (*55*) and clathrin-coated endocytic pits (*41*), and it has been shown to directly bind dynamin *in vitro* through its SH3 domain (*41*). mAbp1 features a cofilin homology domain, which has F-actin-binding activity, and both proline-rich and SH3 domains. Yeast Abp1 features an acidic Arp2/3-interacting region, which appears to be functionally active (*56*); Mammalian Abp1 lacks this domain, however, although it can potentially still interact with an Arp2/3-complex-binding partner: i.e. dynamin itself could link Abp1 to WASp and thus Arp2/3 by interacting with syndapin (*40*), another dynamin-binding protein (see below).

3.2. Other dynamin partners

Finally, a number of other dynamin partners hold potential interest in terms of membrane remodeling and protrusion. These include the actin regulators intersectin and syndapin, and the signal transduction scaffold proteins Grb2 and Nck.

Intersectin is a multidomain scaffolding protein involved in the formation of clathrin-coated vesicles. Interestingly, it shows guanine nucleotide exchange factor activity towards Cdc42 (*57*), a small GTPase known to regulate actin nucleation via WASp and Arp2/3, and it also binds directly to N-WASp.

The ubiquitous syndapin 2 has been shown to interact directly with dynamin and, among others, N-WASp. Consistent with a role in actin dynamics, syndapin localizes to sites of high actin turnover, such as filopodia tips and lamellipodia (*58*).

The scaffold proteins Grb2 and Nck have been characterized as signal-transduction-related proteins with the ability to bind phosphorylated growth-factor receptors. Grb2 features N-terminal and C-terminal SH3 domains, which both bind Dyn-PRD (*35, 59*). Nck, instead, has three SH3 domains, only the third of which binds dynamin (*60*). Recently, both Grb2 and Nck have been shown to cooperate in activating actin-based motility (*61, 62*).

In conclusion, although the role played by these proteins in the regulation of membrane remodeling and protrusion still needs to be defined, they appear to hold great promise as a treasure-trove in the network of interactions that involve dynamin.

3.3. Mechanisms of action

The classical model of dynamin function is based on *in vitro* experiments showing its ability to self-assemble into spirals around membrane tubule templates. These spirals are able to constrict after GTP hydrolysis, reducing the diameter of the membrane tubes and leading to their fragmentation (*63*). An alternative model suggests that the increase in spiral pitch, induced by GTP hydrolysis, starts membrane collapse and consequent fragmentation (*64*).

Recently, novel findings indicate dynamin as an important regulator at the interface between the membrane and

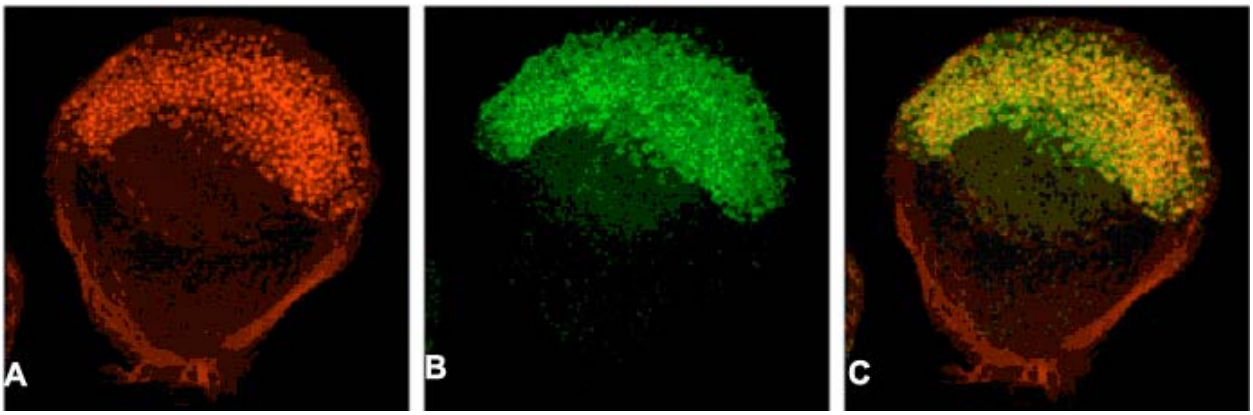

Figure 2. Macrophage podosomes. Fluorescence image of migratory macrophages stained for A) actin (red) and B) vinculin (green) Merged image clearly shows that podosomal structures consist of a central core of actin filaments surrounded by a juxtamembrane ring enriched in vinculin (image courtesy of Dr. Stefan Linder).

the dynamic actin cytoskeleton. In particular, the modular structure of dynamin could link together membranes (via the PH domain) and the actin cytoskeleton (via SH3-containing partners such as cortactin and mAbp1); hence, through its GTPase and self-assembling activities, dynamin could modulate actin nucleation (*54*) and the stability of actin filament networks (*48, 49*).

4. PODOSOMES

As introduced above, cells make contact with the ECM through protrusive contacts extending from the plasma membrane. A small number of cell types form peculiar, poorly understood contact sites that were initially called 'rosettes'; these were later renamed as 'podosomes' to symbolize their morphological appearance and putative function (*65*).

Podosomes were initially observed in Rous-sarcoma-virus-transformed cells (*66*) and in monocyte-derived cells such as osteoclasts (*67*) and macrophages (*68*). Indeed, to date, the only non-transformed cell types that spontaneously express podosomes are those of monocytic lineage. Podosomes consist of dot-shaped, F-actin-rich close contacts, which upon greater scrutiny (electron microscopy), appear as small, 1-2 μm long, 200-400 nm wide plasma-membrane protrusions that assemble during the early stages of adhesion to the substratum (*4*).

Focal adhesion plaques, the most ubiquitous and well-known membrane-ECM contacts, bear a high degree of resemblance to podosomes, although the latter are clearly distinguishable by a number of features. Within each podosome, a central core of actin filaments is surrounded by a juxtamembrane ring enriched in vinculin (Figure 2), talin, α-actinin and p60src, and with a high phosphotyrosine content (*65, 66, 69*). Further to this, podosomes are assembled more rapidly than focal adhesions and their formation does not require serum or ongoing protein synthesis. Indeed, podosomes have been observed to be highly dynamic structures that assemble and disassemble (*18, 70*) at least 10-fold faster than focal adhesions.

Contributions from a large number of laboratories have elucidated the main protein components and some of the functional relationships between these components, which are important in maintaining/establishing a proper podosome structure (for a review see *3*).

4.1. Podosome functions

What, however, is the physiological function of podosomes? Given the similarity between podosomes and focal adhesions and their composition, a role in adhesion is generally considered to be likely, albeit allowing for their different dynamic properties, as described above. For instance, monocytes rely on basic functions such as adhesion, migration, chemotaxis (directed motility) and phagocytosis to perform their biological functions. Podosomes are considered to be important for monocyte adhesion and chemotaxis. Indeed, normal monocytes have an inherent capacity to attach and form podosomes even directly on glass, and this can be stimulated by PKC activation (*69, 71*). The same has also been observed for macrophage adhesion to laminin, where the formation of podosomes is also PKC-dependent (*72*). Of note, smooth muscle cells, which do not spontaneously form podosome-like structures, can be induced to do so by exogenous PKC stimulation (*73*). Finally, diverse subgroups of integrins appear to be essential podosome components. β1- and β2-integrins have been seen to be recruited to podosomes, either at their tips or at the juxtamembrane vinculin-rich ring, depending on the cell type (*69, 71*). Taken together, these findings are consistent with a role for podosomes in adhesion/motility; this interpretation has not been formally proven however, and is based on correlative evidence. Indeed, only very recently has the possibility been discussed that podosomes are associated with degradation of the ECM associated with invasion (*3*).

More recently, a number of studies have rekindled interest in these structures in terms of the actin regulation machinery, among which Dyn2 (*18*). This has occurred specifically with the definition of their cytoskeleton composition in the light of the recent descriptions of the actin polymerization machinery. For instance, Cdc42, WASp and the Arp2/3 complex co-localize with actin bundles at podosomes, defining the machinery regulating dynamic actin involved in their

regulation (*74*). Also, macrophages either from Wiskott-Aldrich syndrome patients, which lack normal WASp, or expressing a mutant WASp are unable to form podosomes (*75*). Again, evidence showing that Wiskott-Aldrich syndrome macrophages have a defect in directed cell motility (*76*) confirms the correlative link between podosomes and chemotaxis.

4.2. The role of dynamin in podosome function

Recently, yet another functional link between actin and Dyn2 has been revealed by studying podosome dynamics (*18*). Indeed, expression of the dominant negative, GTPase-impaired Dyn2 mutant K44A ($Dyn2^{K44A}$) delays actin turnover at podosomes. Dyn-PRD might play an important role in dynamin targeting to these structures, since a green fluorescent protein fusion protein of Dyn2-PRD co-localizes with actin at podosomes and expression of a PRD deletion mutant of Dyn2 almost completely disrupts podosomes (*48*). The dominant-negative behavior of the PRD deletion mutant suggests a scenario in which the PRD of Dyn2 is necessary for proper localization, i.e. via cortactin or other actin-binding protein(s), whereas another Dyn2 domain(s) mediates Dyn2 interactions with other essential partner protein(s) that are critical for actin turnover.

Interestingly a well-known Dyn2 binding partner implicated in endocytosis, endophilin 2, has also been localized to podosomes (*18*). The functional meaning of this is not clear since other typical endocytosis machinery components have not been localized to areas of podosome activity (clathrin, adaptor proteins, transferrin receptor *18*), suggesting that podosomes are not "hot-spots" of endocytic activity. These findings, together with the observations on actin turnover, highlight the non "classical" (i.e. membrane fission) activities of dynamin at podosomes.

5. OSTEOCLAST PODOSOMES

Mineralized bone reabsorption is carried out by osteoclasts. In response to extracellular stimuli, they switch from migratory to resorptive activities. During resorption, osteoclasts remain stationary at sites of bone matrix degradation (*15, 67*) with the characteristic formation of podosome-type contacts. Indeed, for bone resorption to take place, these podosomes must be reorganized into a quite prominent circular sealing or clear zone at the osteoclast periphery. Osteoclast podosomes feature perpendicular actin filaments associated with fimbrins, actinin, gelsolin and vinculin (*67, 77*), and as with those of monocytes and macrophages, are very dynamic, with a turn-over of about 2–12 min during motility (*78*). Their assembly increases during bone resorption and is regulated by cytosolic calcium and PKC activity (*79*). The osteoclast podosome ring has a unique organization of the cortical cytoskeleton, consisting of a dense circumferential band of actin filaments co-localizing with vinculin, talin (*77*) and the proline-rich tyrosine kinase 2 (PYK2, *80*). This ring forms a tight adhesive contact (the sealing zone) that defines a contained subosteoclastic environment for local matrix degradation and formation of a resorption lacuna (*14, 15*). Formation of this sealing zone involves adhesion-activated intracellular signaling events, which are likely to participate in the reorganization of actin and associated proteins from individual podosomes into the continuous ring; again, as with other monocytic cells, there is a lot of evidence for a major regulatory role of various integrins in the functions of osteoclast podosomes. In particular, it is clear that αvβ3-integrin is required for podosome formation and function in bone reabsorption. Indeed, bone marrow macrophages from β3-integrin-knockout mice are able to differentiate into osteoclasts, but these fail to spread properly (*81*). These cells are also not able to correctly reorganize the cytoskeleton into podosome rings, even though it is not clear if the formation of podosomes or only their reorganization into peripheral rings are blocked (*81*).

5.1. Osteoclast podosome functions

The role podosomes are thought to play in osteoclast biology are essentially two, and these both relate to the main stages of differentiation. In the motile phase, podosomes are associated with to motility, whereas during resorption podosomes participate mainly by ensuring the tight seal needed to maintain the low pH within the resorption lacuna. As with non-osteoclast podosomes, the alleged podosome function in motility is speculative and needs to be better defined. Their function in bone resorption is quite clear, however, and is further supported by the finding that membrane-type metalloprotease 1 is localized to osteoclast podosomes (*16*), thus suggesting that this protease might induce focal points of degradation and hence increase the "grip" with the calcified matrix, with a mechanism similar to that seen for invadopodia (see below).

5.2. The role of dynamin in osteoclast podosomes

Needless to say, the physiology of osteoclasts is an intense field of study for the reason that pathological disorders of their function are associated with a number of relevant bone diseases, such as osteoporosis and osteopetrosis, and also because they mediate osteolysis induced by bone metastases. However, little is known in terms of actin cytoskeleton regulation in the functional control of osteoclast podosome activity. Specifically, this does involve Dyn2 (*18*) and, interestingly, two of the dynamin interactors described above, WASp and cortactin (*82*); unfortunately, these have only been described as being localized to osteoclast podosomes and have not been tested for a functional role. These studies do, however, provide us with the indication that at podosomes osteoclasts share common actin regulation machineries with other monocytic cells.

6. INVADOPODIA

When invasive tumoral or transformed cells are grown on an ECM substratum, they extend protrusions into the matrix from their ventral surfaces that display proteolytic activity against ECM components (*5, 83*). Under the optical microscope, invadopodia can be recognized as actin-rich formations (Figure 3A) with underlying ECM degradation areas (Figure 3B). At the ultrastructural level, these structures have been suggested to consist of 200 nm wide and up to 3 μm long

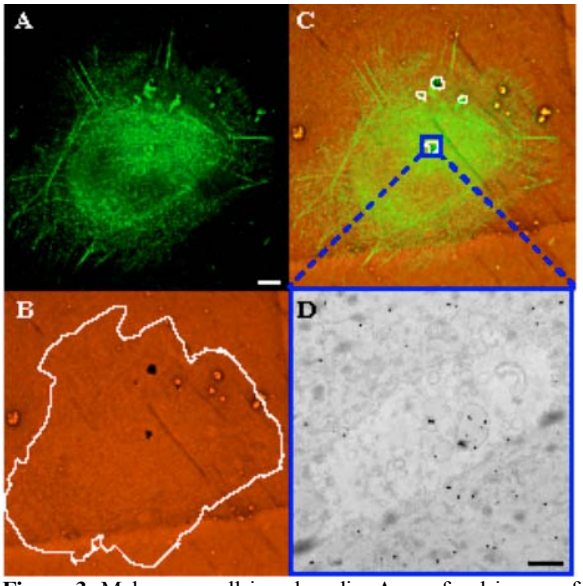

Figure 3. Melanoma cell invadopodia. A: confocal image of invadopodia structures marked by actin-GFP in A375 melanoma cells plated on fluorescent gelatin; B: confocal image of rhodamine-conjugated gelatin showing degradation patches underneath invadopodia structures from the same cell; cell contour is digitally rendered and indicated by the white line; C: merge between actin-GFP staining and fluorescent gelatin; matrix degradation patches are highlighted with a white line. D) correlative immunoelectron microscopy of the area indicated by the blue contour in C. Gold labeling shows concentration of actin in invadopodial protrusions. Size bar is 10 µm in fluorescence image and 1 µm in the electron microscopy image

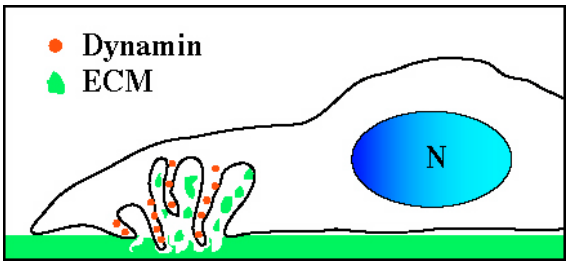

Figure 4. Schematic reconstruction of the invadopodial complex. This diagram is based on correlative light-electron microscopy reconstructions (*7*). ECM is indicated in green and localization of dynamin in red. Invadopodial protrusions are organized into a profound invagination of the ventral surface of the plasma membrane; within the area delimited by the large invagination**,** large fragments of gelatin can often be seen

membrane protrusions extending into the matrix (*5, 11*). Very recently, a detailed ultrastructural analysis based on a correlative light-electron microscopy approach has been performed (Figure 3C, D). The areas of contact of the plasma membrane at sites of degradation were revealed as being profound invaginations of the ventral surface of the plasma membrane that were, on average, 8 µm wide and 2 µm deep. Within the area delimited by the large invaginations**,** large fragments of gelatin could often be seen. Many surface protrusions with diameters ranging from hundreds of nm to a few µm originated from the larger invaginations and sometimes penetrated into the matrix. These protrusions were clearly consistent with the structures originally described as invadopodia, but appeared to be part of a more complex superstructure (Figure 4, 7). Hence, from the start, invadopodia were clearly associated with a well-defined function: focal pericellular degradation of the ECM, a crucial event in physiological ECM remodeling events and in cell invasive behavior (for a review see *84*). Although in terms of molecular characterization, invadopodia have received less attention than podosomes, a clear picture has nevertheless emerged. Invadopodial protrusions are enriched in integrins and the associated tyrosine kinase signaling machinery, metalloproteases, and quite prominently, in actin and actin-associated proteins (*8-12*), clearly defining them as powerhouses for the focal degradation of the ECM.

6.1. Invadopodia functions

There is probably little doubt that the specific function performed by invadopodia is the focalized degradation of the ECM *in vitro*; this holds true in all cell systems where they have been detected and studied. Indeed, invadopodia were first found to be precisely localized at sites of degradation of the ECM (*83*) and their enrichment in metalloproteases that was defined later was entirely consistent with this. Furthermore, interfering with invadopodia components leads to direct effects on matrix degradation (*7, 10, 11, 85*). Correlative light-electron microscopy also indicates that protease activity is prominent at invadopodia tips (Baldassarre and Buccione, unpublished results). As for the physiological role of invadopodia *in vivo*, or at least in a three-dimensional context, very little is known. Experiments will have to be designed to investigate this issue directly by combining both optical and electron microscopy techniques and by directly testing the function of known invadopodia components in three-dimensional invasion models.

6.2. Role of dynamin in invadopodia

Developments have been particularly exciting in this area. Recently, Dyn2 was shown to be localized at invadopodia and to be necessary for focalized matrix degradation at invadopodia. Here, dynamin function was inhibited by expressing dominant-negative, GTPase-impaired $Dyn2^{K44A}$ or the PRD-deleted Dyn2 mutant. In both cases, the number and extension of ECM degradation foci were drastically reduced. Furthermore, immuno-electron microscopy has shown that Dyn2 is concentrated within the invadopodial protrusions (*7*). Previously, the dynamin partner cortactin was seen to be localized to invadopodia at sites of ECM degradation. Further to this, microinjection of anti-cortactin antibodies inhibits ECM degradation by blocking invadopodia formation (*11*).

In this framework, it has also been shown that in v-*src*-transformed fibroblasts, WASp accumulates at punctuate structures at the ventral surfaces of the plasma membrane, in correspondence with ECM degradation sites. Expression of a dominant-negative WASp mutant that is

unable to activate Arp2/3 suppresses the formation of these structures and the ECM degradation (*85*). Although defined by the authors as podosomes, these structures actually present all the typical features of invadopodia, as first described in transformed cells (*83*).

Thus, recent reports and ongoing studies are clearly defining a role for the main actin-regulation players in invadopodia structure/function. A picture is clearly emerging in which an actin-regulating machinery based on the tight coordination between cortactin, WASp and actin itself is at work at invadopodia, possibly in response to a specific signal(s), which represent fundamental processes for ECM degradation to occur. A major function in regulating this machinery might involve dynamin, perhaps by regulating the stability of the actin/cortactin meshwork. Hence, a main role for dynamin would be to coordinate actin assembly/disassembly, to facilitate breaches in the cortical cytoskeleton, and to allow protease secretion in a highly polarized manner. Protrusive activities would thus also be favored by cycles of actin nucleation and F-actin stabilization, similar to those of actin comets (*48, 49*). The role of Arp2/3, which is likely to be involved, has not been investigated at invadopodia; nor has the implication of other potential actin regulators such as Abp1. As a final consideration, the ultrastructural localization of Dyn2 within invadopodial protrusions, together with the lack of endoyctotic activity at invadopodia (*7*) seems to rule out a classical (i.e. membrane fissioning) activity of dynamin in endocytosis at invadopodia. There remains the possibilty that dominant-negative Dyn2 expression could affect secretion of proteases by blocking fission of transport carriers at the distal Golgi.

7. PERSPECTIVES

Clearly, the interactions with the intracellular trafficking machineries on one side, and the actin cytoskeleton on the other (discussed here), provide a distinctly polyhedral role for dynamin in a number of fundamental processes. In particular, the interactions between dynamin and a number of pivotal actin-regulating partners appear to lie at the heart of many membrane-remodeling processes. Also, podosomes and invadopodia appear to be powerful experimental paradigms to study the dynamic interrelationships between membrane transport, cytoskeleton regulation and cell invasion through the ECM. Thus, the potential for exploitation of these particular membrane activities in terms of dynamin function appears vast, and has only just begun. Much thus needs to be done, both in terms of unraveling the intricacies of dynamin action in membrane remodeling, and having a better understanding of the biological roles of invadopodia and, to a greater extent, podosomes.

8. ACKNOWLEDGEMENTS

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Abbreviations: Pleckstrin homology (PH), Proline-rich domain (PRD), GTPase effector domain (GED), Extracellular matrix (ECM), Dynamin 1/2, Dyn1/2

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