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Cortactin Branches Out: Roles in Regulating Protrusive Actin Dynamics

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Abstract

Since its discovery in the early 1990's, cortactin has emerged as a key signaling protein in many cellular processes, including cell adhesion, migration, endocytosis, and tumor invasion. While the list of cellular functions influenced by cortactin grows, the ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. Recently, several advances have been made in our understanding of the interaction between actin and cortactin, providing insight into how these two proteins work together to provide a framework for normal and altered cellular function. This review examines how regulation of cortactin through post-translational modifications and interactions with multiple binding partners elicits changes in cortical actin cytoskeletal organization, impacting the regulation and formation of actin-rich motility structures.

Keywords

cortactin; actin; Arp2/3; N-WASp; motility

Introduction

Cell motility and directional migration are essential for a wide range of cellular processes and disease states, including wound healing, vascular disease, osteoporosis, mental retardation, chronic inflammatory disease, as well as tumor invasion and metastasis (Ridley 2006). The ability of a cell to migrate in response to external stimuli involves several coordinated steps: Formation of lamellipodia at the leading edge, attachment of the lamella to the substratum, forward translocation of the cell body, detachment of adhesions and subsequent retraction of the cell rear (reviewed in (Ridley et al. 2003)). Similarly, invasion requires altered cellular adhesion properties, degradation of the adjacent extracellular matrix, and directional cell migration. The complicated processes of cell migration and invasion require dynamic regulation of the cortical actin cytoskeleton, utilizing a network of signaling proteins that coordinate regulated changes in the actin architecture. One of the key molecules involved in cortical actin regulation is the adaptor protein cortactin. Cortactin is a multi-domain protein that was first identified as a Src substrate (Kanner et al. 1990; Wu et al. 1991). Subsequently, cortactin has been shown to play an essential role in many actin-based cellular processes, encompassing cell migration and invasion (Yamaguchi and Condeelis 2007), axon guidance (Knoll and Drescher 2004), neuronal morphogenesis (Martinez et al. 2003; Gray et al. 2005), and tumor cell metastasis (Li et al. 2001). Several recent reviews have highlighted cortactin's role in a variety of cellular processes, including

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cancer progression, invasion and metastasis, and endocytosis (Daly 2004; Ayala et al. 2006; Cosen-Binker and Kapus 2006; Orth and McNiven 2006; Weaver 2006; Buday and Downward 2007; Yamaguchi and Condeelis 2007; Weaver 2008). In this review we focus on the structure/function relationship between cortactin and actin, and what is known about cortactin and the formation and regulation of dynamic actin-based structures and its role in the formation of motility-related membrane protrusions.

Mechanisms of actin assembly

The cortical actin cytoskeleton is a complex and dynamic structure that participates in key aspects of cellular homeostasis. In response to extracellular stimuli, specific intracellular signaling cascades produce various actin-based protrusions of the plasma membrane, including lamellipodia, filopodia, dorsal waves, and invadopodia/podosomes. Lamellipodia formation can occur in response to treatment with EGF, leading to the activation of several kinase families, including Src, Erk, and PI3K (Yamaguchi and Condeelis 2007). PI3K in turn activates Rac, a member of the Rho family GTPases, which regulates the signaling pathway involved in barbed end generation and dendritic actin nucleation (Yamaguchi and Condeelis 2007). In addition to phosphorylating cortactin, Src mediates the phosphorylation of several downstream effector molecules, facilitating the activation of Arp2/3 complex and actin nucleation (Yamaguchi and Condeelis 2007). Stimulation with PDGF activates PI3K and Src, as well as PAK1, PKA, and Abl kinases (Buccione et al. 2004). The concerted action of these signaling proteins serves to aid in the formation of circular dorsal ruffles (CDRs), invadopodia, and lamellipodia. Regardless of the signaling pathway, initiation of actin polymerization remains the consistent factor responsible for initiating the formation of membrane protrusions required for motility and invasion, allowing the cell to react and adapt to a variety of environmental cues.

A critical step in dynamic remodeling of the cortical actin cytoskeleton is activation of the actin-related protein (Arp)2/3 complex. The Arp2/3 complex consists of seven subunits (Arp2, Arp3, and ARPC1-5) that function together to nucleate and initiate new actin filaments. Arp2/3 binds to the side of a preexisting F-actin (mother) filament and nucleates production of a new (daughter) filament at a 70° angle (Machesky et al. 1999; Higgs and Pollard 2001). Arp2/3-nucleated branched actin networks *in vitro* are very similar in appearance to the cortical actin network present in lamellipodia (Svitkina and Borisy 1999), and subsequent work has demonstrated a requirement of Arp2/3 activity in lamellipodia formation (Mullins et al. 1998; Bailly et al. 2001). Newly branched actin filaments elongate by addition of actin monomers to their barbed ends, and are terminated by the binding of capping protein (CP) to the barbed end. The addition of capping protein ensures that the newly produced filaments are short and rigid, with polymerization concentrated near the leading edge to produce protrusive force (Condeelis 2001). Activation of Arp2/3 results in significant conformational change of several complex members, allowing all seven subunits of the complex to interact with the mother filament (Blanchoin et al. 2000a). Spatially, Arp2/3 is positioned at the pointed end of the newly forming filament (Pollard 2007). The exposed surfaces of the Arp2 and Arp3 subunits assume a conformation similar to that of an F-actin barbed end, and these proteins function to mimic the first two G-actin subunits in daughter filaments (Beltzner and Pollard 2004)(reviewed in (Pollard 2007)). Subsequent network disassembly and actin filament depolymerization involves the inhibition of PAK and LIMK. Kinase inhibition results in ADF/cofilin dephosphorylation by the phosphatase slingshot, promoting ADF/cofilin-mediated severing and disassembly of actin filaments at the network rear, replenishing the actin monomer pool for subsequent rounds of polymerization (Lappalainen and Drubin 1997; Theriot 1997; Bamburg 1999; Carlier et al. 1999; Chen et al. 2000; Niwa et al. 2002). In addition to the Arp2/3 complex, several other proteins have been shown to initiate actin nucleation, including formins, spire and Cordon

blue (reviewed in (Watanabe and Higashida 2004; Baum and Kunda 2005; Pollard 2007; Winckler and Schafer 2007)). The action of these proteins differs from Arp2/3 in that they nucleate straight actin filaments.

Cortactin structure and subcellular localization

Cortactin is a 63–65kDa protein initially characterized as a tyrosine phosphorylated substrate in v-Src-transformed chick embryo fibroblasts (Kanner et al. 1990; Wu et al. 1991). Cortactin was also independently identified as downstream target of FGFR-mediated c-Src activation (Zhan et al. 1993) and as a gene amplified and overexpressed in tumor cells with chromosome 11q13 amplification (Schuuring et al. 1993). When analyzed by SDS-PAGE, cortactin migrates as an 80/85kDa doublet that cannot be attributed to post-translational modification of the protein (Wu and Parsons 1993). The reason for the incongruity between the calculated MW and the observed Mr in SDS-PAGE experiments is currently unclear, but may involve an altered ability to bind SDS inherent in the primary sequence and/or spontaneous reacquisition of secondary structural elements.

Equilibrium sedimentation and deep etch electron microscopy has determined that cortactin is a monomeric rod-shaped protein ~220Å in length, thinner than the diameter of an actin filament (Weaver et al. 2002). This may represent the “open” form of the protein, since recent work indicates that cortactin can assume an alternative confirmation that is partially globular (Cowieson et al. 2008) (see below). Based on primary sequence analysis cortactin is composed of several distinct domains that either bind other proteins or are sites of post-translation modification (Fig. 1) (Wu et al. 1991). In the murine form, these regions include an amino terminal acidic domain (NTA) (amino acids 1–84). The NTA domain contains a conserved DDW region (amino acids 20–22) that is responsible for interaction with Arp3 and subsequent activation of the Arp2/3 complex (Weed et al. 2000; Higgs and Pollard 2001). Carboxyl terminal to the NTA domain is a series of 6 complete and one partial tandemly repeating segments (amino acids 85–326) termed cortactin repeats that comprise the actin binding region (ABR). The cortactin repeats region directly binds F-actin, with maximal binding activity centered on the fourth repeat. Following the repeats region is a α -helical domain (site of calpain cleavage (Huang et al. 1997b; Perrin et al. 2006)) and a proline-rich region (PRR; amino acids 327–494) enriched in sites of tyrosine and serine phosphorylation. The extreme C-terminus contains an Src homology (SH)3 domain (amino acids 495–542) that interacts with proline-rich binding sequences in several cortactin interacting partners. These include WIP (Kinley et al. 2003), N-WASP (Weaver et al. 2002), MLCK (Dudek et al. 2002), and dynamin 2 (McNiven et al. 2000) (reviewed in (Cosen-Binker and Kapus 2006)).

In serum-starved or unstimulated cells, cortactin is normally present in the cytoplasm as a non-phosphorylated protein (Head et al. 2003). Upon growth factor stimulation (EGF, FGF, or PDGF), activation of Rac1 leads to cortactin relocation from internal cytoplasmic regions to the cortical actin network (Zhan et al. 1993; Zhan et al. 1994; Head et al. 2003). In addition to Src, cortactin is also phosphorylated by the cytoplasmic tyrosine kinases Fyn, Fer, cMet, c-Abl, Syk ((Gallet et al. 1999; Kapus et al. 2000; Crostella et al. 2001; Huang et al. 2003) Boyle et al. 2007). The serine/threonine kinases PAK1 and MAPK also directly phosphorylate cortactin (Campbell et al. 1999; Webb et al. 2006). The actions of these kinases enable cortactin to function in enhancing actin polymerization and branched actin network formation through its interaction with the actin nucleating complex Arp2/3, as well as the recruitment and positioning of other signaling proteins through SH2 and SH3-mediated interactions as described below.

Cortactin interactions with Arp2/3 and N-WASp

The formation of motility-associated plasma membrane protrusive structures requires the choreographed actions of specific actin effector proteins responsible for the proper spatio-temporal regulation of actin polymerization. Stabilization of branch points in the actin cytoskeletal network is essential for efficient membrane protrusion and directed cell motility. Arp2/3 branch points are inherently metastable, debranching spontaneously *in vitro* over time (Blanchoin et al. 2000b). Binding of cortactin to Arp2/3 at F-actin branch points stabilizes the branch juncture, inhibiting filament debranching and network breakdown (Weaver et al. 2001).

Cortactin is able to directly influence actin polymerization by serving as a nucleation promoting factor (NPF) for Arp2/3 (Urano et al. 2001; Weaver et al. 2001). NPFs are a class of proteins that can activate Arp2/3 through direct binding, resulting in a conformational change in the Arp2/3 complex that facilitates *de novo* actin nucleation and filament elongation as described above (Welch and Mullins 2002). The Wiskott-Aldrich syndrome protein (WASP) family of proteins, including WASP and N-WASp, and the related WAVE/Scar proteins also serve as NPFs for Arp2/3 (Pollard 2007). Cortactin functions as an NPF for Arp2/3 through its ability to bridge Arp3 with ARPC2, 4 and 5, stabilizing the active conformation of the Arp2/3 complex and facilitating the addition of actin monomers to the Arp2 and Arp3 subunits on the side of the mother filament (Weaver et al. 2002; Pollard 2007) (Fig. 2A). Interestingly, the NPF function of cortactin requires both the NTA and the actin binding region (ABR) of cortactin, demonstrating the importance of interaction with both F-actin and Arp2/3 for efficient actin polymerization (Weaver et al. 2001).

Similar to cortactin, N-WASp and WAVE/Scar proteins bind to F-actin, though this interaction occurs through a basic region and not an ABR (Suetsugu et al. 2001; Kelly et al. 2006). These proteins can bind G-actin monomers and the Arp2/3 complex by virtue of a Wasp Homology-2 (WH2)-central-acidic (WCA) region ((Marchand et al. 2001), reviewed in (Takenawa and Suetsugu 2007)). While F-actin binding is important for WASp-family protein function, the binding of G-actin to the WCA region results in dramatic enhancement of NPF activity by placing actin monomers in close proximity to activated Arp2/3 (Machesky et al. 1999).

Although cortactin-mediated Arp2/3 activation is weaker than that of N-WASp, cortactin synergizes with N-WASp in activating Arp2/3, enhancing N-WASp mediated actin nucleation (Weaver et al. 2001). How N-WASp and cortactin cooperate to initiate Arp2/3 activation is somewhat controversial. The interaction of N-WASp with the side of an actin filament enhances recruitment of and activates Arp2/3, through binding to Arp2, Arp3, and ARPC1 subunits (Suetsugu et al. 2001; Pollard 2007). The binding of the cortactin NTA domain displaces the N-WASp WCA domain from the Arp3 subunit, but not Arp2 or ARPC1 (Weaver et al. 2002). In this manner, N-WASp and cortactin may both bind to and activate Arp2/3 complex concomitantly, forming a ternary complex of Arp2/3/WCA/NTA that results in enhanced NPF activity. Alternately, evidence exists that the activation of Arp2/3 by N-WASp and cortactin occurs in a sequential manner. Cortactin has a higher binding affinity for activated Arp2/3 complex at branch points compared to non-activated, non-actin associated Arp2/3 (Urano et al. 2003). After Arp2/3 is activated by N-WASp, cortactin effectively binds and displaces N-WASp from the Arp2/3, presumably stabilizing the Arp2/3/cortactin/F-actin complex (Urano et al., 2003). Whether one or both of these mechanisms are employed at the cellular level to drive Arp2/3 activity remains to be determined.

In addition to directly interacting with Arp2/3 via its NTA, cortactin also promotes actin-nucleation activity indirectly through the SH3 domain. The SH3 domain of cortactin binds

directly to a proline-rich region on N-WASp, liberating N-WASp from its auto-inhibited state, resulting in N-WASp-mediated Arp2/3 nucleation activity (Mizutani et al. 2002; Martinez-Quiles et al. 2004) (Fig. 2B). The interaction of cortactin and N-WASp promotes the localization of N-WASp to sites of actin polymerization within invadopodia/podosomes (Mizutani et al. 2002) and enhances cell movement (Kowalski et al. 2005). Collectively these studies suggest cortactin and N-WASp are intimately intertwined in regulating Arp2/3 activity responsible for cortical actin assembly.

WASP-interacting protein (WIP) also binds to the SH3 domain of cortactin and enhances cortactin-mediated Arp2/3 activation (Kinley et al. 2003) (Fig. 2C, D). Similar to other SH3 binding partners, localization of WIP to the cell cortex area is dependent upon the cortactin binding domain (Kinley et al. 2003). Maximal Arp2/3 activity is achieved when the cortactin-WIP complex is associated with actin filaments, suggesting a novel role for cortactin in linking WIP to pre-existing filaments. WIP also inhibits the depolymerization of actin filaments (Martinez-Quiles et al. 2001), resulting in the stabilization of actin branch points and inhibition of filament depolymerization.

Characterization of cortactin binding to F-actin

Cortactin was initially identified as an F-actin binding protein in cosedimentation assays using recombinant truncation mutants (Wu and Parsons 1993). Topical mapping analysis determined that the repeat region was responsible for F-actin binding, with a binding stoichiometry of one cortactin molecule per 14 actin monomer subunits. Deletion mapping of the cortactin repeats region indicates that the presence of the fourth repeat is required for optimal F-actin binding (Weed et al. 2000). This finding is supported by F-actin binding studies with different naturally-occurring cortactin splice variants isolated from human squamous cell carcinoma cell lines (van Rossum et al. 2003). Alternate splicing of the cortactin transcript results in the loss of the 6th ABR (termed splice variant 1; SV1-cortactin) or the loss of the 5th and 6th ABR (SV2-cortactin). Both splice variants retain the fourth repeat and display similar F-actin binding activities as compared to wild-type cortactin. In addition, these splice variants retain the ability to activate Arp2/3-mediated actin nucleation, although the SV2-cortactin variant is not as effective as SV1-cortactin. Collectively these studies support the importance of the fourth cortactin repeat in maintaining the actin binding activity of the protein. While other studies have shown cortactin splice variants lacking the fourth repeat are capable of binding F-actin, these isoforms are not as abundant as wild type, SV-1 or SV-2 cortactin (Katsube et al. 2004).

To determine which residues within actin filaments interact with cortactin, Pant et al. (Pant et al. 2006) performed a 3D reconstruction analysis from electron micrographs of actin filaments decorated with a recombinant protein encompassing the cortactin repeats region. Their results indicate that cortactin likely binds to the actin filament near amino acids 338–348 in an individual actin subunit, a known binding region for the F-actin associated proteins gelsolin (McLaughlin et al. 1993), vitamin D binding protein (DBP) (Otterbein et al. 2001), and ADF/cofilin (reviewed in (Dominguez 2004)). Binding of the cortactin repeats region to this area deepens the cleft between adjacent actin monomers. The authors hypothesize that this leads to a transient instability within the helix, allowing for the binding of other proteins to the filament, (i.e.; Arp2/3 complex). In addition, cortactin preferentially binds to F-actin filaments containing ATP or ATP/ADP-Pi, demonstrating a higher affinity for newly polymerized actin filaments as opposed to older ADP-loaded filaments (Bryce et al. 2005). This is in agreement with the requirement for cortactin for maximal incorporation of fluorescently-labeled G-actin monomers within extending lamellipodia (Bryce et al. 2005).

Detailed conformational information on cortactin has not been available due in part to the lack of a published crystal structure. Recently, cortactin was analyzed by a combined approach utilizing chemical cross-linking, circular dichroism and small angle X-ray scattering (Cowieson et al. 2008). This study revealed that recombinant cortactin assumes a globular “lollipop” shape in solution, with the helical domain residing next to the actin-binding region. This conformation results in a folding back of the C-terminal region, placing the second actin repeat in close proximity to the fifth repeat, and SH3 domain near the helical domain and the second repeat. The conformation deduced from these studies has important ramifications regarding how cortactin can interact with F-actin, since binding to F-actin does not induce changes in cortactin secondary structure (Cowieson et al. 2008). Actin binding studies at low temperature reveal that cortactin can assemble F-actin into sheets ~ one filament width wide, thereby bundling F-actin into parallel arrays. Whether or not this activity takes place in a cellular context remains to be evaluated.

One regulatory mechanism recently identified that governs cortactin binding to F-actin involves the histone deacetylase HDAC6 (Zhang et al. 2007). Cortactin is a substrate for the histone acetyltransferase PCAF, which acetylates eleven lysine residues within the protein. It is not clear if PCAF is the only histone acetyltransferase that acetylates cortactin, as there is no significant difference in the acetylation status of cortactin from *PCAF*^{-/-} fibroblasts as compared to controls (Zhang et al. 2007). Eight of the targeted lysines are located within the ABR of cortactin. These eight lysine residues are postulated to form two positively “charged patches” that facilitate the favorable interaction of cortactin with F-actin. Acetylation by PCAF neutralizes the lysine patches, inhibiting binding of the ABR to F-actin. Deacetylation by HDAC6 reverses this process and restores the ability to bind F-actin. The acetylation/deacetylation status of cortactin also has physiological consequences, as acetylated cortactin is unable to translocate to the cell periphery in a Rac-dependent manner, while deacetylated cortactin retains this ability. Cells containing acetylated cortactin also showed decreased cell migration, likely due to impaired cortactin binding to F-actin. The reversible acetylation status of cortactin provides a unique signaling mechanism that regulates F-actin binding activity.

The effects of cortactin phosphorylation on the actin cytoskeleton

The phosphorylation of cortactin and resulting functional consequences have been an intense area of study for many years, with several recent reports providing a better understanding with regard to actin dynamics and cellular behavior. In the case of tyrosine phosphorylation, initial mapping studies identified the major Src phosphorylation sites on murine cortactin as Y421, Y466 and Y482, with Y486 phosphorylated to a lesser extent (Huang et al. 1997a; Huang et al. 1998). Tyrosine 475 has also been identified as a phosphorylation site by mass spectroscopy, although the responsible kinase(s) have not been identified (Martin et al. 2006). Phosphorylation of Y421, Y466 and Y482 has known biochemical and cellular consequences with regards to actin dynamics. Tyrosine phosphorylation of cortactin at these sites is hypothesized to lead to a conformational change in the protein, resulting in more efficient cleavage by calpain proteases (Huang et al. 1997b; Perrin et al. 2006), possibly affecting the ability of cortactin to bind to and cross-link actin filaments. The majority of reports indicate that high levels of tyrosine phosphorylation correlate with elevated cell migration and cancer metastasis (Huang et al. 1998; Liu et al. 1999; Bourguignon et al. 2001; Li et al. 2001; Huang et al. 2003), although recent evidence indicates that cortactin tyrosine phosphorylation inhibits motility in some tumor types (Jia et al. 2008). Cell type or context differences may play a role in explaining these discrepancies. Tyrosine phosphorylation can also regulate cortactin function by enhancing the binding of select SH3-ligands, including MLCK, CD2AP, and dynamin2 (Dudek et al. 2002; Lynch et al. 2003; Zhu et al. 2007). Cortactin was recently identified as a specific substrate for the tyrosine

phosphatase PTP1B (Stuible et al. 2008). PTP1B binds to cortactin primarily at phosphorylated Y446, although surrounding residues may indirectly affect this interaction. EGFR activation leads to phosphorylation of Y446, as does hyperosmolarity, which activates Fyn and Fer (Kapus et al. 2000) resulting in phosphorylation of the Src-targeted sites Y421, Y470, Y486 in addition to Y446. Expression of PTP1B reduces Y421 and Y446 phosphorylation, and a Y-F mutation at codon 446 results in increased hyperosmotic apoptotic signaling, indicating that phosphorylation of Y446 is may be important for regulating apoptotic responses based on its phosphorylation status.

Several recent studies have examined the downstream cellular effects of cortactin tyrosine phosphorylation. Tyrosine phosphorylation of cortactin in osteoclasts is essential for the formation and turnover of podosomes and invadopodia, structures associated with invasive potential (Luxenburg et al. 2006; Tehrani et al. 2006; Ayala et al. 2008). Cortactin tyrosine phosphorylation is also required for efficient metastasis of cancer cells, where breast cancer cell lines expressing a tyrosine phosphorylation-deficient mutant of cortactin demonstrate a marked reduction of osteolytic metastases compared to control cells (Li et al. 2001). The phosphorylation status of cortactin also plays a role in centrosome separation at the G2-M phase in the cell cycle though its ability to link the actin cytoskeleton to the centrosome (Wang et al. 2008). Suprastimulation of pancreatic acini, used to mimic the disease state of pancreatitis, leads to an increase in cortactin tyrosine phosphorylation and the reorganization of the actin cytoskeleton, resulting in the formation of cortactin-rich membrane blebs (Singh and McNiven 2008). This process is dependent on cortactin tyrosine phosphorylation since expression of a phosphorylation-null mutant decreases actin reorganization and diminishes membrane bleb formation. Future work will likely uncover new roles for cortactin tyrosine phosphorylation in normal and pathogenic cellular processes.

While cortactin phosphorylation plays a role in several discrete cellular functions, a link between tyrosine phosphorylation and Arp2/3 activation was initially dismissed since cortactin fragments lacking the α -helical, PRR and SH3 domains activate Arp2/3 *in vitro* nearly as effectively as the full-length protein (Weaver et al. 2001). However, additional work now points to a positive effect for Src-mediated cortactin phosphorylation on Arp2/3 mediated actin polymerization through Nck and N-WASp (Tehrani et al. 2007) (Fig. 2E–G). Nck1 binds to Src-phosphorylated cortactin through its SH2 domain, with the Nck1/phosphocortactin complex in turn interacting with either WIP (Fig. 2E) or N-WASp (Fig. 2F) through its SH3 domain. Trimeric cortactin/Nck1/N-WASp or /WIP complexes enhance Arp2/3 nucleation activity. Interestingly, when cortactin is the only NPF present (i.e., in conditions lacking N-WASp but retaining NCK and WIP), the Arp2/3-binding DDW motif within the cortactin NTA domain is essential for stimulating Arp2/3-mediated actin nucleation. Additionally, the SH3 domain of Src-phosphorylated cortactin is not required for Arp2/3 activation when Nck1 and N-WASP or WIP are present, suggesting that the Nck1 SH3 domain can perform in a surrogate manner to bring N-WASP or WIP to cortactin, resulting in Arp2/3 complex activation. The binding of Nck1 to phosphorylated cortactin therefore provides an indirect link to Arp2/3 complex regulation, as well as involving cortactin in an additional layer of complexity in regulating actin nucleation (Fig. 2G).

In addition to tyrosine phosphorylation, cortactin is also a substrate for several serine/threonine kinases. Serine residues 405 and 418 were first identified to be phosphorylated downstream of MEK and can be directly phosphorylated by Erk1/2 (Campbell et al. 1999; Webb et al. 2006). Phosphorylation of S405 and 418 is associated with an 80 to 85kDa shift in Mr on SDS-PAGE, potentially due to the disruption of intramolecular interactions between the cortactin SH3 domain and amino-terminal residues (Campbell et al. 1999; Martinez-Quiles et al. 2004; Stuible et al. 2008). In support of this, phosphorylation of S405 and S418 corresponds with increased N-WASP binding and activation of Arp2/3 actin

nucleation (Martinez-Quiles et al. 2004). Interestingly, Src-mediated tyrosine phosphorylation inhibits the binding between cortactin and N-WASp in this setting, resulting in decreased Arp2/3 nucleation (reviewed in (Lua and Low 2005)). This may be due to the absence of Nck in these assays. Phosphorylation of S405 and S418 is also required for efficient invadopodia formation and extra cellular matrix degradation (Ayala et al. 2008).

In addition to chemical ligand stimulation, cortactin phosphorylation and translocation can also be affected by mechanical or osmotic stress. Endothelial cell monolayers exposed to shear stress demonstrate enhanced stress fiber formation and Rac-dependent cortactin translocation to the cortical actin network independent of cortactin tyrosine phosphorylation (Birukov et al. 2002). However, monolayer exposure to sphingosine 1-phosphate (S1P) increases barrier function in a cortactin-dependent fashion, as cortactin knock-down significantly inhibits S1P-mediated barrier enhancement (Dudek et al. 2004). Translocation and tyrosine phosphorylation of cortactin is also increased in response to S1P treatment, and is critical to regulating barrier function. In addition, the interaction between MLCK and cortactin also affects barrier function, as cortactin blocking peptides inhibit S1P-mediated effects on MLC phosphorylation and barrier enhancement (Dudek et al. 2004). In the case of osmotic stress, hyperosmolarity results in cell shrinkage, triggering the tyrosine phosphorylation of cortactin independent of changes in osmolarity or intracellular pH (Kapus et al. 1999). Cortactin phosphorylation under these conditions is mediated by the Src family member Fyn. Rac-dependent cortactin translocation in response to hypertonicity is independent of shrinkage-induced phosphorylation, localizing cortactin and Arp2/3 to areas of active cortical actin reorganization known to occur in response to osmotic stress (Di Ciano et al. 2002).

Separate from exogenous stimuli, cortactin phosphorylation can also be effected by the polymerization status of actin (Fan et al. 2004). Depolymerization of actin by latrunculin B leads to enhanced tyrosine phosphorylation of cortactin mediated by Fer. Alternately, filament stabilization by jasplakinolide blocks cortactin phosphorylation induced by either hypertonic stress or treatment with latrunculin B. Stabilization results in the redistribution of cortactin to F-actin rich patches. In this way, the polymerization status of the F-actin network may regulate the actions of cortactin by altering its phosphorylation status, resulting in a feedback loop alternating between network expansion and reorganization (Gunst 2004).

Recently, the Cell Migration Consortium reported a comprehensive mass spectroscopy analysis in an attempt to identify all cortactin phosphorylation sites (Martin, et al 2006). In addition to the sites discussed above, sixteen additional phosphorylation sites were identified, twelve occurring on serine and four on threonine. Of these sites, serine 113 was independently identified as a PAK phosphorylation site, serving to downregulate F-actin binding to cortactin (Webb, et al 2006). Another intriguing phosphorylation site identified from this analysis is threonine 24, which is directly adjacent to the DDW motif in the NTA region (Martin, et al 2006). This raises the possibility that phosphorylation of this site may play a role in the regulation of Arp2/3 binding to the cortactin NTA. Future work will likely address the role of T24 phosphorylation and associated kinase(s) in cortactin function, as well as how other cortactin phosphorylation sites regulate cortactin activity.

Additional cortactin binding proteins and their role in actin organization

Given its involvement in multiple actin-based cellular processes, cortactin is capable of exerting differential effects on cortical actin cytoskeletal organization and assembly. In addition to the proteins discussed above, the ability of cortactin to alternatively regulate cortical actin dynamics stems from its direct association with additional actin-associated

proteins. These interactions provide further insights into how cortactin regulates actin polymerization and organization.

Missing in metastasis (MIM)—MIM is a putative metastatic suppressor protein that is expressed at low levels in tumor cell lines (Lee et al. 2002). MIM contains a proline-rich region that mediates its binding to the cortactin SH3 domain. (Lin et al. 2005). Binding of MIM to cortactin enhances the ability of cortactin to stimulate Arp2/3 mediated actin polymerization, and decreases actin polymerization by N-WASp. However, at high concentrations MIM functions in a dominant negative fashion and inhibits the ability of cortactin to stimulate actin nucleation, possibly due to MIM sequestering G-actin through its WH2 domain (Mattila et al. 2003; Woodings et al. 2003; Woodring et al. 2003). The ability of MIM to bind to G-actin is also responsible for the inhibition of N-WASp-mediated Arp2/3 activation in the same system (Lin et al. 2005). MIM binds F-actin (Yamagishi et al. 2004), which may enhance cortactin-mediated Arp2/3 activation by placing MIM at the site of active polymerization, allowing MIM to recruit and bind to other key proteins (Lin et al. 2005). MIM proteins also contain an IRSp53 MIM domain (IMD) that shows structural similarities to the Bin-Amphiphysin-RSV (BAR) domain that plays a role in both sensing and inducing membrane curvature (Millard et al. 2005). Similar to other BAR-containing proteins, MIM is able to bind to and deform negatively-charged membranes composed of either phosphatidylinositol 4,5 bisphosphate (PIP2) or phosphatidylserine (Suetsugu et al. 2006; Mattila et al. 2007). The IMD of MIM may function to link the actin cytoskeleton and the plasma membrane by inducing outward protrusions of the plasma membrane similar to filopodia (Machesky and Johnston 2007). However, MIM proteins appear to play a role in lamellipodia, not filopodia formation (Bompard et al. 2005; Machesky and Johnston 2007). While it has not been identified as a component in invadopodia to date, the binding of MIM to cortactin may position MIM to play a role in membrane protrusion associated with invadopodia.

Dynamin-2—Dynamin-2 is a large GTPase that performs fundamental roles in vesicle formation, trafficking, and invadopodia formation (Urrutia et al. 1997; McNiven 1998; van der Blik 1999; McNiven et al. 2000; Weaver 2006). The proline-rich region of dynamin-2 binds to the cortactin SH3 domain, and both proteins colocalize at the lamellipodial edge and within invadopodia (McNiven et al. 2000; McNiven et al. 2004; Zhu et al. 2005). Dynamin-2 binding to cortactin enhances Arp2/3-mediated actin polymerization to a moderate degree, and influences the architecture of resultant Arp2/3-F-actin networks (Schafer et al. 2002). The interaction between cortactin and dynamin is also essential for CDR and invadopodia formation (Baldassarre et al. 2003; Krueger et al. 2003). Cortactin and dynamin play a central role in receptor-mediated endocytosis, with efficient clathrin-dependent endocytosis requiring the concerted activities of the Arp2/3 complex, dynamin, and cortactin (McNiven et al. 2000; Cao et al. 2003; Zhu et al. 2005). Expression of a cortactin mutant deficient in Arp2/3 binding decreases the interaction between cortactin and dynamin as well as blocking transferrin uptake (Zhu et al. 2005). The cortactin/dynamin complex is essential for vesicle formation at the plasma membrane (Cao et al. 2003), and binding is enhanced by the Src-mediated tyrosine phosphorylation of cortactin (Zhu et al. 2007). These studies indicate that the interaction between dynamin-2 and cortactin plays an important role in multiple actin-based processes that involve movement and remodeling of cellular membranes.

p120 Catenin—p120 catenin (Ctn) is an armadillo-family protein and a Src substrate involved in cell-cell junction maintenance, transcriptional regulation and cell migration (Sarrio et al. 2004; Shibata et al. 2004; Bellovin et al. 2005; Yanagisawa and Anastasiadis 2006; Daniel 2007; Reynolds 2007). p120 Ctn binds to the amino-terminal portion of

cortactin encompassing the NTA and cortactin repeats region (Boguslavsky et al. 2007). The binding of p120 Ctn to cortactin regulates lamellipodia persistence, as MCF-7 cells with reduced p120 Ctn expression display defective persistence that is phenotypically similar to cells with reduced cortactin expression (Bryce et al. 2005). While the loss of p120 Ctn expression leads to a reduction in the amount of cortactin and Arp2/3 from the leading edge, loss of cortactin expression does not affect the localization pattern of p120 Ctn (Boguslavsky et al. 2007). These data indicate that p120 Ctn may serve to sequester and stabilize cortactin within lamellipodia, allowing for subsequent cortactin-based regulation of lamellipodia dynamics.

Alix—Alix, (AIP1 or Hp95), is a conserved F- and G-actin binding adaptor protein that is associated with regulation of fibroblast morphology (Wu et al. 2002; Pan et al. 2006). Reduced Alix expression due to siRNA silencing results in decreased cellular F-actin and fewer stress fibers. In accordance with the effects on actin organization, cells lacking Alix expression contain fewer membrane protrusions and display an altered morphology (Pan et al. 2006). Alix binds to cortactin through a central region containing tandem binding sites (Pan et al. 2006), positioning Alix to function in F-actin filament bundling and stress fiber formation. Loss of Alix expression results in decreased cortactin localization within lamellipodia and a reduced association of cortactin with F-actin (Pan et al. 2006). Although these data suggest that Alix may play a role in regulating cortactin function within lamellipodia, how the interaction between these two proteins function together to influence actin dynamics is still not clear.

Caldesmon—Caldesmon is an F-actin-binding protein well characterized in smooth muscle that is also present in non-muscle cells (reviewed in (Hodgkinson 2000; Kordowska et al. 2006)). Caldesmon binds to the cortactin NTA region, resulting in attenuated Arp2/3-mediated actin nucleation (Huang et al. 2006). However, the actual biological significance and potential downstream effects of the caldesmon/cortactin interaction remains unknown. In non-dividing rat aortic fibroblasts (RAF) cells, caldesmon binds and stabilizes actin filaments (Kordowska et al. 2006). Upon cell division, caldesmon colocalizes with cortactin at the cell periphery where it is subsequently phosphorylated. Phosphorylation of caldesmon functions to decrease the binding affinity to F-actin, facilitating efficient actin cytoskeletal remodeling required for cell division and migration. Likewise, expression of a non-phosphorylatable caldesmon mutant results in the inability of RAFs to disassemble large actin bundles when induced to migrate by phorbol 12-myristate 13-acetate (PMA) (Kordowska et al. 2006). The differential binding of caldesmon to actin upon phosphorylation, coupled with its interaction with cortactin, positions these proteins to play an essential role in regulating actin cytoskeletal remodeling in response to mitotic signals.

Hip1R—Hip1R is an F-actin and clathrin binding protein that functions in receptor-mediated endocytosis (Engqvist-Goldstein et al. 1999; Engqvist-Goldstein et al. 2001; Cao et al. 2003; Engqvist-Goldstein et al. 2004; Veiga and Cossart 2005). Hip1R contains a conserved talin-HIP1R/Slp2p actin-tethering C-terminal homology (THATCH) domain that mediates binding to F- or G-actin. Adjacent to the THATCH domain is a proline-rich region (PRR) that interacts with the SH3 domain of cortactin, positioning Hip1R to regulate actin polymerization dynamics during endocytic events (Brett et al. 2006; Le Clainche et al. 2007). In accordance with this, recent findings demonstrate that Hip1R expression negatively regulates actin assembly (Kaksonen et al. 2003; Engqvist-Goldstein et al. 2004), while a loss of Hip1R promotes cortactin-mediated actin polymerization at sites of clathrin coated pit formation (Le Clainche et al. 2007). Expression of a Hip1R construct defective in cortactin binding results in the formation of abnormal F-actin structures. *In vitro* actin assembly assays lacking Arp2/3 indicate that Hip1R and cortactin in combination inhibit

actin assembly, while either protein alone has no effect. The inhibition of actin assembly is attributed to blocked barbed end filament elongation, similar to the effects of capping protein. These studies suggest that binding of Hip1R to cortactin may function as a filament barbed end cap at sites of endocytic vesicle formation, limiting filament elongation and facilitating endocytic vesicle internalization (Le Clainche et al. 2007).

Cortactin function in the formation of actin-based protrusive structures

The localization of cortactin with the cortical actin cytoskeleton combined with its role in regulating actin polymerization positions it to play a governing role in a variety of cellular events involving actin-based protrusion of the cell membrane. Consequently, the contribution of cortactin in the formation and function of lamellipodia, invadopodia and CDRs has been the subject of much recent investigation, leading to a better understanding about the molecular events and signaling pathways that regulate these structures.

Lamellipodia formation and adhesion—Regulation of the actin network during lamellipodia extension is one of the best characterized cellular actin-based processes. Lamellipodia are thin, flat membrane extensions containing a polarized array of actin filaments that form an orthogonal cross-linked actin network due to the distinctive nucleation signature of the Arp2/3-complex activity (Small 1988; Ponti et al. 2004). Directly behind the lamellipodium is a thicker, actin- and myosin II-rich region termed the lamella (Ponti et al. 2004; Gupton et al. 2005). This region separates the leading edge from the cell body, and is the site where integrin-rich cell-substrate adhesions first couple to the actomyosin contractile network to transmit translocating force (Ponti et al. 2004). The orthogonal arrangement of actin filaments allows filaments to move laterally across the cell front as actin polymerization progresses (Small 1994; Small and Resch 2005). The lamellipodium also initiates new sites of adhesion with the substratum, providing points of traction required for forward motility to occur (Small et al. 2002). Several proteins regulate the formation and maturation of lamellipodia; most notable are Rho, Rac, and Cdc42, members of the Rho family of GTPases (Ridley and Hall 1992; Hall 1998; Hall 2005). Rho regulates the formation of actin-myosin filaments and stress fiber assembly, while Rac and Cdc42 regulate the formation of lamellipodia and filopodia, respectively. All three GTPases contribute to the development of integrin-based focal contact formation, a process that is essential for migration (reviewed in (Raftopoulou and Hall 2004)).

Lamellipodia protrusion is initiated by actin filament elongation, a process which requires the production of free barbed ends. Free barbed ends within lamellipodia are generated by cofilin-mediated filament severing, uncapping of existing filament ends, or de novo nucleation by Arp2/3 (Condeelis 2001; Zigmond et al. 2003). Actin monomer addition to free barbed ends generates the protrusive force that pushes the membrane forward. Following extension, lamellipodia must adhere to the substratum in order to be functional in initiating and maintaining motility. Newly formed adhesions within the lamellipodia induced by Rac are termed focal “contacts”, while the more stable focal adhesions in the lamella and cell body are controlled by Rho activity (Ridley et al. 2003). Linkage of the lamellipodia to the substrate through focal contacts allows the cell to generate the traction force necessary for cell translocation. In the absence of adhesion, lamellipodia can lift and fold back towards the cell body, generating what have been classically termed “membrane ruffles”.

Recently, it has been shown that cortactin plays a role in regulating both actin dynamics and the formation of adhesive structures within the lamellipodia. Despite the ability of cortactin to influence Arp2/3 activity and actin network stability, cortactin does not appear to play a role in the initial protrusion of lamellipodia, since cortactin depletion from cells by siRNA does not affect the overall rate of protrusion (Bryce et al. 2005; Kempia et al. 2005;

Yamaguchi and Condeelis 2007). Cortactin is involved in actin network assembly and formation of lamellae-associated adhesion structures subsequent to initial lamellipod extension. Suppression of cortactin expression by siRNA in fibrosarcoma cells also reduces the rate of new adhesion formation in lamellipodia, as measured by altered paxillin dynamics in nascent focal contacts, leading to decreased lamellipodia persistence (Bryce et al. 2005). Similarly, Boguslavsky et al. (Boguslavsky et al. 2007) reported that siRNA-mediated reduction of cortactin expression in MCF-7 cells reduces lamellipodial persistence, mirroring the phenotype of cells lacking p120 catenin expression. siRNA-mediated depletion of cortactin also appears to impair cell spreading on fibronectin (Illes et al. 2006).

In contrast to these studies, several other groups have determined that cortactin depletion by siRNA has opposing effects on lamellipodia behavior. In one study, reduced cortactin expression in MTLn3 rat adenocarcinoma cells results in enhanced lamellipodia formation in MTLn3 cells exposed to EGF-coated beads (Kempiak et al. 2005). Stable down-regulation of cortactin expression in breast epithelial cells results in decreased cell migration and invasion, enhanced cell spreading, and elevated cell-cell adhesion (van Rossum et al. 2006). Finally, we have found that cortactin depletion in MTLn3 and 1483 head and neck squamous cell carcinoma cells results in impaired lamellipodia retraction following stimulation with soluble EGF and alters the lamellipodia F-actin architecture¹. The apparent differences in observed effects between these studies may be due to a variety of factors, including cell type, matrix involved, and chemotactic stimulus. While potentially conflicting, these studies do highlight a putative functional role for cortactin in matrix adhesion and lamellipodial stability. Expansion of these studies with further investigation will help delineate a more precise role for cortactin function in regulating lamellipodia dynamics.

Additional evidence for cortactin in lamellipodia adhesion can be inferred from work demonstrating direct binding of Arp2/3 complex to vinculin, a protein found in focal contacts that targets Arp2/3 to sites of matrix adhesion (DeMali et al. 2002). The interaction between Arp2/3 and vinculin raises the possibility that a cortactin/Arp2/3/vinculin ternary complex may be present within newly forming focal contacts, providing a simultaneous means to regulate Arp2/3 activation and actin network stability coupled to nascent adhesion formation. Additionally, FAK binds directly to Arp3, and this interaction enhances Arp2/3-mediated actin polymerization required for the formation of nascent lamellipodia and subsequent cell spreading (Serrels et al. 2007). The release of Arp3 from FAK at the periphery of the cell places the Arp2/3 complex away from more mature adhesion structures and is associated with the formation of lamellipodia from initial transient adhesion structures (Serrels et al. 2007). FAK can also bind to and phosphorylate N-WASP (Wu et al. 2004), suggesting that FAK may be responsible for the correct positioning of multiple components of the actin polymerization machinery required for efficient lamellipodia formation.

In addition to a potential role in adhesion, other work has shown that cortactin binds directly to endothelial cell (EC) MLCK, inhibiting cortactin-mediated Arp2/3 actin polymerizing activity and reducing the affinity of MLCK for F-actin (Dudek et al. 2002). MLCK and cortactin undergo retrograde flow from the cell periphery towards the lamellar region (Kaksonen et al. 2000; Giannone et al. 2004), where myosin-dependent contractile complexes are required for lamellipodia formation and adhesion (reviewed in (Small and Resch 2005)). Therefore, an additional function for cortactin is that it may serve to chaperone MLCK to distinct areas within the lamella, aiding to promote MLCK activation required for the myosin-dependent contractile force utilized in loco-regional focal contact assembly. While experimental validation for this is needed in a cellular context, evidence to date suggests that cortactin has the capability to link actin polymerization and lamellipodia

¹A.G. Ammer and S.A. Weed, unpublished data

formation to adhesion and force generation, thereby influencing critical actin-based processes essential for leading edge protrusion and directional cell migration.

Formation and function of invadopodia—While the precise role(s) of cortactin in lamellipodia and adhesion formation remains somewhat unclear, it is well established that cortactin is critical for the formation and function of invadopodia. Invadopodia are ventral membrane protrusions that extend into the extracellular matrix and facilitate cellular invasion by focally sequestering membrane-bound matrix metalloproteases. Invadopodia also function as sites of exocytosis for secreted matrix metalloproteases, thus serving a dual role in directing enzymatic digestion of the ECM (reviewed in (Weaver 2006)). Invadopodia and similar structures (alternatively termed podosomes, invadosomes, invadopods or ipods) are present in several cell types, including osteoclasts, macrophages, vascular smooth muscle cells and metastatic tumor cells (Tarone et al. 1985; Linder et al. 1999; Fultz et al. 2000; Hai et al. 2002; Gimona et al. 2003; Linder and Aepfelbacher 2003; Buccione et al. 2004; Gimona and Buccione 2006; Clark et al. 2007). Invadopodia are characterized by several distinctive properties that separate them from other actin-based protrusive structures. These include a reliance on Src kinase activity for their formation, the assembly of focal adhesion proteins in close proximity to actin branching machinery, and the localized degradation of ECM at the site of development (reviewed in (Weaver 2006)). siRNA-mediated knockdown of cortactin in Src-transformed fibroblasts, breast, head and neck and melanoma cancer cells prevents the formation of invadopodia and subsequent ECM degradation (Artym et al. 2006; Clark et al. 2007; Webb et al. 2007; Ayala et al. 2008). Other actin-regulatory proteins required for invadopodia formation and function reported to date include Arp2/3 complex (Yamaguchi et al. 2005), N-WASp (Mizutani et al. 2002; Yamaguchi et al. 2005), cofilin (Yamaguchi et al. 2005) and dynamin2 (McNiven et al. 2004).

Several recent studies have analyzed the molecular events required for invadopodia formation and activity. A step-wise model explaining the sequence of events involved in invadopodia formation in breast carcinoma cells derived from live cell imaging has been reported (Artym et al. 2006). Based on this work, actin and cortactin initially accumulate at areas where the cell membrane is in contact with the ECM, with cortactin potentially serving as an adaptor to recruit other essential proteins to sites of formation. After this initiation step, recruitment of the membrane-bound matrix metalloproteinase MT1-MMP to the initiation sites determines a “preinvadopodia” stage. Subsequent degradation of the ECM by MT1-MMP and other proteases marks the onset of invadopodia maturation, with mature invadopodia enriched in cortactin, actin, and MT1-MMP. A final late stage of mature invadopodia is marked by the dissolution of cortactin and F-actin from mature invadopodia. These late stage invadopodia still retain MT1-MMP and continue to degrade matrix. Additional studies examining the role of cortactin and matrix metalloprotease secretion from invadopodia (Clark et al. 2007) show a link between the expression level of cortactin and the amount of MMP-2 and MMP-9 release in HNSCC cells. Suppressed cortactin expression in these cells affects ECM degradation to a larger extent than can be reconciled to the focal action of invadopodia alone, with matrix degradation occurring at regions not intimately associated with the invadopodia interface. Overexpression of cortactin in these cells results in increased secretion of MMP-2 and MMP-9, while reduced cortactin expression correlates with decreased metalloprotease secretion. Cortactin protein levels also regulate the secretion of non-invadopodial protein ApoA1, suggesting that cortactin is involved in governing additional secretory pathways apart from metalloprotease delivery (Clark et al. 2007).

Recent work in v-Src transformed fibroblasts examined the specific cortactin domains responsible for invadopodia formation (Webb et al. 2007). Using deletion mutants of cortactin in transient transfection assays, the actin-binding region of cortactin is the sole

determinant required for v-Src induced invadopodia formation and matrix degradation in cells where endogenous cortactin expression is suppressed by siRNA. However, Src-mediated cortactin phosphorylation is also involved, since expression of a construct containing tyrosine-phenylalanine mutations codons 421, 466 and 482 partially restores the invasive phenotype. Similar experiments in A375MM melanoma cells indicate that the cortactin NTA and SH3 domain are required for invadopodia formation (Ayala et al. 2008). This same study also demonstrated that multiple different phosphorylation sites on cortactin are responsible for optimal invadopodia matrix degradation activity, including tyrosine 421, 466 and 482 and serines 113, 405 and 418 (Ayala et al. 2008). These data indicate that cortactin function in invadopodia is highly regulated by multiple signaling pathways. A role for tyrosine phosphorylation of cortactin in invadopodia function is further supported by the correlation of tyrosine phosphorylated cortactin with matrix degradation (Bowden et al. 2006), possibly reflecting a role in regulating podosome turnover similar to that reported in osteoclasts (Luxenburg et al. 2006).

Role in Circular Dorsal Ruffles—Circular dorsal ruffles (CDRs, or “dorsal waves”) are another protrusive membranous structure dependent on cortactin activity for proper formation. CDRs are dynamic actin-rich structures that consist of a ridge of extended dorsal plasma membrane and form along peripheral cell edges of migrating cells in response to growth factor stimulation ((Mellstrom et al. 1983; Mellstrom et al. 1988; Dowrick et al. 1993); reviewed in(Buccione et al. 2004)). Unlike peripheral lamellipodia-associated ruffles that typically exhibit continuous rapid membrane dynamics, CDRs form only once in resting cells several minutes after stimulation and persist for 5–20 minutes (Dowrick et al. 1993; Dharmawardhane et al. 1997). Functionally, CDRs are hypothesized to participate in cytoplasmic remodeling (Krueger et al. 2003), establishment of polarity in migrating cells, and internalization of activated receptor tyrosine kinases (Orth and McNiven 2003). CDRs also facilitate the development and formation of lamellipodia, as there is a high correlation between the development of CDRs and lamellipodia production following growth factor stimulation (Krueger et al. 2003). Localization of MMP-2 at the tips of CDRs suggests a potential additional role in matrix degradation during three-dimensional cell translocation (Suetsugu et al. 2003).

CDRs contain several of the same signaling and structural proteins that are found in lamellipodia and invadopodia. The main structural element of CDRs is F-actin, as treatment of cells with cytochalasin D or latrunculin A to disrupt assembly dynamics prevents CDR formation (Hedberg et al. 1993; Warn et al. 1993; Westphal et al. 2000). CDRs also contain Arp2/3 complex, indicating that nucleation of branched actin networks are involved in their formation (Krueger et al. 2003). N-WASp, WAVE-1 and -2, WIP, dynamin, Abl/Arg tyrosine kinases, and cortactin are also found within CDRs, providing an assemblage of signaling and actin regulatory proteins needed for actin-based CDR regulation (Westphal et al. 2000; Anton et al. 2003; Krueger et al. 2003; Suetsugu et al. 2003; Kovacs et al. 2006).

Cortactin plays an essential role in CDR formation. Disruption of the interaction between cortactin and dynamin using either an inhibitory monoclonal antibody against the Dyn2 PRR or a dominant-negative construct of Dyn2 that is missing the PRR domain alters the formation of CDRs by affecting cortactin-mediated activation of Arp2/3 (Schafer et al. 2002; Krueger et al. 2003). Cortactin is also able to influence CDR formation through phosphorylation by and binding to Abl and Arg, cytoplasmic tyrosine kinases that play an active role in actin regulation and CDR formation (McWhirter and Wang 1991; Van Etten et al. 1994; Woodring et al. 2003; Boyle et al. 2007). Abl/Arg phosphorylates cortactin at the Src-targeted Y421/Y466/Y482 sites, and mutation of these residues inhibits PDGF-induced CDR formation (Boyle et al. 2007). This requirement for cortactin tyrosine phosphorylation, coupled with the dependence for dynamic actin assembly in CDR formation, may suggest

that phospho-tyrosine-mediated binding of NCK1/N-WASp or NCK1/WIP complexes to cortactin may serve as a fundamental step in the genesis of CDRs.

Concluding Remarks

Cortactin has emerged as an essential protein in regulation of the cortical actin cytoskeleton through its ability to influence cytoskeletal reorganization in response to extra- and intracellular stimuli. By interacting with a variety of binding partners, cortactin bridges the actin cytoskeleton to many effector proteins that control actin-based cellular processes. Although much of the recent focus on cortactin function has biochemically centered on its role in regulating Arp2/3 complex activity and F-actin interactions, how these functions tie into regulating individual or multicellular behavior remains to be comprehensively discerned. Several important questions, including roles for unstudied phosphorylation sites, additional post-translational modifications and potential participation in other actin-based events remain unevaluated. How cortactin impacts actin dynamics during vertebrate development is also unknown, hampered in part by the lack of transgenic knock-out rodent models. Precise roles of cortactin in tumor progression and other disease types are becoming rapidly expanded or just beginning to be identified. The field remains replete with opportunities for further exciting and important discoveries.

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Abbreviations used

ABR	actin binding region
ADF	Actin depolymerization factor
Alix	ALG-2 interacting protein X
Arp2/3	Actin related proteins 2 and 3
BAR	Bin-Amphiphysin-RSV domain
CDR	circular dorsal ruffle
CP	Capping protein
DBP	Vitamin D binding protein
ECM	extracellular matrix
EGF	Epidermal growth factor
ERK	Extracellular signal Regulated Kinase
FGF	Fibroblast growth factor
HDAC6	Histone deacetylase 6
Hip1R	Huntingtin-interacting protein 1 related
HNSCC	head and neck squamous cell carcinoma
IMD	IRSp53 MIM domain
IRSp53	insulin receptor tyrosine kinase substrate p53

LIMK	LIM domain kinase
MEK	MAP kinase or ERK kinase
MIM	missing in metastasis
MLCK	myosin light chain kinase
MMP	matrix metalloprotease
MT1-MMP	Membrane type-1 matrix metalloproteinase
NPF	nucleation promoting factor
NTA	N-terminal acidic region
p120 ctn	p120 catenin
PAK	p21 activated kinase
PCAF	p300/CBP-associated factor
PDGF	Platelet-derived growth factor
PIP2	phosphatidylinositol 4,5 bisphosphate
PMA	phorbol 12-myristate 13-acetate
PRR	proline-rich region
S1P	Sphingosine 1-phosphate
Scar	Suppressor of cyclic AMP receptor
SH	Src-homology
THATCH	talin-HIP1/R/Sla2p actin-tethering C-terminal homology
WASP	Wiskott-Aldrich syndrome protein
WAVE	WASP family Verprolin-homologous protein
WCA	Wasp Homology-2 (WH2)-central-acidic region
WIP	WASp-interacting protein

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Figure 1. Domain structure of cortactin and associated binding proteins. Specific cortactin domains are described in the text. Proteins outlined in red represent binding partners with known interaction sites. Proteins outlined in blue represent known binding partners with undetermined binding sites. Proteins outlined in green represent kinases known to phosphorylate cortactin at the indicated sites.

Figure 2.

Cortactin-mediated activation of Arp2/3 actin nucleation and phosphorylation-independent interactions with N-WASp and WIP. **A.** Cortactin functions as an NPF for Arp2/3 complex. Cortactin binds the Arp2/3 through its NTA domain and F-actin through the fourth tandem repeat, resulting in direct activation Arp2/3 actin nucleation activity. **B.** Cortactin activates N-WASp. Cortactin enhances N-WASp mediated Arp2/3 activation by disrupting N-WASp autoinhibition through binding of the cortactin SH3 domain to the PRR region of N-WASp. The CA domain of N-WASp binds to and activates Arp2/3, while the WH2 domain binds ATP-loaded G-actin. In this scenario, N-WASp appears to be the dominant activator of Arp2/3 complex. **C.** WIP enhances cortactin-mediated Arp2/3 activation. The cortactin SH3 domain interacts with the WIP cortactin binding domain (CBD), presumably bringing tandem WH2 domains with bound G-actin in close proximity with Arp2/3 complex activated by the cortactin NTA, serving to enhance cortactin-mediated Arp2/3 activation.

Figure 3.

Increased complexity and role of tyrosine phosphorylation in cortactin-mediated Arp2/3 complex activation. **A.** Activation of Arp2/3 complex by a cortactin/WIP/N-WASP complex. Binding of the cortactin SH3 domain to the CBD of WIP, coupled with the interaction of the WIP WASp binding domain (WBD) to the WH1 domain of N-WASP creates a trimeric complex capable of activating Arp2/3 actin nucleation activity. **B.** Tyrosine phosphorylation of cortactin influences Arp2/3 activation. The adaptor NCK binds cortactin through an SH2-phosphotyrosine mediated interaction. An SH3 domain of Nck1 in turn interacts with proline-rich regions on WIP, thereby positioning WIP to enhance Arp2/3 activation in an analogous manner to the positioning of WIP in Fig. 2C. **C.** N-WASP induced Arp2/3 activation mediated by an Nck1/cortactin complex. Nck1 binding to tyrosine phosphorylated cortactin as in **B** can also interact with and activate N-WASP through binding of one or more Nck1 SH3 domains binding to the proline rich region (PRR) of N-WASP, resulting in Arp2/3 activation. **D.** Multiprotein complexes of tyrosine phosphorylated cortactin, Nck1, N-WASP and WIP incorporating aspects of Fig 2 and 3 are conceivable, with the possible binding of WIP to the cortactin SH3 domain and/or N-WASP in addition to the Nck1/N-WASP complex described in **C**. In instances where N-WASP is

present, cortactin appears to function as an adaptor rather than an NPF. Abbreviations: N-WASp-WH1: WASP-homology 1; GBD: GTPase-binding domain; PRR: proline-rich region; W: WASP-homology 2 domain (WH2); CA: Arp2/3 binding connector and acidic domain. WIP – CBD: cortactin binding domain; NBD: Nck binding domain; WBD: N-WASp binding domain. Nck1 – SH3: Src-homology 3 domain; SH2: Src-homology 2 domain. Cortactin – NTA: N-terminal acidic domain; Helix: alpha-helical region; P-rich: proline-rich region. For simplicity, actin filaments are shown where the Arp2/3 complex is located to demonstrate nucleation activity.

Figure 4. Schematic illustration of membrane- and cytoplasmic-based signaling molecules and their post-translational modifications on cortactin function. Kinase-based signaling cascades initiated by adhesion or growth factor receptors are mediated by select serine/threonine and tyrosine kinases that serve to modify cortactin at the indicated amino acids. Cortactin function is also regulated by a cycle of acetylation/deacetylation. Functional consequences of each specific modification are indicated.

Table 1
Summary of cortactin binding proteins that regulate actin cytoskeletal dynamics

Summary of cortactin-binding proteins that interact directly with the actin cytoskeleton, including binding characteristics to cortactin and actin, as well as functional and physiological effects.

Protein	Ctn interaction site	F or G actin binding	Function	Resulting effects on cortactin/F-actin	References
Alix	Undetermined	F	Regulation of fibroblast morphology	Involved in localization of cortactin to lamellipodia	(Wu et al. 2002; Pan et al. 2006)
Arp2/3	NTA (aa 20–22)	F	<i>de novo</i> actin nucleation	Activation of Arp2/3-mediated actin nucleation	(Urano et al. 2001; Weaver et al. 2001)
Caldesmon	NTA (aa 4–47))	F	Non-muscle - Assembly and stabilization of microfilaments Muscle - with troponin - Mediating factor for Ca ²⁺ dependent inhibition of smooth muscle contraction	Attenuates Arp2/3-mediated actin polymerization	(Sobue et al. 1981; Huang et al. 2006)
CD2AP	SH3	-	Scaffolding protein involved in endocytosis	Link between endocytic machinery and the actin cytoskeleton for trafficking of receptor-containing vesicles	(Lynch et al. 2003)
Shank2	SH3	-	Scaffolding protein	Potential link between the postsynaptic NMDA receptor-PSD-95 complex and the actin cytoskeleton	(Du et al. 1998; Naisbitt et al. 1999)
Dynamin 2	SH3	-	GTPase associated with endocytosis and cell migration	Vesicle formation at the plasma membrane and the trans-Golgi network	(McNiven et al. 2000; Cao et al. 2003; Cao et al. 2005; Kruchten and McNiven 2006)
Fgd1	SH3	-	Cdc42 GEF	Enhances cortactin-mediated actin polymerization through Arp2/3 activation	(Zheng et al. 1996; Hou et al. 2003; Kim et al. 2004)
HDAC6	repeat region (aa 84–330)	-	Deacetylation of target proteins	Promotes cortactin association with F-actin	(Zhang et al. 2007)
HIP1R	SH3	F	Negative regulator of actin assembly in clathrin-mediated endocytosis and clathrin-coated vesicle budding from TGN	Forms a complex with cortactin that blocks actin filament barbed end elongation	(Engqvist-Goldstein et al. 1999; Kaksonen et al. 2003; Engqvist-Goldstein et al. 2004; Le Clairche et al. 2007)
MIM	SH3	G	Putative metastatic suppressor	Enhances cortactin-mediated actin polymerization through Arp2/3 activation	(Lee et al. 2002; Mattila et al. 2003; Lin et al. 2005)
Nck	pY421, pY466 and/or pY482	-	Adaptor protein	Enhances Arp2/3-mediated actin polymerization	(Okamura and Resh 1995; Tehrani et al. 2007)
p120 Ctn	N-terminal region (aa 1–330)	-	Stabilization of cell-cell junctions	Decrease in lamellipodial extension and focal adhesion formation	(Boguslavsky et al. 2007)
N-WASP	SH3	G/F	Activator of Arp2/3 complex	Enhances Arp2/3-mediated actin polymerization	(Suetsugu et al. 2001; Takenawa and Itoh 2001; Pollard and Borisy 2003; Takenawa and Suetsugu 2007)

Protein	Ctn interaction site	F or G actin binding	Function	Resulting effects on cortactin/F-actin	References
PTP1B	Y446	-	Tyrosine-specific phosphatase	Dephosphorylates cortactin on Y421 and Y446	(Stuible et al. 2008)
WIP	SH3	F and G	Regulation of actin polymerization	Enhances cortactin-mediated actin polymerization through Arp2/3 activation; stimulates membrane protrusion	(Kinley et al. 2003; Aspenstrom 2004; Anton and Jones 2006)
ZO-1	SH3	-	Scaffolding protein between transmembrane and cytoplasmic proteins	Regulation of chemotactic response	(Katsube et al. 2004; Lee et al. 2006)