

## Regulated Interactions between Dynamin and the Actin-binding Protein Cortactin Modulate Cell Shape

Mark A. McNiven,\* Leung Kim,‡ Eugene W. Krueger,\* James D. Orth,\* Hong Cao,\* and Tai Wai Wong‡

\*Department of Biochemistry and Molecular Biology, and the Center for Basic Research in Digestive Diseases, Mayo Clinic, Rochester, Minnesota 55905; and ‡Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

**Abstract.** The dynamin family of large GTPases has been implicated in the formation of nascent vesicles in both the endocytic and secretory pathways. It is believed that dynamin interacts with a variety of cellular proteins to constrict membranes. The actin cytoskeleton has also been implicated in altering membrane shape and form during cell migration, endocytosis, and secretion and has been postulated to work synergistically with dynamin and coat proteins in several of these important processes. We have observed that the cytoplasmic distribution of dynamin changes dramatically in fibroblasts that have been stimulated to undergo migration with a motagen/hormone. In quiescent cells, dynamin 2 (Dyn 2) associates predominantly with clathrin-coated vesicles at the plasma membrane and the Golgi apparatus. Upon treatment with PDGF to induce cell migration, dynamin becomes markedly associated with membrane ruffles and lamellipodia. Biochemical and morphological studies using antibodies and GFP-tagged dynamin demonstrate an interaction with cortactin. Cortactin is an actin-binding protein that contains a well defined SH3 domain. Using a variety of biochemical methods we demonstrate that the cortactin-

SH3 domain associates with the proline-rich domain (PRD) of dynamin. Functional studies that express wild-type and mutant forms of dynamin and/or cortactin in living cells support these *in vitro* observations and demonstrate that an increased expression of cortactin leads to a significant recruitment of endogenous or expressed dynamin into the cell ruffle. Further, expression of a cortactin protein lacking the interactive SH3 domain (Cort $\Delta$ SH3) significantly reduces dynamin localization to the ruffle. Accordingly, transfected cells expressing Dyn 2 lacking the PRD (Dyn 2(aa) $\Delta$ PRD) sequester little of this protein to the cortactin-rich ruffle. Interestingly, these mutant cells are viable, but display dramatic alterations in morphology. This change in shape appears to be due, in part, to a striking increase in the number of actin stress fibers. These findings provide the first demonstration that dynamin can interact with the actin cytoskeleton to regulate actin reorganization and subsequently cell shape.

**Key words:** dynamin • actin • cortactin • cell shape • lamellipodia

### Introduction

Dynamins are a family of large mechanochemical GTPases that have been implicated in vesicle formation from multiple cellular compartments (Urrutia et al., 1997; McNiven, 1998; van der Blik, 1999; McNiven et al., 2000). The participation of dynamin in diverse membrane trafficking processes suggests that it may interact with numerous membrane-associated and cytoskeletal proteins at distinct

cellular locations (McNiven, 1998; Schmid et al., 1998; McNiven et al., 2000). We have observed that antibodies to dynamin, in addition to staining clathrin-coated vesicles at the plasma membrane and trans-Golgi network (Henley and McNiven, 1996; Jones et al., 1998), also stain the cell cortex. In addition, we have found, dynamin 2 (Dyn 2)<sup>1</sup>-GFP expressed in cultured cells is also recruited to the cortical ruffles (Cao et al., 1998). Together, these findings sug-

Address correspondence to Mark A. McNiven, Department of Biochemistry and Molecular Biology, 1721 Guggenheim Building, Mayo Clinic, Rochester, MN 55905.

Tai Wai Wong's present address is Department of Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

<sup>1</sup>Abbreviations used in this paper: Cort $\Delta$ SH3, cortactin protein lacking the interactive SH3 domain; Dyn 2, dynamin 2; Dyn 2(aa) $\Delta$ PRD, dynamin 2 lacking the PRD; GFP, green fluorescent protein; GST, glutathione S-transferase; IP, immunoprecipitation; PRD, proline-rich domain; wt, wild-type.

gested that dynamin may interact with the actin cytoskeleton. Using multiple biochemical methods, we now demonstrate a direct physical interaction between Dyn 2 and the actin-binding protein, cortactin. This 80-kD protein has been shown to localize within membrane ruffles in cultured cells (Wu and Parsons, 1993) and was originally identified as a substrate for the protein tyrosine kinase, pp 60<sup>src</sup> (Wu et al., 1991; Okamura and Resh, 1995). In addition, cortactin is known to undergo tyrosine phosphorylation in response to various peptide growth factors, such as PDGF (Downing and Reynolds, 1992; Maa et al., 1992; Zhan et al., 1993). Cortactin contains an SH3 domain, a proline-rich region, and a series of tandem repeats that form multiple F-actin-binding domains (Wu et al., 1991; Wu and Parsons, 1993). These unique features make it likely that cortactin participates in the transduction of mitogenic signaling and cytoskeletal alterations. To test the premise that cortactin and dynamin interact within intact cells, we performed double immunofluorescence staining of NIH/3T3 fibroblasts or a rat hepatocellular cell line (clone 9) with antibodies to dynamin and cortactin. We find that dynamin does not localize to all cytoplasmic actin structures, but does show an exact and precise colocalization with cortactin in PDGF-stimulated cells. This striking colocalization is markedly increased in cells expressing either wild-type (wt) cortactin or Dyn 2, and is significantly reduced in cells expressing either a Dyn 2 construct where the proline-rich domain (PRD) has been deleted (Dyn 2(aa) $\Delta$ PRD-GFP), or a cortactin protein lacking the SH3 domain (Cort $\Delta$ SH3). Further, these Dyn 2(aa) $\Delta$ PRD cells display dramatic shape changes concomitant with a marked increase in the formation of actin stress fibers. Thus, this study provides novel *in vivo* and *in vitro* evidence for an interaction between dynamin and an SH3 domain-containing, actin-binding protein, that appear to act together to regulate actin organization, and subsequently, cell shape.

## Materials and Methods

### Subcellular Fractionation and Immunoprecipitation

NIH/3T3 cells were grown on 150-mm dishes in DME supplemented with 10% newborn calf serum. Cells were rinsed with PBS and scraped into 2 mL of cold hypotonic lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 1 mM sodium orthovanadate, and 40 mM ammonium molybdate) supplemented with 1 mM PMSF, and leupeptin and pepstatin at 20 mg/mL. After a 10-min incubation on ice, the cells were lysed in a tight-fitting glass homogenizer. Nuclei and unbroken cells were removed by low-speed centrifugation, and the lysate was further centrifuged at 150,000 g for 1.5 h to yield a cytosolic fraction (S100) and a membrane pellet. By immunoblot analyses, all of the cortactin and >95% of the dynamin in these cells are recovered in the cytosolic fraction. For analyzing the effect of growth factor stimulation on the dynamin-cortactin complex, confluent cultures were starved for 24 h in DME supplemented with 0.2% calf serum and 10 mM Hepes (pH 7.2). Half of the cultures were treated with PDGF (30 ng/mL) for 10 min at 37°C before they were lysed. Immunoprecipitation (IP) and immunoblot analyses were carried out as described previously (Kim and Wong, 1995). For all IPs, 0.5 mg of total cell protein was used as starting material, whereas 30  $\mu$ g of cell protein was added per lane for Western blot analysis. Antibodies used for IP were monoclonal anticortactin (Upstate Biotech), polyclonal antidynamin (MC63; Henley and McNiven, 1996), and anti-Dyn 2 COOH-end (Dyn 2) antibodies. The anti-Dyn 2 antibodies were raised against the synthetic peptide, SHSPTPQRRPVSSVHPPGRPPAVRP, that corresponds to residues 762–786 of Dyn 2 (Cook et al., 1994). The anti-Dyn 2 mAb used for blotting was from Transduction Labs.

### Immunofluorescence Localization

NIH/3T3 cells were plated on glass coverslips at a density of 10<sup>4</sup> cells per 35-mm dish. After 24 h, the culture medium was replaced with DME supplemented with 0.2% calf serum, and the cells were cultured an additional 24 h before stimulation with PDGF. Cells were treated with PDGF (30 ng/mL in media) for 10–15 min at 37°C and were rinsed twice with 37°C PBS, submerged in 37°C fixative (100 mM Pipes, pH 6.95, 3 mM MgSO<sub>4</sub>, 1 mM EGTA, 3% formaldehyde), and incubated 20 min at room temperature. For indirect immunocytochemistry, fixed cells were permeabilized with PBS containing 0.1% Triton X-100 for 2 min, and incubated with antibodies as described (Henley and McNiven, 1996). For F-actin localization, rhodamine-phalloidin (Sigma-Aldrich) was included with the secondary antibody step. Labeled cells were rinsed three times with PBS, once with distilled water, and were then mounted in Prolong antifade reagent (Molecular Probes). Digital images were acquired using a cooled charged coupled device camera (Photometrics) attached to a Zeiss Axiovert 35 microscope equipped with a 100W mercury arc lamp and processed as described previously (Henley and McNiven, 1996). Clone 9 cell culture and immunofluorescence were as described (Cao et al., 1998).

### Protein Interaction Mapping

A glutathione S-transferase (GST) fusion protein of the SH3 domain of cortactin was isolated as described (Wu and Parsons, 1993) and immobilized to glutathione-Sepharose (Amersham Pharmacia Biotech). The beads were incubated with a cytosolic extract of NIH/3T3 cells in the presence or absence of a proline-rich peptide. Proteins that bound were fractionated by SDS-PAGE and were analyzed by immunoblotting. A GST fusion protein containing the SH3 domain of PLC $\gamma$ -1 (Santa Cruz Biotechnology) was used similarly to analyze its interaction with dynamin. Peptides used in the competition analyses were synthesized by Chiron Mimotopes and were purified by reverse-phase HPLC. For blot overlay assays, dynamin was immunoprecipitated from a cytosolic extract using the anti-Dyn 2 antibody and fractionated by SDS-PAGE. The proteins were electrophoretically transferred to a piece of Immobilon filter, which was subsequently blocked with a solution of 4% BSA in TBST at 4°C overnight. The filter was incubated with either GST or the GST-cortactin SH3 fusion protein (10 mg/mL) in blocking solution at room temperature for 1 h. After extensive washing with TBST, the filter was incubated with polyclonal anti-GST antibodies to detect the binding of GST or GST-SH3 bound to the filter. Antibody binding was detected as in immunoblot analyses.

### Construction of Dyn 2(aa) $\Delta$ PRD-GFP and Cort $\Delta$ SH3

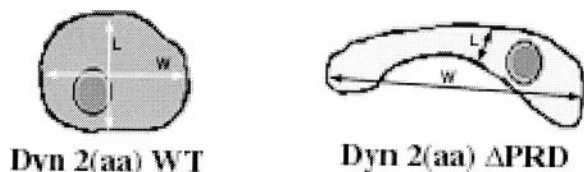
A DNA fragment corresponding to Dyn 2(aa) $\Delta$ PRD was PCR amplified from Dyn 2(aa) WT-pEGFP N<sub>1</sub> (Cao et al., 1998) template DNA using the following primers: Dyn 2N5' (Cao et al., 1998) and Dyn 2  $\Delta$ PRD3', GAATTCCTGGACACGGTGCTGGTGCTGATGTCCCAA. The PCR conditions used were 94°C for 1 min, 60°C for 3 min, for 28 cycles followed by a 4°C soak. These primers incorporate HindIII and EcoRI restriction site onto the 5' and 3' ends of the PCR product, respectively. The reaction products were analyzed by agarose gel electrophoresis. The Perkin Elmer XL PCR kit was used for the amplification reaction. The Dyn 2(aa) $\Delta$ PRD product was ligated with the pCR 3.1 vector (Invitrogen). The Dyn 2(aa) $\Delta$ PRD insert was excised from the pCR3.1 subclone and ligated with the Clontech expression vector pEGFP-N<sub>1</sub>. The Dyn 2(aa) WT-pEGFP N<sub>1</sub> and Dyn 2-pCR3.1 constructs used in this work were constructed previously (Cao et al., 1998).

Full-length cortactin and Cort $\Delta$ SH3 were amplified from rat liver cDNA using the following primers: cortTA.su, ATGATGTGGAAAGCCTCTGCAGG; cortTA.as, GCCGCAGCTCCACATAGTTGG; and cortTA $\Delta$ SH3.as, CACTCTCATACCCATCGTAGGT. The PCR conditions used were 94°C for 5 min, 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, for 30 cycles followed by a 72°C, 7 min final extension and a 4°C soak. Roche Molecular Biochemicals PCR reagents were used. The cortTA.su primer was used as the sense upper primer to amplify both full-length and  $\Delta$ SH3 cortactin. The  $\Delta$ SH3 product encompasses amino acids 1–450 of cortactin. The products were visualized using agarose gel electrophoresis and purified using the QIAquick PCR purification kit. Purified full-length and  $\Delta$ SH3 products were subcloned into the pcDNA3.1/CT-GFP-TOPO and pCR3.1 vector, respectively (Invitrogen). The sequence, orientation, and reading frame of insert DNAs was confirmed by restriction digest analysis and automated DNA sequencing. Oligomers and DNA sequencing were synthesized and performed by the Mayo Molecular Biology Core facility (Applied Biosystems 3948 Nucleic Acid Synthesis and Pu-

rification System and ABI PRISM 377 DNA Sequencer, Perkin Elmer). Sequences were analyzed using DNA Star, Sequencher 3.1.1 (Gene Codes Corporation), and MacVector 6.5.3 (Oxford Molecular Group PLC) DNA analysis software. The Dyn 2(aa)WT-GFP, and Dyn 2(aa) $\Delta$ PRD-GFP, cortactin wt, and Cort $\Delta$ SH3 constructs were transfected into cells using the lipofectamine plus<sup>TM</sup> reagent kit (Life Technologies). Transfection conditions were according to the manufacturer.

### Quantitation of Cell Shape and Recruitment of Dynamin and Cortactin

Images for quantitation were acquired using a cooled CCD camera (Photometrics) driven by the image acquisition program MetaMorph (Universal Imaging). Images of Dyn 2(aa) WT-GFP and Dyn 2(aa) $\Delta$ PRD-GFP cells were taken at full resolution (1,400  $\times$  1,000) using the same acquisition settings (exposure time = 4 s, 12 bit grayscale). Both the wt dynamin cells, and the Dyn 2(aa) $\Delta$ PRD cells (average of 100 cells for each) were measured for length and width (Scheme 1). Using width divided by length (W/L), the data was then moved to Excel 98 (Microsoft Corp.), where average values were calculated for each set, and graphs were generated from the averaged values.



Scheme 1.

The effect of overexpressing wt dynamin, wt cortactin, Dyn 2(aa) $\Delta$ PRD, and Cort  $\Delta$ SH3 in NIH/3T3 and Clone 9 cells was determined by visual inspection. 100 transfected cells for each construct were scored in a double-blind assay and the level of immunofluorescence intensity at the cell periphery was assigned compared with that of untransfected cells. Cell shape was recorded as migratory or aberrant as compared with untransfected cells. Motile cells are polarized with a large, extended lamellipodia, whereas aberrantly shaped cells demonstrate an elongated, spindle-shaped or arborized appearance.

## Results

### Dynamin Binds to Cortactin In Vitro

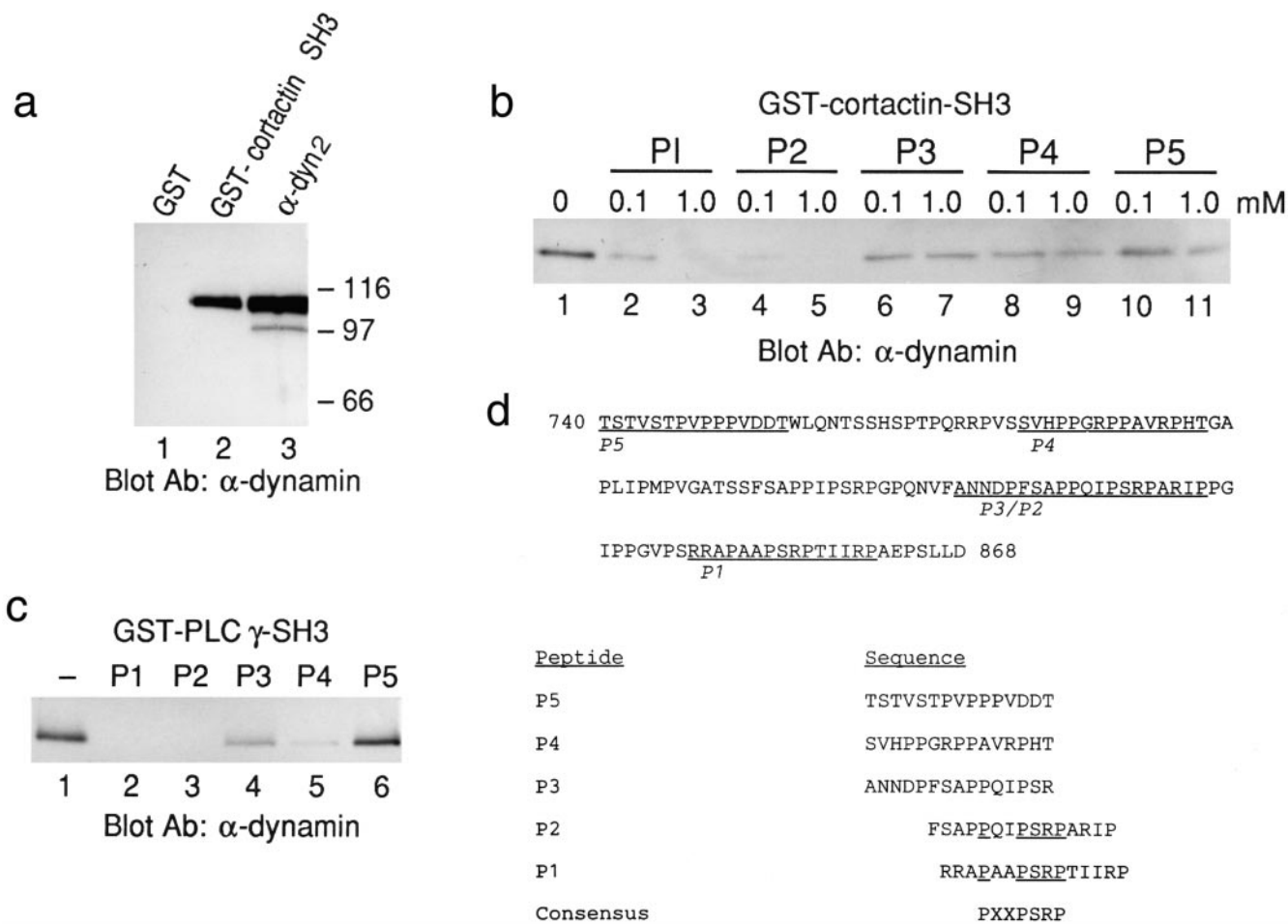
Immunostaining of cultured NIH/3T3 fibroblasts with two well characterized, polyclonal antibodies to either the conserved NH<sub>2</sub> terminus (MC63) or the proline-rich tail of Dyn 2 (Henley and McNiven, 1996) produced an unexpected localization to the cell periphery (Fig. 1, a and b). Most strikingly, this cortical labeling was increased dramatically in fibroblasts treated with growth factors, such as PDGF, which are known to induce cell migration. In these stimulated cells, dynamin staining was localized to clathrin-coated vesicles at the plasma membrane and Golgi apparatus, as well as actin-containing cortical ruffles of the leading edge (Fig. 1, c–e'). Whereas Golgi labeling was observed routinely, in some cells it was absent due to a more rigorous extraction procedure that optimized staining of the cytoskeleton. To define the components of the actin cytoskeleton with which dynamin associates, we conducted an IP-based screening of fibroblast homogenates with antibodies to dynamin and actin-associated proteins. Specifically, Dyn 2 immunoprecipitates were subjected to SDS-PAGE and immunoblot analysis with antibodies to either vinculin, paxillin, cortactin, fimbrin, talin, or zyxin. Whereas the dynamin antibodies did pull down some paxillin protein, (data not shown) only cortactin appeared to

bind Dyn 2 in a consistent way. The actin-binding protein cortactin, which is a target for tyrosine kinases and may link growth signals with the actin cytoskeleton (Wu et al., 1991; Downing and Reynolds, 1992; Maa et al., 1992), was a particularly attractive candidate for a dynamin binding partner for several reasons. First, cortactin is known to contain an SH3 domain that may have an affinity for the COOH-terminal PRD of dynamin, and second, cortactin has been shown to localize to the cortical leading ruffles of migrating, PDGF stimulated cells, where it is believed to modulate actin assembly and dynamics (Wu and Parsons, 1993). The localization of cortactin is similar to that seen in PDGF-treated cells stained with the dynamin antibodies (Fig. 1, c–e). Thus, reciprocal IP experiments were performed (Fig. 1, f and g) on cultured fibroblast homogenates (NIH/3T3 cells) using cortactin and dynamin antibodies. The cortactin antibody precipitated a 105-kD polypeptide, which was recognized by the MC63 Pan-dynamin antibody. The immunoblot also detected an additional polypeptide band that was suspected to be a proteolytic fragment of dynamin or a minor spliced variant (Sontag et al., 1994). Conversely, cortactin was detected in a Dyn 2 immunoprecipitate with the Pan-dynamin antibody. Interestingly, an isoform-specific antibody made to the COOH terminus of the ubiquitously expressed Dyn 2 failed to immunoprecipitate cortactin (Fig. 1 g, lane 5), although the same antibody detected the coimmunoprecipitated dynamin by Western blotting (data not shown). The fact that the Pan-dynamin antibody (MC63) was competent to pull down the cortactin-dynamin complex, whereas the tail-specific (Dyn 2) antibody did not, suggested that cortactin and dynamin may form a stable complex mediated by the COOH-PRD of Dyn 2. Because both of these proteins are likely to participate in a variety of distinct cellular functions, it is likely that only a portion of the total cytoplasmic pool of these proteins form a complex with each other. Indeed, Western blot analysis was implemented to compare the amounts of coimmunoprecipitated Dyn 2–cortactin complexes with that of total cellular protein levels. We estimate that 20–30% of the total Dyn 2 and cortactin are complexed together (data not shown).

### Identification of a Peptide Sequence in the Dynamin COOH-terminal PRD that Mediates Cortactin-binding

Based on the interaction of dynamin with other proteins, it is likely that an association between dynamin and cortactin is mediated by a direct physical interaction of the SH3 domain of cortactin with the proline-rich COOH terminus of dynamin. To test this, a recombinant GST-fusion protein containing the cortactin SH3 domain was immobilized to glutathione-Sepharose and incubated with a cytosolic extract of NIH/3T3 cells. As shown in Fig. 2 a, the GST–cortactin SH3 domain-coupled beads bound significant amounts of dynamin, whereas GST beads alone did not. To define the specific peptide regions by which dynamin binds cortactin, synthetic peptides corresponding to individual polyproline sequences of the dynamin C-tail were included in the fibroblast homogenate before addition of the homogenate to the GST column. Two of the peptides used (P1 and P2) competed away the cortactin–dynamin interaction thereby preventing dynamin from binding to the beads whereas three other proline-rich peptides had





**Figure 2.** The SH3 domain of cortactin binds dynamin. **a**, A cytosolic extract of NIH/3T3 cells was incubated with GST or GST-cortactin SH3 domain that was immobilized on glutathione Sepharose beads (lanes 1 and 2) or immunoprecipitated with the Dyn 2-specific antibody (lane 3). The bound proteins were fractionated and analyzed by blotting with an antidynamin mAb. As shown by the prominent dynamin band in lane 2, a substantial amount of dynamin was retained on the GST-cortactin SH3 domain beads as compared with the GST beads alone (lane 1). **b**, The GST pull-down assay was carried out in the absence or presence of one of the five synthetic peptides (P1–P5) at 0.1- or 1-mM concentrations. Binding of dynamin to the immobilized GST-cortactin SH3 domain was measured by blotting with an antidynamin mAb. Note that the P2 and P1 peptides were the most effective in blocking dynamin binding to the GST-SH3 domain beads (**c**). As a control the GST pull-down assay was carried out using a GST fusion protein containing the SH3 domain of PLC $\gamma$ -1, in the absence (lane 1) or presence (lanes 2–6) of 1 mM of each of the five peptides. Binding of dynamin by the PLC $\gamma$ -1 SH3 domain was assayed by blotting with antidynamin antibodies. As for the cortactin-SH3 domain-associated beads, peptides P2 and P1 inhibited dynamin binding, and some inhibition was demonstrated by the P4 peptide. **d**, Peptide sequence of the Dyn 2 COOH terminus. The four proline rich sequences are underlined. The bottom shows the sequences of the five synthetic peptides used to test the inhibition of SH3-dynamin interactions. The consensus sequence PXXPSRP was found to inhibit Dyn 2 binding to the GST-cortactin SH3 domain.

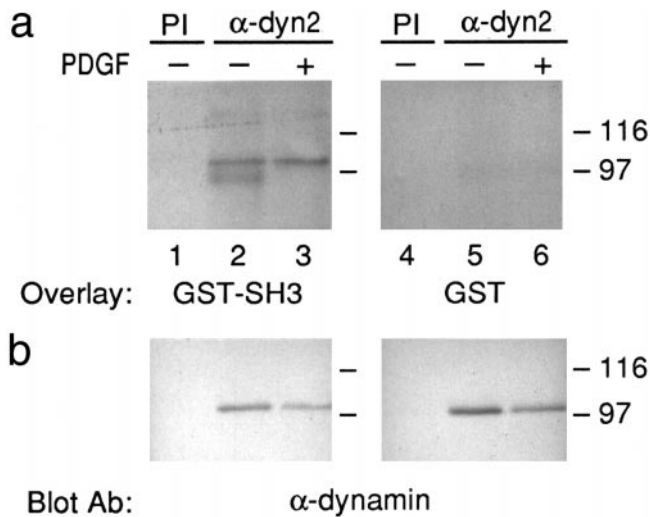
which partially resembles the putative cortactin-binding consensus sequence. Thus, there is overlapping, yet non-identical sequence specificity in the binding of the dynamin PRD to the SH3 domains of two distinct proteins.

To test for direct binding of dynamin to the SH3 domain of cortactin, a blot overlay assay was used. Dynamin was immunoprecipitated from cell lysates, separated by SDS-PAGE, and immobilized on a filter that was then incubated with the GST fusion protein containing the cortactin SH3 domain. Specific binding of dynamin to the GST-SH3 fusion protein, but not GST alone, was detected (Fig. 3 a). This observation indicated that the interaction between cortactin and dynamin may be direct and not mediated by other proteins. In addition to the major 105-kD dynamin band, an additional 95-kD polypeptide was detected in the blot overlay. This dynamin-immunoreactive band was also

isolated by coimmunoprecipitation of cortactin and dynamin with a cortactin antibody (Fig. 1 f) and by IP with the Dyn 2-specific antibody from fibroblast homogenates (Fig. 1 g). Detection of this lower molecular weight band by multiple methods and antibodies is consistent with our prediction that it represents a proteolytic fragment or spliced variant of Dyn 2 that is recognized by the dynamin antibodies and retains an ability to bind the GST-SH3 domain in this assay.

#### Colocalization of Dynamin and Cortactin in the Membrane Ruffles of Motile Cells

The observations described above demonstrate that antibodies to dynamin label membrane ruffles and lamellipodia in cultured fibroblasts (Fig. 1) and that Dyn 2 binds directly to cortactin by its proline rich tail domain (Figs. 2



**Figure 3.** Dynamin binds directly to the cortactin-SH3 domain. Cytosolic extracts of quiescent or PDGF-stimulated NIH/3T3 cells were immunoprecipitated with preimmune serum (PI; lane 1) or with the Dyn 2-specific tail antibody (lanes 2 and 3). The immunoprecipitated proteins were fractionated by SDS-PAGE and transferred to a filter (a). Duplicate filters were incubated with either GST or a fusion protein containing the SH3 domain of cortactin (GST-SH3). Binding of the GST or GST-SH3 to the filters was detected using anti-GST antibodies, followed by peroxidase-conjugated goat anti-rabbit IgG. The bottom panels show the same filters that were stripped and reprobed with antibodies to dynamin. Note that while all of the lanes containing immunoprecipitates using immune serum contained dynamin protein (b), the GST-cortactin SH3 domain probe, but not the GST probe alone, bound to the filter (a).

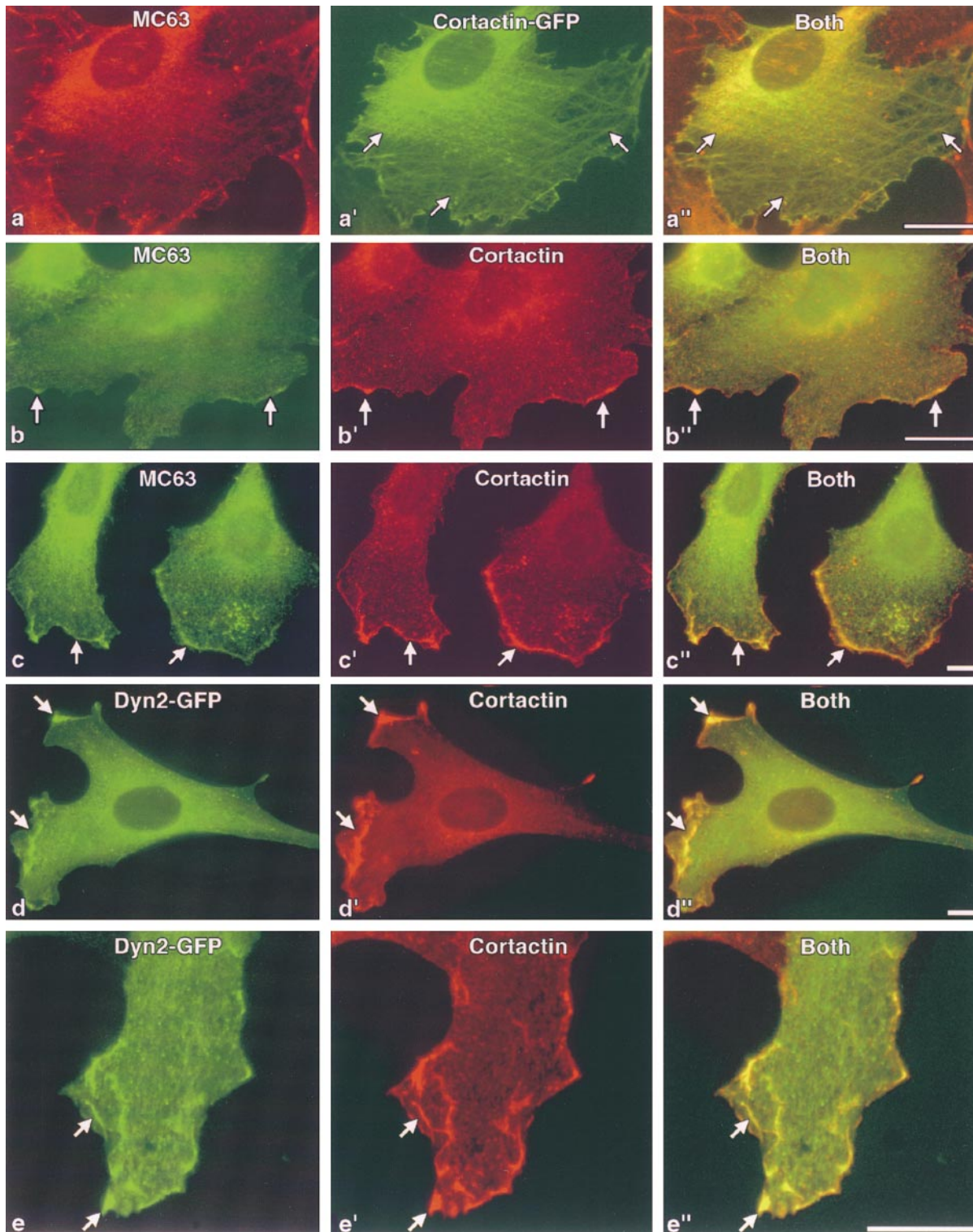
and 3). To test if Dyn 2 colocalizes with cortactin, double immunofluorescence staining of resting and PDGF-stimulated NIH/3T3 cells was conducted using antibodies to dynamin and cortactin (Fig. 4). In resting fibroblasts, cortactin was localized to a fine cortical rim along the cell periphery and punctate spots on the plasma membrane. Expression of exogenous cortactin, or cortactin-GFP in these cells showed a localization identical to that of untransfected cells with an additional localization to numerous stress fibers (Fig. 4, a-a'') that also stained positive with rhodamine phalloidin (data not shown). Resting cells displayed only a modest colocalization between dynamin and cortactin in the cortex (Fig. 4, b-b''). However, upon stimulation with PDGF (Fig. 4, c-c''), fibroblasts assumed a polarized morphology characteristic of motile cells (Bray and White, 1988; Stossel, 1993). Most dramatic was the change in the distribution of dynamin staining, which became concentrated in a thin veil at the ruffling edge of the cell. Concomitant with this rearrangement of dynamin was a striking and precise colocalization with cortactin that was shown previously to accumulate in the periphery of PDGF-treated cells (Wu and Parsons, 1993).

To further confirm the translocation of dynamin to the leading ruffles of migrating fibroblasts, cells were transfected with green fluorescent protein (GFP)-tagged Dyn 2(aa). Dyn 2-GFP has been used previously to provide dependable localization of this protein in living or fixed cells without the use of antibodies (Cao et al., 1998; Jones et al., 1998). Additionally, we have observed Dyn 2-GFP in the

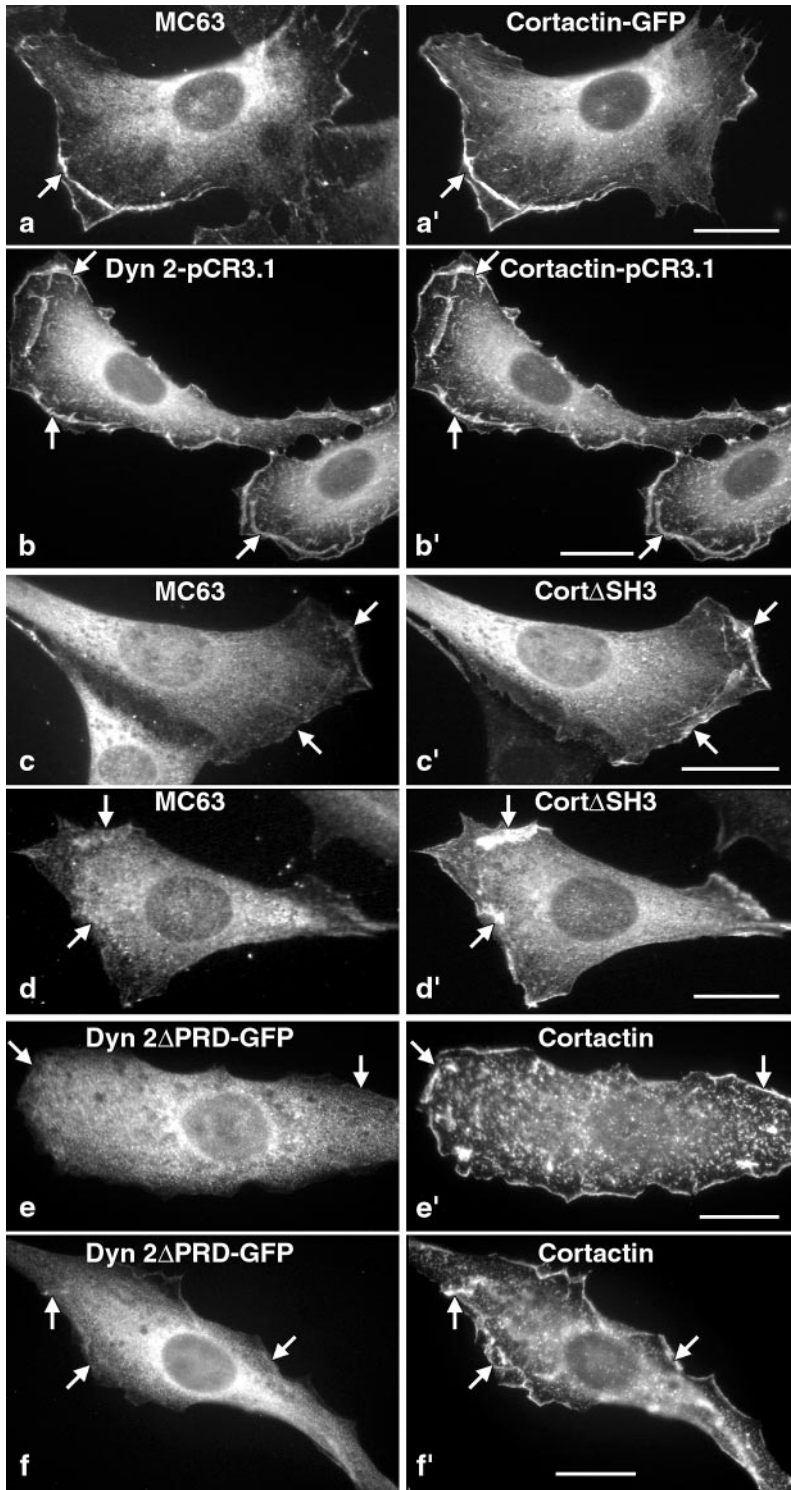
cortex of epithelial cells in a previous study (Cao et al., 1998). Whereas fibroblasts and most epithelial cells express at least four different spliced variants of the Dyn 2 gene, we initially coupled GFP to the Dyn 2(aa) form, which has been shown to localize with clathrin-coated structures at the cell surface and the Golgi apparatus (Jones et al., 1998). Fig. 4 shows representative images of NIH/3T3 cells transfected to express Dyn 2(aa)-GFP, which were subsequently double stained with antibodies to cortactin. Resting cells expressing Dyn 2(aa)-GFP were indistinguishable from resting untransfected cells stained with dynamin antibodies (data not shown), however, upon stimulation with PDGF (Fig. 4, d and e), transfected cells underwent a characteristic change in shape whereas the Dyn 2(aa)-GFP became concentrated within the ruffles at the leading edge of the cell. In support of the double immunofluorescence staining, the Dyn 2(aa)-GFP colocalized precisely with cortactin at the cell ruffles. To extend this observation, we expressed the three other identified forms of Dyn 2 in these cells to test if one or all Dyn 2 proteins associated with the cell cortex. We found that Dyn 2(ba)-GFP also localized to ruffles in stimulated cells, whereas the recruitment of the Dyn 2(bb)-GFP and Dyn 2(ab)-GFP forms was significantly less (data not shown). Thus, studies with either antibodies or fluorescently tagged protein expressed in living cells are consistent with the biochemical data presented in the previous figures that Dyn 2 interacts with the actin-binding protein, cortactin, at the leading edge of stimulated cells. The fact that Dyn 2 localization was perfectly aligned with cortactin in the cortex, and not with other cytoplasmic actin structures, suggests that this interaction with cortactin is specific. Further, cortical colocalization of only two of the four Dyn 2-GFP forms expressed was observed, which also suggests a specific interaction between these two proteins.

#### **Functional Tests for Dynamin-Cortactin Interaction in Living Cells**

To further define if Dyn 2 and cortactin truly interact *in vivo*, we performed two additional experiments. We tested if a modest overexpression (three- to fivefold) of one partner would increase the recruitment of the other protein to the ruffle; and second, whether expression of truncated Dyn 2 or cortactin, lacking their putative interaction domains (PRD and SH3, respectively), would alter the cytoplasmic colocalization of these proteins when expressed in cells. The results of these experiments are summarized in Table I, and Fig. 5 displays representative images of NIH/3T3 cells expressing various Dyn 2 and cortactin constructs. To test if increased levels of cortactin would recruit additional dynamin to the lamellipodia, cells were transfected with Cort-GFP, allowed to recover for 24-48 h, and were then stained with Dyn 2 antibodies to localize endogenous dynamin. Remarkably, cells expressing Cort-GFP displayed many more ruffles and lamellipodia in which a marked increase in endogenous dynamin protein was apparent. This dramatic increase in dynamin at the ruffle was observed even in the absence of PDGF stimulation (Fig. 5, a and a'). The recruitment of Dyn 2 to the cortex was increased further in cells cotransfected with both cortactin and Dyn 2(aa) expression constructs when stimulated (Fig. 5, b and b'). As shown in Fig. 4, an increased expression of



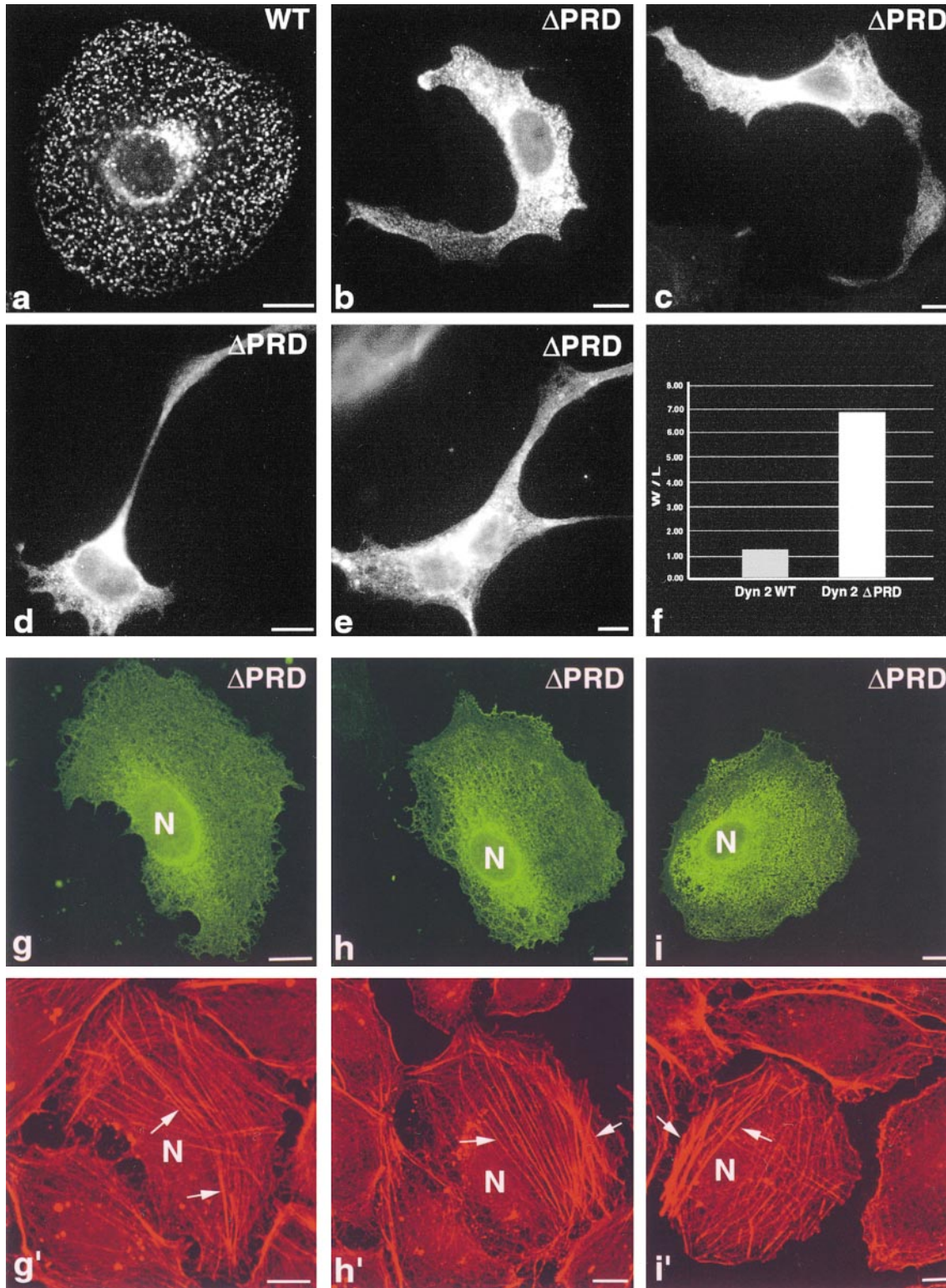
**Figure 4.** Dynamin colocalizes with cortactin in the lamellipodia of growth factor-stimulated fibroblasts via the PRD. Immunofluorescence localization of dynamin and cortactin in quiescent, and PDGF-treated NIH/3T3 cells. Labels indicate whether cells were transfected. Resting cells were transfected with a full-length cortactin-GFP construct and double-labeled for dynamin (a-a''), or untransfected cells were fixed and double-labeled with antibodies to dynamin (MC63) and cortactin (b-b''). Resting cells (a-a'') transfected with the Cort-GFP construct displayed a nonpolarized phenotype with cortactin localized around the cell cortex and on actin stress fibers (arrows). The distribution of cortactin in untransfected cells was identical to that of cells transfected with cort-GFP, although there was a reduced localization to stress fibers (b-b''). Upon stimulation with PDGF, there was a marked recruitment of both Dyn 2 (c) and cortactin (c') into large cortical ruffles (arrows) situated at the leading edges of the actively migrating cells. An identical localization was observed in PDGF-stimulated NIH/3T3 cells expressing Dyn 2(aa)-GFP (d and e) that were fixed and stained for cortactin (d' and e'). Similar to untransfected cells stained with antibodies, Dyn 2(aa)-GFP became concentrated in lamellipodia at the leading edge of PDGF stimulated, motile cells (d and e, arrows). Bars, 10  $\mu$ m.



**Figure 5.** Alterations in cortactin change the distribution of Dyn 2 in living cells. NIH/3T3 cells were transfected with constructs encoding GFP-tagged (a), wt (b), or truncated (c–f) forms of cortactin and/or Dyn 2, allowed to recover for 24–48 h, then prepared for immunofluorescence microscopy to monitor changes in protein distribution. Labels denote if cells were transfected. a and b, Overexpression of cortactin–GFP induced a marked increase in ruffle formation and the recruitment of endogenous Dyn 2, even in unstimulated cells (a and a'). Lamellipodia formation and Dyn 2 recruitment was induced to high levels in stimulated cells that coexpressed both cortactin and Dyn 2 (b and b'). Stimulated cells expressing a mutant cortactin (Cort $\Delta$ SH3) protein lacking the interactive SH3 domain (c' and d') show high levels of cortactin in the lamellipodia and cortical ruffles, with low levels of endogenous Dyn 2 (c and d) recruited to these structures, particularly when compared with the cells expressing wt cortactin (a and b). Stimulated cells expressing a mutant Dyn 2 (Dyn 2 $\Delta$ PRD–GFP) lacking the interactive PRD show cortactin is recruited to the lamellipodia and cortex of these cells (e' and f'), whereas little of the expressed mutant protein (e and f) was found in these structures. Note the peculiar elongate shape of the Dyn 2 $\Delta$ PRD–GFP expressing cell (f). A summary of these localization patterns is in Table I. Bar, 10  $\mu$ m.

**Figure 6.** Expression of a truncated dynamin (Dyn 2(aa) $\Delta$ PRD) induces profound changes in cell shape with a concomitant proliferation of actin stress fibers. Fluorescence micrographs of cultured clone 9 cells expressing either wt Dyn 2(aa)–GFP (a) or Dyn 2(aa) $\Delta$ PRD–GFP (b–e). Cells expressing the wt protein (a) display a normal discoidal shape and a punctate distribution of dynamin at both the plasma membrane and Golgi apparatus, identical to that of untransfected cells. In strong contrast, cells expressing the Dyn 2(aa) $\Delta$ PRD–GFP became elongated, sprouting long peculiar neurite-like appendages (b–e). Morphological measurements of >200 wt and mutant cells confirmed this shape change showing a six- to sevenfold increase in width versus length (f). To test if changes in actin





organization might be responsible for these changes in shape, clone 9 cells expressing the Dyn 2(aa) $\Delta$ PRD-GFP (g-i) were fixed and stained for actin using rhodamine phalloidin (g'-i'). Whereas surrounding untransfected cells possessed a cortical ring of filamentous actin with few stress fibers, mutant cells displayed a reduction in cortical actin with a dramatic alteration in the actin cytoskeleton. Specifically, these cells possessed an extensive number of stress fibers that traversed the long axis and contributed to the shape malformation seen in isolated cells that were not grown in a monolayer (b-e). It should be noted that changes in the shape of these mutant cells are minimized in the confluent cultures shown here to provide a comparison of actin organization with the surrounding, untransfected cells (g-i). Shape changes are most prevalent in sparsely plated cells (b-e). Bars, 10  $\mu$ m.

Table 1. Effect of Dyn 2, Cortactin, Dyn 2ΔPRD, and CortΔSH3 Overexpression in Cultured Cells

Construct	Dyn 2 in Ruffle	Cortactin in Ruffle	Migratory morphology	Aberrant morphology	Stress fiber formation*
Dyn 2 (wt)	++	+	NC	NC	NC
Cortactin (wt)	++	+++	+++	+	NC
Dyn 2ΔPRD	--	NC	-	+++	+++
CortΔSH3	--	++	++	++	NC
Dyn 2 (wt) & cortactin (wt)	+++	+++	+++	NC	NC

+ or -, Indicates level of protein staining by fluorescence compared with untransfected cells. NC, Indicates no change.

\*Actin stress fiber formation was quantitated in clone 9 cells. The remainder of Table 1 was generated using PDGF-stimulated NIH/3T3 cells.

Dyn 2 through the transfection of a Dyn 2(aa)-GFP construct in fibroblasts did not increase cell ruffling or the levels of cortactin sequestered in these structures. These observations suggest that cortactin is first localized to the lamellipodia and subsequently recruits its dynamin binding partner. Additional studies expressing a CortΔSH3 construct in cells supports this prediction. Cells expressing this truncated cortactin continued to display substantial levels of this protein in the cell ruffles that were significantly depleted of endogenous Dyn 2 (Fig. 5, c and d). Thus, altering the cortactin protein in cells had a marked effect on Dyn 2 localization. Finally, expression of a truncated Dyn 2 protein, missing the COOH-terminal 124 amino acids comprising the PRD (Dyn 2(aa)ΔPRD) that mediates cortactin binding (Figs. 2 and 3) also greatly reduced the levels of dynamin in the leading ruffle (Fig. 5, e and f). Interestingly, although cortactin remained localized to the ruffles of these mutant cells, their polarity and shape appeared markedly altered. Generally, control stimulated fibroblasts displayed an obvious leading edge filled with cortactin and Dyn 2 with only modest levels of these proteins localized to the lateral and posterior cortices. In contrast, mutant cells expressing truncated Dyn 2 or cortactin proteins sequestered little Dyn 2 in the leading ruffle, whereas cortactin appeared distributed in the ruffle and also along most of the cell circumference. Further, these cells also appeared to take on peculiar elongate shapes, with long appendages. Because fibroblasts are generally irregular in shape, we tested whether the Dyn 2(aa)ΔPRD protein, when expressed in a cell line of more uniform size and form, might induce more obvious changes in cell shape. Clone 9 cells, a normal, rat hepatocyte cell line with a consistent discoidal shape were transfected to express Dyn 2(aa)ΔPRD-GFP, and were then allowed to recover for 24–48 h before fixation and viewing with the fluorescence microscope. Fig. 6, a–e, depicts representative pictures of clone 9 cells expressing either wt (Fig. 6 a) or truncated (Fig. 6, b–e) Dyn 2(aa)-GFP. Wt cells show a typical discoid morphology with Dyn 2 localized to clathrin-coated buds both at the plasma membrane and the trans-Golgi network as previously published (Cao et al., 1998). In contrast, mutant cells distributed Dyn 2(aa)ΔPRD-GFP diffusely throughout the cytoplasm. Although these cells appear healthy and survive for extended periods (weeks) in culture, they did possess peculiar, elongated or moon-shaped morphologies. Measurement of 200 cells revealed a consistent six- to sevenfold elongation of mutant cells compared with wt cells (Fig. 6 f). Staining of these malformed cells with rhodamine-phalloidin revealed an extraordinary number of large stress fibers coursing through the cytoplasm along the long axis of the cell. This was in striking

contrast to the strong peripheral band of cortical actin observed in wt clone 9 cells (Fig. 6, g'–i'). Cells in these images displayed less of a shape change when cultured to confluency to allow comparison of actin staining with surrounding cells. Much greater changes in shape were observed in cells grown more sparsely (Fig. 6, b–e). As discussed below, these changes in actin organization and cell shape in mutant cells are consistent with the concept that the dynamin PRD binds to the SH3 domain of cortactin to regulate actin cytoskeleton dynamics.

## Discussion

In this study, we have performed biochemical, morphological, and functional studies to demonstrate a physical interaction between the actin binding protein cortactin and the mechanochemical GTPase dynamin. The biochemical methods we have employed include coimmunoprecipitation (Fig. 1), immunofluorescence microscopy (Figs. 1, 4–6), GST-SH3 domain affinity chromatography (Fig. 2), and filter overlay assays (Fig. 3). Further, we have used peptides to the COOH-terminal PRD of dynamin to compete for binding of native dynamin to a GST-cortactin SH3 domain column to identify the peptide sequence motif (PxxPSRP) in the dynamin PRD that mediates this structural interaction with cortactin (Fig. 2). Morphological studies using two distinct dynamin antibodies and an expressed Dyn 2-GFP protein resulted in a striking and near precise colocalization of Dyn 2 and cortactin in select regions of the leading lamellipodial extension of migrating NIH/3T3 fibroblasts (Fig. 4). Finally, functional studies using expressed wt and truncated cortactin, and Dyn 2 proteins in cultured cells (Figs. 5 and 6) demonstrates that cortactin levels alter the recruitment of dynamin into the leading edge ruffle, and that the SH3 domain of cortactin and PRD of Dyn 2 are required to mediate this interaction. Surprisingly, altering this interaction induced profound changes in actin organization, and subsequently, cell morphology.

### Regulated Recruitment of Dyn 2 and Cortactin into the Lamellipodia of Motile Cells

A striking observation made during this study was the translocation of dynamin to the leading edge of an advancing cell in response to the mitogen/motogen PDGF (Figs. 1, 4, and 5). This response suggests that Dyn 2 and cortactin may play an important role in the process of cell polarization and ruffling initiated by a signaling cascade mediated by tyrosine phosphorylation. This association of Dyn 2 with cortactin is supported by several different biochemical criteria and morphological studies using Pan- or isoform-specific dynamin antibodies and, most importantly,

Dyn 2–GFP. Currently, it is unclear if the interaction between the PRD of dynamin and the SH3 domain of cortactin is regulated by PDGF stimulation. It is well established that cortactin is phosphorylated on both serine and threonine residues and is tyrosine phosphorylated in response to growth factor stimulation or transformation by activated Src (Okamura and Resh, 1995). We have confirmed that cortactin is tyrosine phosphorylated in our system using a phosphotyrosine antibody, although no tyrosine phosphorylation of dynamin was detected (data not shown). Further, using biochemical methods, we were unable to detect any changes in the amount or affinity of cortactin for Dyn 2 in response to PDGF treatment. This may be explained by the fact that only modest levels of the total Dyn 2 and cortactin pools are altered during stimulation, making it difficult to measure any significant changes in binding. This is supported by the fact that a large percentage of total cellular cortactin is phosphorylated in resting cells with only a modest increase induced by PDGF treatment (Kim and Wong, 1995). Alternatively, rather than changing the affinity of the cortactin–dynamin interaction, growth factor treatment may change the distribution of these already complexed proteins. Biochemical studies measuring dynamin–cortactin binding under various conditions *in vitro* will prove informative.

While this report is the first to identify a physical and functional link between dynamin and cortactin, there is precedence for dynamin participating in other actin-mediated processes. For example, a recent study has examined the interactions between dynamin, actin, and a dynamin-binding protein amphiphysin in developing hippocampal neurons (Mundigl et al., 1998). It was found that all three proteins accumulate together in the leading edge of the growth cone. A separate study that overexpressed high levels of a dominant negative mutant of a neuronal-specific dynamin (Dyn 1) in HeLa cells revealed some alterations in cytoskeletal organization, cell shape, and cell adhesiveness (Damke et al., 1994). Consistent with these findings is the observation that hippocampal neurons treated with Dyn 1 antisense oligonucleotides are prevented from forming neurites in culture (Torre et al., 1994). Further, a recent study has suggested that the actin-binding protein, profilin, interacts with a host of protein components of the endocytic machinery, including dynamin (Witke et al., 1998). Finally, dynamin has been shown to localize to the phagocytic cup of mouse macrophages and is required for complete inclusion of the phagosome (Gold et al., 1999). It is interesting that Dyn 2 appears to be associated with actin-mediated processes, such as membrane ruffling or phagocytosis, which require the active extension of membrane. It will be important to define how Dyn 2 participates in these processes.

### *Specificity of the Dynamin–Cortactin Complex in Cells*

The PRD of dynamin has been implicated in binding to the SH3 domain of no less than a dozen different structural and signaling proteins in a variety of cells (for reviews see Schmid et al., 1998; McNiven et al., 2000). Is the interaction with cortactin genuine and can the dynamin PRD distinguish between these numerous effectors in the confines of a living cell? Multiple observations using intact cells support the biochemical findings described in Figs. 1–3. First,

the immunolocalization of Dyn 2 with actin in cultured fibroblasts (Fig. 1) and epithelial cells (not shown) reveals a specific colocalization with some, but not all, actin structures. For example, Dyn 2 is localized with cortical actin in the leading edge of migrating cells, but not actin stress fibers toward the cell posterior. In fact, the cortical localization of dynamin is in perfect alignment with that of cortactin, not actin, suggesting a specific interaction. Second, the localization of Dyn 2 to the cortex appears to be restricted to two of the four forms of the Dyn 2 protein found in fibroblasts and epithelium. Both sites reside in the middle of the protein and represent either a 46-amino acid substitution starting at residue 399 or a modest deletion of only four amino acids starting at residue 516 (Cook et al., 1994; Sontag et al., 1994). A detailed study (Cao et al., 1998) has closely examined the distribution of all four of these Dyn 2 proteins in epithelial cells using GFP expression constructs. These forms have some association with clathrin, either at the plasma membrane and/or the Golgi apparatus. However, we have observed that the Dyn 2(aa) and (ba) forms that include the four-amino acid insert at the second splicing region show the strongest affinity for the ruffle of the four forms tested. Thus, defined sequences within the dynamin protein may provide specific information for targeting to distinct cellular organelles or to cortactin in the membrane cortex. It should be noted that neither of the splicing regions for Dyn 2 reside within the PRD, the region we have demonstrated interacts with cortactin (Figs. 2 and 3). Therefore, it is assumed that alternative splicing may alter dynamin folding and in turn change the accessibility or affinity of the Dyn 2 PRD to cortactin. Third, increased expression of wt cortactin protein significantly increases the size and number of membrane ruffles in transfected cells, and most importantly, induces a marked increase in the levels of either endogenous or exogenous Dyn 2 incorporated into these structures (Table 1; Fig. 5, a and b). This suggests that an increase in the levels of cytoplasmic cortactin acts to recruit Dyn 2 to lamellipodia even in resting cells not exposed to PDGF (Fig. 5, a and a'). Consistent with this finding is the observation that cells expressing a truncated cortactin protein lacking the interactive SH3 domain, continued to form lamellipodia with associated cortactin but little, if any, Dyn 2 was present in these structures. This suggests that this truncated cortactin maintains its ability to bind actin in the lamellipodia, but is unable to recruit Dyn 2. Interestingly, whereas there is a modest reduction of cortactin recruitment in these cells (Table I), the distribution of this protein became less polarized, associating with the leading edge, as well as the cell posterior in some cells (Fig. 5 d'). Finally, we performed an experiment reciprocal to those described above through the expression of a truncated Dyn 2 protein lacking the PRD (Fig. 5, e and f). We observed that while these mutant cells formed leading lamellipodia filled with cortactin, very low levels of Dyn 2 were observed in these structures (Table 1). It should be noted that, as for all dominant negative expression experiments, it is unlikely that the mutant protein will compete away all of the endogenous protein from the cortex. Most interestingly, however, many of these cells displayed peculiar, aberrant shapes (Fig. 6, f and f'), which is discussed further below.

Thus, all of the observations discussed above are consis-

tent with the concept that the Dyn 2 and cortactin proteins interact in the confines of living cells. We are unaware of any previous study of dynamin binding partners that has provided evidence using both biochemical and morphological methods, while demonstrating that altering the expression and/or fidelity of each protein affects the distribution of its partner.

### ***Putative Functions of the Dynamin–Cortactin Complex in Regulating Cytoskeletal Organization and Cell Shape***

How might dynamin–cortactin interactions modulate actin organization and, subsequently, cell shape? Huang and co-workers (Huang et al., 1997, 1998) have demonstrated that Src-mediated phosphorylation of several specific COOH-terminal tyrosines of cortactin significantly reduces its ability to bundle actin filaments. This is consistent with the general premise that nonmotile cells may possess many stress fibers, whereas stimulation with growth factors such as PDGF leads to a disassembly of these structures along with the concomitant assembly of actin within lamellipodia. Currently, the association of cortactin along stress fibers is controversial due to contrasting results from different groups (Wu and Parsons, 1993; Huang et al., 1997). Our cortactin antibody rarely labels stress fibers in untransfected cells, though we did observe an association with stress fibers in cells expressing modest levels (two- to threefold) of untagged, or GFP-tagged, cortactin (Figs. 4 a' and 5 a'). Whether the increased levels of cortactin induced an artifactual association, or enhanced a preexisting interaction, is undefined. Thus, it is unclear whether Dyn 2 and cortactin interact along stress fibers in resting cells to regulate their formation and/or disassembly. We have not observed Dyn 2 on these structures, although we have observed a significant colocalization of Dyn 2 and cortactin at punctate, clathrin-like pits, along the plasma membrane (not shown). In addition, Dyn 2 and cortactin have recently been shown to colocalize at podosomes along the cell base (Ochoa et al., 2000). Thus, Dyn 2 and cortactin may interact at multiple cytoplasmic locations depending on the activation state of the cell. Future studies will test how these proteins work together to regulate lamellipodial formation and extension, and motility.

The authors thank Dr. Tom Parsons for the cDNA expressing the cortactin SH3 sequence.

This research was supported by grants from the National Institutes of Health and the American Cancer Society to M.A. McNiven and T.W. Wong.

Submitted: 19 April 2000

Revised: 21 July 2000

Accepted: 8 August 2000

### **References**

Bray, D., and J.G. White. 1988. Cortical flow in animal cells. *Science*. 239:883–888.  
 Cao, H., F. Garcia, and M. McNiven. 1998. Differential distribution of dynamin isoforms in mammalian cells. *Mol. Biol. Cell*. 9:2595–2609.  
 Cook, T.A., R. Urrutia, and M.A. McNiven. 1994. Identification of dynamin 2,

an isoform ubiquitously expressed in rat tissues. *Proc. Natl. Acad. Sci. USA*. 91:644–648.  
 Damke, H., T. Baba, D.E. Warnock, and S.L. Schmid. 1994. Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. *J. Cell Biol.* 127:915–934.  
 Downing, J.R., and A.B. Reynolds. 1992. PDGF, CSF-1, and EGF induce tyrosine phosphorylation of p120, a pp60<sup>src</sup> transformation-associated substrate. *Oncogene*. 6:607–613.  
 Gold, E.S., D.M. Underhill, N.S. Morrissette, J.B. Guo, M.A. McNiven, and A. Aderem. 1999. Dynamin 2 is required for phagocytosis in macrophages. *J. Exp. Med.* 190:1849–1856.  
 Henley, J.R., and M.A. McNiven. 1996. Association of a dynamin-like protein with the Golgi apparatus in mammalian cells. *J. Cell Biol.* 133:761–775.  
 Huang, C., N. Yansong, T. Wang, Y. Gao, C.C. Haudenschild, and X. Zhan. 1997. Down-regulation of the filamentous actin cross-linking activity of cortactin by Src-mediated tyrosine phosphorylation. *J. Biol. Chem.* 272:13911–13915.  
 Huang, C., J. Liu, C.C. Haudenschild, and X. Zhan. 1998. The role of tyrosine phosphorylation of cortactin in the locomotion of endothelial cells. *J. Biol. Chem.* 273:25770–25776.  
 Jones, S.M., K.E. Howell, J.R. Henley, H. Cao, and M.A. McNiven. 1998. Role of dynamin in the formation of transport vesicles from the trans-Golgi network. *Science*. 279:573–577.  
 Kim, L., and T.W. Wong. 1995. The cytoplasmic tyrosine kinase FER is associated with the catenin-like substrate pp120 and is activated by growth factors. *Mol. Cell. Biol.* 15:4553–4561.  
 Maa, M.C., L.K. Wilson, J.S. Moyers, R.R. Vines, J.T. Parsons, and S.J. Parsons. 1992. Identification and characterization of a cytoskeleton-associated epidermal growth factor sensitive pp60<sup>src</sup> substrate. *Oncogene*. 7:2429–2438.  
 McNiven, M. 1998. Dynamin: A molecular motor with pinchase action. *Cell*. 94:151–154.  
 McNiven, M.A., H. Cao, K.R. Pitts, and Y. Yoon. 2000. Pinching in new places: multiple functions for the dynamin family. *TIBS*. 25:115–120.  
 Mundigl, O., G.C. Ochoa, D.C. Slepnev, A. Kabanov, and P. Decamilli. 1998. Amphiphysin I antisense oligonucleotides inhibit neurite outgrowth in cultured hippocampal neurons. *J. Neurosci.* 18:93–103.  
 Ochoa, G.-C., V.I. Slepnev, L. Neff, N. Ringstad, K. Takei, L. Daniell, H. Cao, M. McNiven, R. Baron, and P. De Camilli. 2000. A functional link between dynamin and the actin cytoskeleton at podosome. *J. Cell Biol.* 150:377–390.  
 Okamura, H., and M.D. Resh. 1995. p80/85 cortactin associates with the Src SH2 domain and colocalizes with v-Src in transformed cells. *J. Biol. Chem.* 270:26613–26618.  
 Scaife, R., I. Gout, M.D. Waterfield, and R.L. Margolis. 1994. Growth factor-induced binding of dynamin to signal transduction proteins involves sorting to distinct and separate proline-rich dynamin sequences. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:2574–2582.  
 Schmid, S.L., M.A. McNiven, and P. De Camilli. 1998. Dynamin and its partners: a progress report. *Curr. Opin. Cell Biol.* 10:504–512.  
 Seedorf, K., G. Kostka, R. Lammers, P. Baskku, R. Daly, W.H. Burgess, A.M. van der Blik, J. Schlessinger, and A. Ullrich. 1994. Dynamin binds to SH3 domains of phospholipase C $\alpha$  and GRB-2. *J. Biol. Chem.* 269:16009–16014.  
 Sontag, J.-M., E.M. Fykse, Y. Ushkaryov, J.-P. Liu, P.J. Robinson, and T.C. Sudhof. 1994. Differential expression and regulation of multiple dynamins. *J. Biol. Chem.* 269:4547–4554.  
 Stossel, T.P. 1993. On the crawling of animal cells. *Science*. 260:1086–1094.  
 Torre, E., M.A. McNiven, and R. Urrutia. 1994. Dynamin 1 antisense oligonucleotide treatment prevents neurite formation in cultured hippocampal neurons. *J. Biol. Chem.* 269:32411–32417.  
 Urrutia, R., J.R. Henley, T. Cook, and M.A. McNiven. 1997. The dynamins: redundant or distinct functions for an expanding family of related GTPases? *Proc. Natl. Acad. Sci. USA*. 94:377–384.  
 van der Blik, A.M. 1999. Functional diversity in the dynamin family. *Trends Cell Biol.* 9:96–102.  
 Witke, W., A.V. Podtelejnikov, A. Di Nardo, J.D. Sutherland, C.B. Gurniak, C. Dott, and M. Mann. 1998. In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:967–976.  
 Wu, H., and J.T. Parsons. 1993. Cortactin, an 80/85-kilodalton pp60<sup>src</sup> substrate, is a filamentous actin-binding protein enriched in the cell cortex. *J. Cell Biol.* 120:1417–1426.  
 Wu, H., A.B. Reynolds, S.B. Kanner, R.R. Vines, and J.T. Parsons. 1991. Identification of a novel cytoskeleton-associated pp60<sup>src</sup> substrate. *Mol. Cell. Biol.* 11:5113–5124.  
 Zhan, X., X. Hu, B. Hampton, W.H. Burgess, R. Friesel, and T. Maciag. 1993. Murine cortactin is phosphorylated in response to fibroblast growth factor-1 on tyrosine residues late in the G1 phase of the Balb/c 3T3 cell cycle. *J. Biol. Chem.* 268:24427–24431.