Cortactin, an 80/85-Kilodalton pp60src Substrate, Is a Filamentous Actin-binding Protein Enriched in the Cell Cortex

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Abstract. Two related cellular proteins, p80 and p85 (cortactin), become phosphorylated on tyrosine in pp60src-transformed cells and in cells stimulated with certain growth factors. The amino-terminal half of cortactin is comprised of multiple copies of an internal, tandem 37-amino acid repeat. The carboxy-terminal half contains a distal SH3 domain. We report that cortactin is an F-actin-binding protein. The binding to F-actin is specific and saturable. The amino-terminal repeat region appears to be both necessary and sufficient to mediate actin binding, whereas the SH3 domain had no apparent effect on the actin-binding activity. Cortactin, present in several different cell types, is enriched in cortical structures such as membrane ruffles and lamellipodia. The properties of cortactin indicate that it may be important for microfilament–membrane interactions as well as transducing signals from the cell surface to the cytoskeleton. We suggest the name cortactin, reflecting the cortical subcellular localization and its actin-binding activity.

Cells undergo structural rearrangement during cell division, cell migration, oncogenic transformation, and in response to stimulation with a variety of extracellular ligands (Albert et al., 1989). Vertebrate cells exhibit a cortical cytoskeletal network that resides beneath, and is associated with, the inner surface of the plasma membrane (Bray et al., 1986). This cortical cytoskeleton is comprised of a dense network of actin filaments and associated actin-binding proteins (Small et al., 1981; Stossel et al., 1981). Components of both the cell cortex and the plasma membrane are important for the integration of cell surface signals, facilitating transfer of these signals to the interior of the cell. The alterations in cortical cytoskeleton accompany a variety of cellular processes, including phagocytosis, cytokinesis, cell locomotion, cell–cell and cell–substratum interaction, transmembrane signaling, secretion, and endocytosis (for reviews see Tilney, 1983; Albert et al., 1989; Bretscher, 1991).

The structure and physical properties of the cortical cytoskeleton are determined, in large part, by cooperative and competitive interactions among many actin-binding proteins (Albert et al., 1989). Based on both in vitro and in vivo studies, a large number of actin-binding proteins have been identified that control assembly and length of actin filaments, link actin filaments into bundles or networks, define the three-dimensional organization of actin filaments and link filaments to other cytoplasmic and membrane components (for reviews see Stossel et al., 1985; Pollard and Cooper, 1986; Albert et al., 1989; Stossel, 1989; Vandekerckhove, 1990; Hartwig and Kwiatkowski, 1991). Subcellular localization studies have shown that many of the actin-binding proteins, such as filamin, villin, gelsolin, and ezrin, are enriched in the submembranous cortical region (Bretscher, 1991). In addition, the interaction of many actin-binding proteins with either monomeric actin or filamentous actin (F-actin) is regulated by common intracellular signals, including calcium, calmodulin, phospholipids, and protein phosphorylation (Vandekerckhove, 1990; Yamashiro et al., 1990; Hartwig et al., 1992). Studies on the regulation of gelsolin and profilin by Ca2+ and phosphatidylinositides have provided models for the reorganization of actin filament network (Stossel, 1989). In vivo studies have documented changes in the activity of certain actin-binding proteins concomitant with cellular events such as receptor stimulation and mitosis (Yamashiro et al., 1990; Goldschmidt-Clermont et al., 1991; Hartwig et al., 1992). The functional significance of actin-binding proteins is further emphasized by the diverse phenotypes exhibited in cell lines or transgenic organisms which either lack or overexpress actin-binding proteins (for review see Noegel and Schleicher, 1991).

Cellular transformation by oncogenic tyrosine kinases is characterized by overt changes in cellular growth control and dramatic changes in cell morphology and cell surface topology (for reviews see Jove and Hanafusa, 1987; Parsons and Weber, 1989). Chicken embryo (CE) cells transformed by pp60src, the oncogenic tyrosine kinase encoded by Rous sarcoma virus, are morphologically round. This phenotype is accompanied by loss of actin stress fibers and aggregation of F-actin into podosomes (David-Pfeuty and Singer, 1980; 1. Abbreviations used in this paper: CE, chicken embryo; F-actin, filamentous actin; GST, glutathione-S-transferase.)
The importance of the cytoskeleton in cellular transformation by protein tyrosine kinase oncogenes has been supported by the study of src mutants. The transforming activity of the various pp60^src mutants correlates with the degree of their association with detergent-insoluble cytoskeleton elements (Hamauchi and Nanao, 1987). The study of cellular substrates of pp60^src has also pointed to the involvement of cytoskeletal proteins in the process of cellular transformation and cell signaling (for reviews see Hunter, 1989; Schaller et al., 1993). Several newly characterized pp60^src substrates are either cytoskeletal proteins or associated with the cytoskeleton. These include FAK, a focal adhesion protein tyrosine kinase (Schaller et al., 1992); pp120, a tyrosine-phosphorylated protein that shares sequence similarity with plakoglobin and armadillo (Kanner et al., 1992; Reynolds et al., 1992); pp110, a phosphotyrosine-containing protein that localizes to stress fibers in CE cells (Kanner et al., 1992; Flynn, D. C., and J. T. Parsons, manuscript in preparation); tensin, an F-actin capping and bundling protein (Davis et al., 1991); p22 caveolin, a coating protein associated with vinculin (Turner et al., 1990).

Recently, we reported the identification of two proteins, designated p80/85, which are substrates for activated pp60^src protein tyrosine kinase (Wu et al., 1991). p80/85 is also tyrosine phosphorylated in cells stimulated with EGF, PDGF, colony stimulating factor I (Downing and Reynolds, 1992; Maa et al., 1992), and in platelets stimulated with thrombin (Wong et al., 1992; Lipert, L., and J. S. Brugge, personal communication). p80/85 exhibits several unique structural features. The amino-terminal half of the protein is comprised of multiple copies of an internal, tandem 37-amino acid repeat (referred to as “repeat region”). The number of repeat units appears to be specified by alternative splicing since, in CE cells, cDNAs have been identified that encode proteins with five (p80) or six copies (p85) of the tandem repeat (Hildebrand, J. D., and J. T. Parsons, manuscript in preparation). The carboxyl-terminal half of the protein contains a distal SH3 (src homology 3) domain, preceded by a region of 50 amino acids with a predicted helical structure and a region rich in proline, serine, and threonine. Here we report that p80/85 protein is an F-actin-binding protein. The binding to F-actin is specific and saturable. The amino-terminal repeat region of the protein appears to be both necessary and sufficient to mediate actin binding. Deletions removing the SH3 domain, the helical region, and the proline/serine/threonine region, had no apparent effect on the actin-binding activity. p80/85 is present in several different cell types and in each is enriched in cortical structures such as membrane ruffles and lamellipodia. The submembranous location of p80/85 indicates that the protein may be important for microfilament-membrane interaction as well as transducing signals from the cell surface to the cytoskeleton. Based on the cortical localization and actin-binding properties of p80/85, we propose the name “cortactin.”

Materials and Methods

Preparation of Cell Extracts

Primary CE cells were prepared from 10-d embryos (SPA/AS Inc., Nor-
Immunofluorescence Microscopy

Immunofluorescence microscopy was carried out as previously described (Wu et al., 1991). Cells were seeded and grown overnight on glass coverslips, washed with PBS, fixed, and permeabilized. Permeabilized cells were incubated with primary anticortactin mAbs and then with rabbit anti-mouse IgG, and detected with FITC-conjugated donkey anti-rabbit IgG as previously described (Wu et al., 1991). For double immunostaining experiments, cells were also incubated with rhodamine-phalloidin as previously described (Wu et al., 1991). Samples were visualized with a Leitz Orthoplan fluorescence microscope. For cytochalasin D treatment, cells were incubated with 2 μM cytochalasin D in growth media for 15 min at 37°C before fixation (Cooper, 1987).

Results

Localization of Cortactin to the Cell Cortex

Previous experiments have shown that p80/85 or cortactin colocalizes with F-actin in the peripheral extensions of normal cells and podosomes (rosettes) of pp60src-transformed cells (Wu et al., 1991). Indirect immunofluorescence of spreading CE cells using anticortactin mAbs revealed intense staining of cortical structures such as the membrane ruffles and lamellipodia (Fig. 2, a and b). The same staining pattern was observed in several other adherent cell types, including rat aortic smooth muscle cells and rat endothelial cells (Fig. 2, c and d). The staining pattern is very similar to that of cortical actin as judged by costaining experiments with phallloidin (data not shown). Cells incubated with the microfilament disrupting agent cytochalasin D exhibited a dramatic alteration in the staining pattern of cortactin. The resulting cytoplasmic aggregates of cortactin colocalized with F-actin aggregates, as demonstrated by costaining of the cells with anticortactin mAb and phalloidin (Fig. 2, e and f). The result of such immunostaining experiments indicated a colocalization of cortactin with F-actin and suggested a possible direct interaction of cortactin with F-actin.
Figure 2. Immunostaining of cortactin. CE cells (a and b), rat aortic smooth muscle cells (c), and rat endothelial cells (d) were grown overnight on glass coverslips, fixed, and immunostained with mAb 4F11 as described in Materials and Methods. In e and f, CE cells were pretreated with cytochalasin D before fixation, and then co-stained with mAb 4F11 (e) and rhodamine-phalloidin (f). Arrows in a–d denote intense staining of the cell cortex. Bar, 10 μm.

Binding of Cortactin to F-actin

To test the possible binding of cortactin to F-actin, CE cell lysates were incubated with polymerized rabbit muscle actin and an F-actin cosedimentation assay was carried out. After sedimentation of F-actin, >95% of the cortactin present in the cell lysate cosedimented with F-actin (Fig. 3, a, lanes 2 and 3). In the absence of F-actin, cortactin remained in the supernatant fraction (Fig. 3, a, lanes 1). As shown in b, the majority of the F-actin sedimented to the pellet fraction under conditions of the assay, indicating a quantitative polymerization of the monomeric actin. In control incubations, BSA, which does not have actin-binding activity, remained in the supernatant fraction (Fig. 3 b, lanes 3). Similar binding of cortactin to F-actin was observed when purified chicken muscle F-actin was used in the assay (data not shown).

The results of the cosedimentation assay indicated that cortactin, present in CE cell lysates, may bind directly to F-actin. To further test this hypothesis, [35S]methionine-labeled cortactin, synthesized by in vitro translation of cortactin mRNA in a reticulocyte lysate, was used in an F-actin cosedimentation assay. A significant portion of the in vitro-translated 80-kD form of cortactin cosedimented with F-actin, providing evidence for the direct interaction of cortactin with F-actin (see Fig. 6, lanes 1b).

Since it was possible that the interaction of cortactin with F-actin might be mediated through a cellular component present in either the CE cell lysate or the reticulocyte lysate, cortactin was expressed as a GST fusion protein in Escherichia coli and its actin-binding properties were investigated. The GST:p80 fusion protein contains amino acid residues 55–526 of p80 fused in frame to sequences encoding GST. As shown in Fig. 4 (lanes 2), the purified GST:p80 protein...
Figure 5. Binding curve of cortactin to F-actin. Different amounts of purified GST.p80 fusion protein were incubated with 15 μg of F-actin and the mixture was centrifuged, as described in Materials and Methods. Supernatant and pellet fractions were resolved by SDS-PAGE and stained with Coomassie blue (as in Fig. 4). The concentrations of bound and free fusion protein were determined by quantitation of individual bands on a densitometer. The curve represents a theoretical binding isotherm for $K_d = 0.43 \pm 0.08$ μM. The inset shows a Scatchard analysis of the same data.

cosedimented with F-actin. Moreover, addition of increasing amounts of GST.p80 protein to a fixed amount of F-actin in the cosedimentation assay revealed that the binding of cortactin to F-actin was saturable (Fig. 4, lanes 3 and 4). Best fit analysis of the binding data indicated the presence of a single binding site (Fig. 5). At saturation, the binding stoichiometry is ~1 GST.p80 protein per 14 actin monomers (Fig. 5). Taken together, these data demonstrate that cortactin from three different sources binds efficiently to F-actin, providing evidence that cortactin interacts directly with F-actin.

Mapping of F-actin Binding Site to Repeat Region of Cortactin

The specificity of the interaction of cortactin with F-actin was further assessed by mapping the region of cortactin that is necessary for the binding. $[^{35}S]$Methionine-labeled cortactin was synthesized by directing in vitro transcription-translation reactions with individual p80 cDNA constructs, illustrated in Fig. 1 b. To generate a series of carboxyl-terminal truncated cortactin molecules, p80 cDNAs were digested with restriction enzymes that cleave within the 3' portion of the coding sequence (Fig. 1 b). Internal deletions were created by in-frame ligation of p80 cDNA fragments generated by cleavage with specific restriction enzymes (Fig. 1 b).

Binding to F-actin was tested in an F-actin cosedimentation assay (Fig. 6). Truncated cortactin, lacking the SH3 domain (d1486–526 and d1392–526, lanes 2b and 3b) or lacking the carboxyl-terminal half of the protein (d1293–526, lanes 6b) cosedimented with F-actin, mimicking the activity of the full-length protein (lanes 6b). In contrast, an internal deletion of amino acid residues 108–382, which deleted the complete repeat region, as well as the following helical region and most of the proline-rich region (Fig. 1 b), did not cosediment with F-actin (Fig. 6, lanes 5b). A cortactin variant, with a smaller internal deletion which removed amino acid residues 142–210 within the repeat region, showed a significantly decreased binding to F-actin (lanes 4b). These results indicated that the amino acid sequences comprising the carboxyl-terminal half of the protein, including the SH3 domain, were not required for the binding of cortactin to F-actin. Instead, the tandemly repeated sequences comprising the repeat region appeared to be necessary for the efficient binding of cortactin to F-actin.

To assess which sequences of cortactin were sufficient to reconstitute the F-actin binding activity, the amino- and carboxyl-terminal halves of p80 were fused separately to GST and expressed as GST fusion proteins in E. coli.
Figure 7. Interaction of GST-cortactin fusion proteins with F-actin. Purified GST fusion proteins (illustrated in Fig. 1 c) were either preincubated with F-actin and centrifuged (lanes 2, 4, and 6) or centrifuged alone (lanes 1, 3, and 5). Supernatant (lanes S) and pellet (lanes P) were separated by SDS-PAGE and stained with Coomassie blue. (Lanes 1 and 2) GST.p80; (lanes 3 and 4) GST.55–292; (lanes 5 and 6) GST.294–526. The positions of the fusion proteins and the major proteolytic product are indicated. The molecular mass markers are shown in lanes M and the sizes are indicated at the left in kD.

GST.55–292 and GST.294–526 contain, respectively, amino acid residues 55–292 and 294–526 (Fig. 1 c). As shown in Fig. 7, GST.55–292 cosedimented with F-actin, whereas the majority of GST.294–526 remained in the supernatant. These results confirmed that the repeat region of cortactin participated in F-actin binding and indicated that this region may be sufficient for the binding. Although the repeat region appears to be both necessary and sufficient for F-actin binding, we cannot rule out the possibility that there are other weaker actin-binding sites. The small amount of GST.294–526 observed in the pellet fraction may be indicative of such a site in the carboxyl-terminal half of the protein (lanes 6).

Inhibition of Cortactin–F-actin Interaction with mAbs

As previously reported, several mAbs appear to interact with different epitopes within cortactin (Wu et al., 1991). Four different mAbs were tested for their ability to inhibit the binding of cortactin to F-actin (Fig. 8). Preincubation of cell lysates with anticortactin mAb 4F11 before mixing with F-actin blocked the association of cortactin with F-actin (Fig. 8, lanes 3 and 4). In contrast, preincubation of cell lysates with either mAb 1H3 (lanes 6), mAb 3B12, or 3D9 (data not shown) did not affect the cosedimentation of cortactin with F-actin. To better understand the nature of the mAb inhibition, we mapped the epitopes of mAb 4F11 and 1H3. GST fusion proteins containing portions of cortactin were tested for their reactivity with the two mAbs in a Western blot. As shown in Fig. 9, mAb 4F11 reacted with GST.p80 (lane J) as well as GST.55–292 (lane 2), but not with GST.294–526 (lane 3). Conversely, mAb 1H3 reacted with GST.p80 (lane 4) and GST.294–526 (lane 6), but not with GST.55–292 (lane 5). This localized the binding sequences for 4F11 and 1H3 to the amino-terminal half and the carboxyl-terminal half of the protein, respectively. The epitopes for 4F11 and 1H3 were further mapped by immunoprecipitation of the individual, in vitro-translated cortactin proteins (summarized in Fig. 1). A cortactin variant with a deletion of amino acid residues 108–382 was not immunoprecipitated by mAb 4F11, whereas variants with a deletion of amino acid residues 142–210 or lacking the carboxyl-terminal region were readily im-

Figure 8. Inhibition of cortactin binding to F-actin by anticortactin mAb. CE cell lysate (lanes 1) and lysates pretreated with mAb 4F11 (lanes 2–4) or mAb 1H3 (lanes 5 and 6) were either incubated with F-actin and centrifuged (lanes 1, 3, 4, and 6) or centrifuged alone (lanes 2 and 5). Supernatant (lanes S) and pellet (lanes P) were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with mAb 4F11. The arrow denotes the position of the major bands of cortactin.

Figure 9. Mapping of epitopes of anticortactin mAbs. Purified GST.p80 (lanes 1 and 4), GST.55–292 (lanes 2 and 5), and GST.294–526 (lanes 3 and 6) were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with either mAb 4F11 (lanes 1–3) or mAb 1H3 (lanes 4–6). The positions of the fusion proteins and the major proteolytic product are indicated. Molecular mass markers are indicated in kD.
Lysates from pp60^v-src-transformed CE cells were incubated with bodies (a) or mAb 4F11 (b). As controls, the same amount of cell F-actin and centrifuged (lanes 2 and 3). Supernatant (lanes 4FI 1 and the immunocomplexes were resolved by SDS-PAGE and immunoblotted with either phosphotyrosine antibodies. The position of the major cortactin bands is indicated by an arrowhead. Molecular mass markers are indicated in kD.

Remodeling of the cellular actin/cytoskeletal network occurs in response to a variety of extracellular and intracellular signals. In this paper we demonstrate that p80/85, a substrate for activated forms of pp60^v-src, other src family protein tyrosine kinases, and several growth factor receptor tyrosine kinases, is a filamentous actin-binding protein. p80/85, which we have termed cortactin, is present in multiple cell types and is enriched in the submembranous cortical region of adherent cells. The F-actin-binding region within cortactin maps to a domain comprised of five or six copies of a tandemly repeated sequence. Deletion of a highly conserved carboxyl-terminal SH3 domain, a structural motif common to many cytoskeleton-associated proteins, did not affect the binding of cortactin to F-actin. The F-actin-binding activity, its cortical localization, and tyrosine phosphorylation suggest that cortactin may play a role in mediating microfilament–membrane interactions in response to signals propagated by receptor and/or nonreceptor protein tyrosine kinases.

Cortactin Has a Specific, Saturable F-actin Binding Activity

Cortactin from three separate sources, i.e., CE cell lysates, programmed reticulocyte lysates or purified as a GST fusion protein from E. coli, bound specifically to polymerized actin in an F-actin cosedimentation assay. These data argue strongly for a direct interaction of cortactin with F-actin, and make it unlikely that the interaction with F-actin is mediated through another cellular protein present in the cortactin preparations. Although cortactin binds only to F-actin (not to monomeric actin; see below) and binds efficiently to purified actin from several sources, at present, we can not rule out the possibility that the binding to polymerized actin may be mediated by another F-actin–binding protein present in the actin preparations.

Using a GST–cortactin fusion protein produced in E. coli, we showed that the binding of cortactin to F-actin is saturable. We estimate that the stoichiometry of binding at saturation is \(\sim 1\) GST.p80 protein per 14 actin monomers. A similar stoichiometry has been reported for other actin-binding proteins, such as brain \(\alpha\)-actinin, macrophage actin-binding protein, and caldesmon (Pollard and Cooper, 1986). Analysis of the binding data yielded an apparent dissociation constant of \(k_d = 0.43 \pm 0.08\) \(\mu\)M, a value similar to that reported for the actin-binding protein spectrin. ABP 120, \(\alpha\)-actinin, and MARCKS (Stossel et al., 1985; Hartwig et al., 1992). Removal of the GST portion of the GST.p80 protein by cleavage with thrombin increased the affinity of bacterially expressed cortactin for F-actin by approximately twofold (data not shown). To date, we have been unable to purify sufficient cortactin from CE cell lysates to perform kinetic measurements similar to those carried out with E. coli-

Figure 10. Binding of tyrosine-phosphorylated cortactin with F-actin. Lysates from pp60^v-src-transformed CE cells were incubated with F-actin and centrifuged (lanes 2 and 3). Supernatant (lanes 2S) and pellet (lanes P) were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with either phosphotyrosine antibodies (a) or mAb 4F11 (b). As controls, the same amount of cell lysates were centrifuged without F-actin (lanes 1). In lanes 2S and 3S, the supernatant fractions were immunoprecipitated with mAb 4F11 and the immunocomplexes were resolved by SDS-PAGE and immunoblotted with either phosphotyrosine antibodies (a) or mAb 4F11 (b). In a, lysates from CE cells (lane C) or pp60^v-src-transformed CE cells (lane F) were also resolved by SDS-PAGE and immunoblotted with phosphotyrosine antibodies. The position of the major cortactin bands is indicated by an arrowhead. Molecular mass markers are indicated in kD.
derived cortactin. Therefore it remains possible that post-translational modification of cortactin (serine/threonine phosphorylation, or tyrosine phosphorylation, see below) may directly alter the affinity or stoichiometry of cortactin binding to F-actin (Yamashiro et al., 1990; Hartwig et al., 1992).

The Repeat Region, but Not the SH3 Domain of Cortactin, Is Responsible for F-actin Binding

The evidence presented above indicates that the repeat region of cortactin is both required and sufficient for F-actin binding. The amino acid sequences comprising the individual repeats are highly conserved among each of the internal repeats (Fig. 1 d). Using both computer sequence analysis and visual inspection, we have been unable to identify sequence similarities with known actin-binding proteins or reported actin-binding sequences (Vanderkerkhove and Vancompernolle, 1992). However, analysis of the secondary structure of the individual tandem repeats predicts an α-helical region within the carboxyl-half of each repeat (Fig. 1 d). Computer modeling of the helical region revealed that the highly conserved lysine residues can be positioned on the same face of the predicted α-helix, contributing to a positively charged helical surface (data not shown). Such a lysine-rich helix has been reported in several actin-binding proteins (Friederick et al., 1992; Vanderkerkhove and Vancompernolle, 1992). Furthermore, the presence of the epitope for mAb 4P11 (a mAb that blocks actin binding and recognizes cortactin from avian, rodent, primate, and invertebrate species) in the repeat region suggests that this region of cortactin is likely to be exposed on the surface of the protein and may be available for protein–protein interaction.

Many F-actin–binding proteins also have monomeric actin-binding activity (Stossel et al., 1985; Pollard and Cooper, 1986). Cortactin was not retained on either a DNaseI affinity column or a DNaseI–actin affinity column, and thus does not appear to bind G-actin (data not shown).

Cortactin, present in several cell types, exhibits a characteristic colocalization with F-actin in the peripheral cortical region. In spite of its in vitro F-actin binding activity, little cortactin is observed to be associated with actin stress fibers. Several factors might explain the selective binding to cortical actin. For example, cortactin may bind specifically to an isoform of actin present in the cortical region or interact with an F-actin–binding protein present (or enriched) in the cell cortex. Alternatively, the presence of a membrane localization signal in cortactin may target cortactin to the submembranous region. The evidence discussed above shows that the carboxyl-terminal SH3 domain, a domain conserved in the src family of protein tyrosine kinases as well as in several proteins with diverse cellular functions (Pawson, 1988) is not directly involved in F-actin binding of cortactin. Several SH3-containing proteins associate either directly or indirectly with the plasma membrane, including myosin I, PLC-γ, spectrin, fodrin,dlgA, and NADPH oxidase-associ- ated proteins (for review see Roadway et al., 1989). Thus the SH3 domain in cortactin may serve as a localization signal to link cortactin to integral or peripheral membrane proteins. Cortactin, together with the proposed SH3-binding proteins, may form complexes in the submembranous region, participating in the interaction of microfilaments with the plasma membrane.

Cortactin Is a Major Tyrosine-phosphorylated F-actin-binding Protein in pp60src Transformed Cells

Previous studies have shown that cortactin becomes tyrosine phosphorylated to a high stoichiometry (∼30% of the total cortactin) in pp60src-transformed avian and rodent cells (Wu et al., 1991), as well as in cells stimulated with EGF, PDGF, and colony stimulating factor I (Downing and Reynolds, 1992; Maa et al., 1992). The data presented above indicate that tyrosine phosphorylation of cortactin does not influence F-actin binding. Therefore, the question remains how (if at all) tyrosine phosphorylation of cortactin might influence the actin remodeling observed in src-transformed cells. Previous studies have shown that cortactin codistributes with F-actin into podosomes in pp60src-transformed cells (Wu et al., 1991). pp60src-transformed cells, in comparison with untransformed cells, have a significant increase in the amount of actin in the Triton-insoluble cytoskeletal fraction (Holme et al., 1986). This partitioning of actin into the cytoskeletal fraction is correlated with a change in filamentous actin assemblies from “stress fiber” pattern to punctative filament aggregates, podosomes (Throne et al., 1985; Marchisio et al., 1987). Although the molecular mechanism for the codistribution of cortactin with F-actin aggregates in transformed cells remains unclear, several possibilities can be entertained. There is growing evidence for specific and stable interaction between phosphoryroside-containing proteins and proteins with SH2 domains. While many such proteins have specific catalytic functions (GTPase-activating protein for p21ras, phospholipase C-γ, pp60src), other proteins appear to function as “bridging” proteins (crk, SHC, and GRB2) (Mayer and Hanafusa, 1990; Cantley et al., 1991; Koch et al., 1991; Lowenstein et al., 1992; Pelicci et al., 1992). It is possible that tyrosine phosphorylation of cortactin may promote its binding to cellular proteins containing SH2 domains. Thus increased levels of tyrosine phosphorylation of cortactin may induce aberrant clustering/aggregation of cortactin–F-actin complexes with such proteins. Alternatively, tyrosine phosphorylation may affect the interaction of cortactin with F-actin by changing the binding affinity. Although such differences in binding have not been observed to date in our studies, the actin-binding properties of several proteins are affected by serine/threonine phosphorylation; e.g., MARCKS protein phosphorylated by protein kinase C and caldesmon phosphorylated by pp34cat2 kinase both exhibit a decreased affinity to microfilaments (Yamashiro et al., 1990; Hartwig et al., 1992). Serine/threonine phosphorylation of cortactin is observed in normal and src-transformed cells and may be, along with tyrosine phosphorylation, an important regulatory signal (Wu et al., 1991).

Cortactin Has a Submembranous Localization in Cell Cortex

Indirect immunofluorescent staining of multiple cell types with anticortactin mAbs revealed a strong staining of the cortical structures such as membranous ruffles and lamel- lipodia. The actin network present in the cell cortex is a more transitory and dynamic structure in comparison with stress fibers. The structure, and in turn the mechanical properties of this actin network, is determined by the interaction of various classes of actin-binding proteins with actin. Several actin-binding proteins are reported to be enriched in the
cell cortex, including filamin, villin, gelsolin, and ezrin (Bretscher, 1991). Many of these proteins are also regulated by cellular signals, such as Ca²⁺, phospholipids, and protein phosphorylation. The presence in the cell cortex of cortactin, an F-actin–binding protein that becomes tyrosine phosphorylated in response to ligand stimulation as well as in cellular transformation, makes cortactin an interesting candidate for a signal-transducing molecule or an effector molecule in the integration of extracellular stimulation with cytoplasmic changes. We note that cortactin is highly homologous to the product of EMS1, a gene located within the amplified chromosome 1q3 region in human carcinomas (Schuuring et al., 1992). The EMS1 gene product, therefore, may be the human counterpart of avian cortactin.

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