Regulation of F-actin-dependent processes by the Abl family of tyrosine kinases

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Journal of Cell Science 116, 2613-2626 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00622

Summary

The F-actin cytoskeleton is a fundamental component of all eukaryotic cells. It provides force and stability and plays an integral role in a diverse array of cellular processes. The spatiotemporal regulation of F-actin dynamics is essential for proper biological output. The basic molecular machinery underlying the assembly and disassembly of filamentous actin is conserved in all eukaryotic cells. Additionally, protein tyrosine kinases, found only in multicellular eukaryotes, provide links between extracellular signals and F-actin-dependent cellular processes. Among the tyrosine kinases, c-Abl and its relative Arg are unique in binding directly to F-actin. Recent results have demonstrated a role for c-Abl in membrane ruffling, cell spreading, cell migration, and neurite extension in response to growth factor and extracellular matrix signals. c-Abl appears to regulate the assembly of F-actin polymers into different structures, depending on the extracellular signal. Interestingly, c-Abl contains nuclear import and export signals, and the nuclear c-Abl inhibits differentiation and promotes apoptosis in response to genotoxic stress. The modular structure and the nuclear-cytoplasmic shuttling of c-Abl suggest that it integrates multiple signals to coordinate F-actin dynamics with the cellular decision to differentiate or to die.

Key words: *abl*$^{-/-}$ *arg*$^{-/-}$ fibroblasts, F-actin, Lamellipodia, Filopodia, Focal adhesions

Introduction

Many essential cellular functions are linked to alterations in the F-actin cytoskeleton. For example, cell division, endocytosis, axon pathfinding, embryonic development, wound healing and T-cell antigen recognition all depend on the highly dynamic nature of the actin cytoskeleton. In recent years we have acquired much insight into regulation of F-actin dynamics. Actin-ATP, Arp2/3, cofilin/ADF and actin-capping proteins have been identified as the minimal cellular components required to propel *Listeria moncytogenes* in the cytoplasm of infected host cells (Loisel et al., 1999). Indeed, these proteins are also essential regulators of the F-actin cytoskeleton during eukaryotic cell translocation (Pollard et al., 2000). However, directed cell movement in mammalian cells involves the coordinated integration of multiple complex signaling pathways to instruct a series of critically timed and spatially separated F-actin-based events (Webb et al., 2002). How this is achieved and regulated remains largely elusive.

Recent investigations have focused on cytoskeletal structures observed in motile cells, including those within lamellipodia, filopodia and at points of cell adhesion (e.g. focal complexes and focal adhesions/contacts). Lamellipodia and filopodia are protrusive structures that permit the cell to search and explore its surrounding environment prior to navigation. Lamellipodia are flat, broad cell-surface extensions that consist of branched F-actin (Svitkina et al., 1997), whereas filopodia are thin, elongated extensions that consist of parallel bundles of F-actin (Small et al., 1978). F-actin microspikes are precursors to filopodia, and the pseudopodia of migrating cells resemble lamellipodia (Kozma et al., 1996; Small et al., 2002; Cho and Klemke, 2002). Migrating cells extend and retract protrusions for long periods before a protrusion is stabilized for movement (Knight et al., 2000). Stabilization occurs when focal complexes are established at the leading edge, thereby linking the extracellular matrix (ECM) to the F-actin cytoskeleton.

The initial events of cell migration (i.e. exploration, adhesion and polarization) require regulated assembly and disassembly of filopodia, lamellipodia and focal adhesions/complexes. These dynamic F-actin structures can be found in migrating cells, spreading cells and growth cones of advancing neurons. Thus, the diverse array of stimuli that individually stimulate chemotaxis, cell spreading and neurite extension promote the formation of similar F-actin structures. Another type of dynamic F-actin structure is observed during PDGF stimulation of fibroblasts: plasma membrane ruffles. Ruffling refers to the highly dynamic curling action of the dorsal plasma membrane where there is rapid actin polymerization and depolymerization. This type of cell surface activity is thought to serve a role in pinocytosis and phagocytosis (Small et al., 2002).

Characterization of the molecular modulators that govern the formation of the different types of F-actin structure is an area of intense research. On the molecular level, the Arp2/3 complex might provide the driving force for formation of membrane protrusions by nucleating actin polymerization from the sides of existing filaments. Integrin receptors provide the adhesive connection between the F-actin cytoskeleton and the ECM. Cells must coordinate signals for actin polymerization,

depolymerization, bundling, severing, capping, focal adhesion turnover and actin-myosin contraction to drive processes that require cell movement. Recent studies have implicated the c-Abl tyrosine kinase in such coordination as a regulator of F-actin-based cellular events.

The Abl family of tyrosine kinases

The c-*abl* proto-oncogene was isolated as the normal cellular counterpart to the Abelson murine leukemia virus oncogene, v-*abl* (Wang et al., 1984). Homozygous mutation of mouse c-*abl* results in neonatal lethality, indicating its importance in development (Tybulewicz et al., 1991). Roughly 75% of *abl*$^{-/-}$ mice die postpartum. Some of the surviving mice develop lymphopenia, thymic atrophy and/or osteoporosis, and a lower percentage exhibit defects in eye development or spermatogenesis (Li et al., 2000; Schwartzberg et al., 1991; Tybulewicz et al., 1991). Interestingly, truncation of c-Abl by gene disruption results in similar sporadic phenotypes (Schwartzberg et al., 1991). Because the *abl*$^{-/-}$ phenotypes show low penetrance and are pleiotropic, the specific role for c-Abl during development has remained unclear. The c-*abl*-related gene, *arg*, was identified in 1986 (Kruh et al., 1986). c-Abl and Arg are widely expressed, closely related (Fig. 1) and appear to be regulated similarly in vitro (Tanis et al., 2003). *arg*$^{-/-}$ mice exhibit behavioral phenotypes (Koleske et al., 1998), but unlike the *abl*$^{-/-}$ mice, they do not exhibit lethality. However, mice die at a much earlier developmental stage (E9-11) when both *abl* and *arg* genes are disrupted (Koleske et al., 1998). Together these data suggest functional redundancy between c-Abl and Arg during early embryonic developmental stages and non-redundant functions later in development.

In mammals the Abl family consists of two c-Abl isoforms (designated 1a and 1b in humans or I and IV in mice) and two Arg isoforms (1a and 1b). The type 1b/IV isoform of c-Abl and the 1b isoform of Arg each contain a myristoylation site in the N-terminus, which is missing in the 1a/I isoforms owing to use of alternative promoters. Abl homologues have been identified in *Drosophila* and *C. elegans* (Goddard et al., 1986; Hoffman-Falk et al., 1983). The N-terminal SH3, SH2, kinase domains

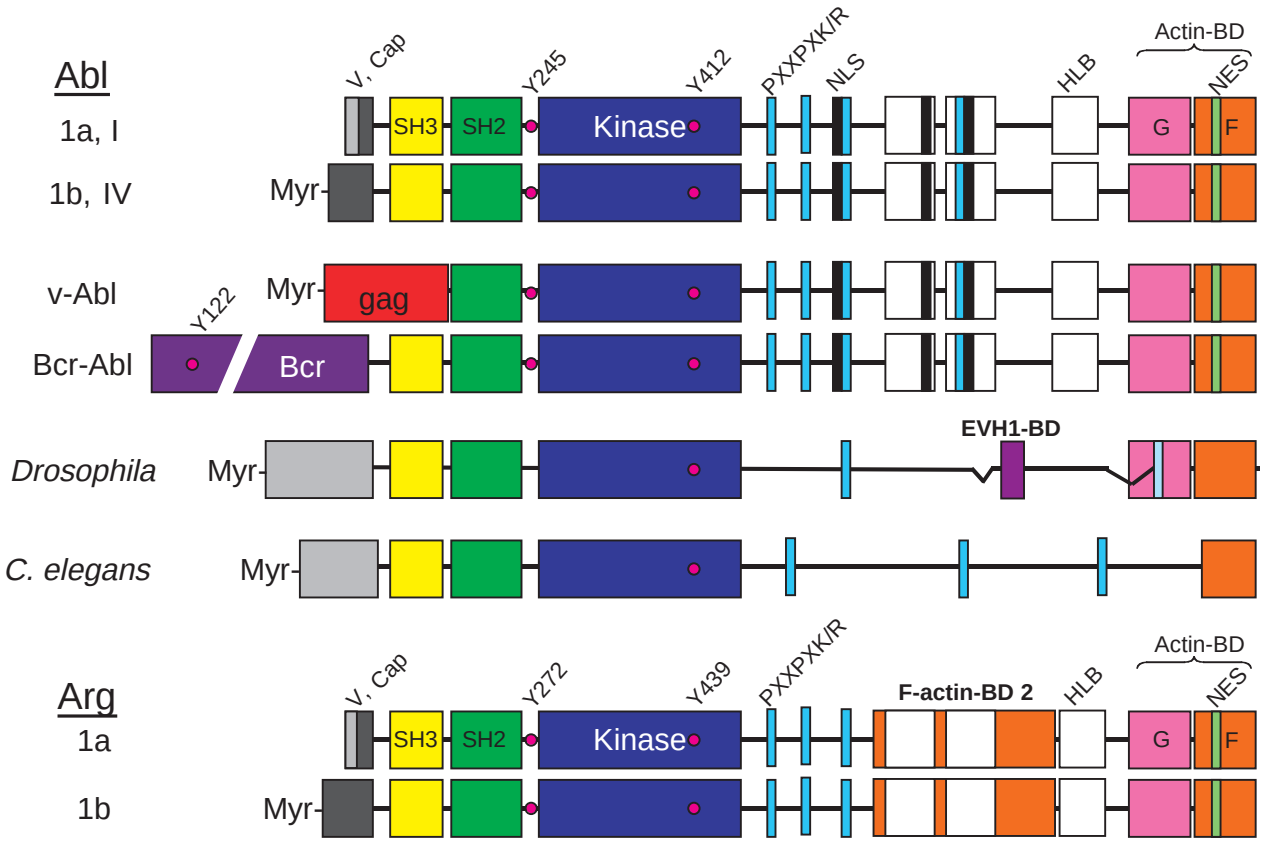

Fig. 1. Modular domains in the Abl family of tyrosine kinases. The extreme N-terminus of the c-Abl protein contains a variable region (V, light and dark gray), which in some family members contains a Cap region (dark gray) and/or a consensus motif for N-terminal myristoylation (Myr-). The Src homology-3 domain (SH3, yellow), Src homology-2 domain (SH2, green) and the catalytic domain (Kinase, dark blue) make up the remaining N-terminal half of c-Abl. In the C-terminal half there are four PXXPXK/R sequences (light blue), three nuclear localization sequences (NLS, black), one nuclear export sequence (NES, light green) and three high mobility group-like boxes (HLB, white). In addition, at the extreme C-terminus there is an actin-binding domain (Actin-BD), which contains a region that mediates binding to monomeric actin (G, pink) and a consensus motif that mediates binding to filamentous actin (F, orange). The regulatory Y245 and Y412 are indicated as red circles. The sequence conservation for these domains, regions and residues in other members of the Abl family is depicted here (in some cases not yet supported experimentally). The oncogenic forms of Abl contain modified N-termini: v-Abl contains a viral gag sequence and Bcr-Abl contains the N-terminal portion of the breakpoint cluster region protein, as labeled. Unique to *Drosophila* Abl is a consensus motif for binding to EVH1 domains (EFPPPPXD, purple) and unique to Arg is an additional C-terminal F-actin binding region (F-actin-BD 2).

and the proline-rich adapter-binding sites are highly conserved among the Abl family members (Fig. 1). The C-terminal regions of c-Abl and Arg contain a conserved nuclear export signal (NES) and an F-actin-binding motif. Between the conserved N-terminal and the C-terminal domains is a region that is divergent among Abl family members. In mouse and human c-Abl, this region contains three nuclear localization signals (NLS) (Wen et al., 1996) and three high-mobility-group-like boxes (HLB) that cooperatively bind to A/T-rich DNA (Miao and Wang, 1996; David-Cordonnier et al., 1998). In Arg this region contains an additional F-actin-binding domain (Wang et al., 2001). In *Drosophila* Abl this region contains an EVH1-binding domain, which interacts with *Drosophila* Enabled (Lanier and Gertler, 2000).

The mammalian c-Abl can shuttle between the nuclear and cytoplasmic compartments because of its NLS and NES (Taagepera et al., 1998) (Fig. 1). The oncogenic Bcr-Abl and v-Abl proteins do not enter the nucleus despite the fact that they each contain the three nuclear localization signals (Van Etten et al., 1989; McWhirter and Wang, 1991; Vigneri and Wang, 2001). Nuclear c-Abl plays a role in transcription regulation, particularly in response to DNA damage (Shaul, 2000; Wang, 2000a; Puri et al., 2002; Barilá et al., 2003), and activation of the nuclear pool of c-Abl can induce apoptosis (Vigneri and Wang, 2001; Wang, 2000a). Cytoplasmic c-Abl is activated by growth factors and cell adhesion, localizing to dynamic regions of the cytoskeleton, including membrane ruffles, the leading edges and F-actin protrusions found in actively spreading fibroblasts or the neurites of cortical neurons. Here, we focus on current understanding of the role of cytoplasmic c-Abl in F-actin dynamics.

Regulation of c-Abl tyrosine kinase

The c-Abl catalytic domain contains an activation segment that is found in all tyrosine kinases. A conserved tyrosine residue in the c-Abl catalytic domain (Y412) is phosphorylated in oncogenic forms of Abl, such as ΔSH3-Abl, Abl-P131L, Bcr-Abl and v-Abl (Barilá and Superti-Furga, 1998; Franz et al., 1989; Jackson and Baltimore, 1989; Mayer and Baltimore, 1994; McWhirter et al., 1993; Van Etten et al., 1995) and, as in other tyrosine kinases, its phosphorylation is associated with an increase in catalytic activity (Dorey et al., 2001; Brasher and Van Etten, 2000). Mutation of Y412 to phenylalanine can interfere with the activation of c-Abl by Src kinases (Brasher and Van Etten, 2000). However, the Y412F mutant does not prevent c-Abl autoactivation (Tanis et al., 2003), which suggests that phosphorylation of other tyrosine residues may contribute to autoactivation of c-Abl. In this regard, phosphorylation of Y245 (Fig. 1) or other tyrosine residues might contribute to the autoactivation of c-Abl kinase. Although phosphotyrosine (pY) is readily observed in the oncogenic Abl kinases in vivo, it has not been detected on the endogenous c-Abl protein under physiological conditions even when its catalytic activity is elevated. pY-c-Abl is detected when cells are dually stimulated with PDGF plus pervanadate (a phosphatase inhibitor, which itself can increase phosphotyrosine on c-Abl, see Table 1). pY-c-Abl might be rapidly targeted for ubiquitin-mediated degradation by the proteasome, hence, making it difficult to detect in vivo (Echarri and Pendergast, 2001; Soubeyran et al., 2003). Alternatively, although phosphorylation at Y412 is essential to achieve maximal c-Abl activity, it is likely that graded levels of kinase activation can be achieved without this phosphorylation event (Fig. 2).

The disruption of the inactive conformation can be achieved by displacement of the N-terminus, SH3 or SH2 domains, as suggested by the crystal structure of c-Abl and in vitro experiments (Nagar et al., 2003; Hantschel et al., 2003; Tanis et al., 2003; Brasher and Van Etten, 2000). The association of

Table 1. Signals that activate cytoplasmic c-Abl tyrosine kinase

Stimulus	Fold activation*	Cell type	References
FN adhesion	1.9-6.8	NIH3T3 fibroblasts, 10T1/2 fibroblasts; MEFs: littermate, c-Abl-reconstituted *abl*$^{-/-}$ or *abl*$^{-/-}$ *arg*$^{-/-}$	Lewis et al., 1996; Woodring et al., 2001; Woodring et al., 2002
PDGF (–/+ pervanadate)	1.3-3.5	NIH3T3 fibroblasts, 10T1/2 fibroblasts; c-Src-reconstituted *syf*$^{-/-}$ MEFs, Ph cells overexpressing PDGFR, c-Abl-reconstituted *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs	Plattner et al., 1999; Dorey et al., 2001; Furstoss et al., 2002
EGF	1.7-3.3	10T1/2 fibroblasts overexpressing EGFR	Plattner et al., 1999
HGF+collagen	1.5-1.6	Ca18/3 and WRO thyroid cancer cells	Frasca et al., 2001
B-cell antigen	1.7 ↑c-Abl protein	Ramos cells	Zipfel et al., 2000
TrkA+CrkII coexpression	n.d.	c-Abl-reconstituted *abl*$^{-/-}$ MEFs	Escalante et al., 2000
Abi coexpression	↑pTyr lysates	*Drosophila* S2 cells overexpressing c-Abl and Abi	Juang and Hoffmann, 1999
Myr-Nck coexpression	↑pTyr lysates	HEK-293T overexpressing c-Abl and myr-Nck	Smith et al., 1999
CrkY221F coexpression	↑pTyr lysates	HEK-293T overexpressing c-Abl and CrkY221F	Shishido et al., 2001
v-Src, c-SrcY527F or FynY528F expression	2-11	BaF3, 10T1/2 fibroblasts, HEK-293T and HEK-293 (endogenous and overexpressed c-Abl)	Plattner et al., 1999; Dorey et al., 2001; Furstoss et al., 2002
Pervanadate	up to 17 ↑pTyr-Abl	BaF3 cells overexpressing c-Abl, c-Abl-reconstituted *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs	Allen and Wiedemann, 1996; Dorey et al., 2001; Furstoss et al., 2002
Hydrogen peroxide	n.d.	COS-7 (endogenous or co-overexpression of c-Abl and PKCδ), wildtype and Arg-reconstituted *arg*$^{-/-}$ MEFs	Sun et al., 2000a; Sun et al., 2000b; Cao et al., 2001

*Fold activation of c-Abl kinase as reported by immune complex kinase assays unless otherwise indicated; n.d., not determined.

substrates with the SH3 or SH2 domain of c-Abl (e.g. binding of a phosphotyrosine ligand to the SH2 domain or a PXXP ligand to the SH3 domain) might increase the catalytic activity in situ to allow substrate phosphorylation without the need for Y412 phosphorylation. Indeed, a substrate-dependent mechanism for activation of c-Src has been proposed (Hanks and Polte, 1997; Hubbard, 1999; Alexandropoulos and Baltimore, 1996). A similar in situ activation of c-Abl kinase may explain the inability to detect pY412 on endogenous c-Abl.

The tyrosine kinase activity of c-Abl is under stringent control within the cell (Smith and Mayer, 2002). Table 1 summarizes stimuli that have been reported to activate cytoplasmic c-Abl in cells. Many of the stimuli cause very low to moderate levels of kinase stimulation, which may reflect a localized transient activation of a subset of the total c-Abl. c-Abl is likely to be held in an inactive conformation through intramolecular and intermolecular restraints (Fig. 2). Current evidence suggests the involvement of the SH3 domain, the proline rich SH2-CAT linker, and the extreme N-terminus, including the myristate group, in retaining the kinase domain in a repressed state (Barilá and Superti-Furga, 1998; Pluk et

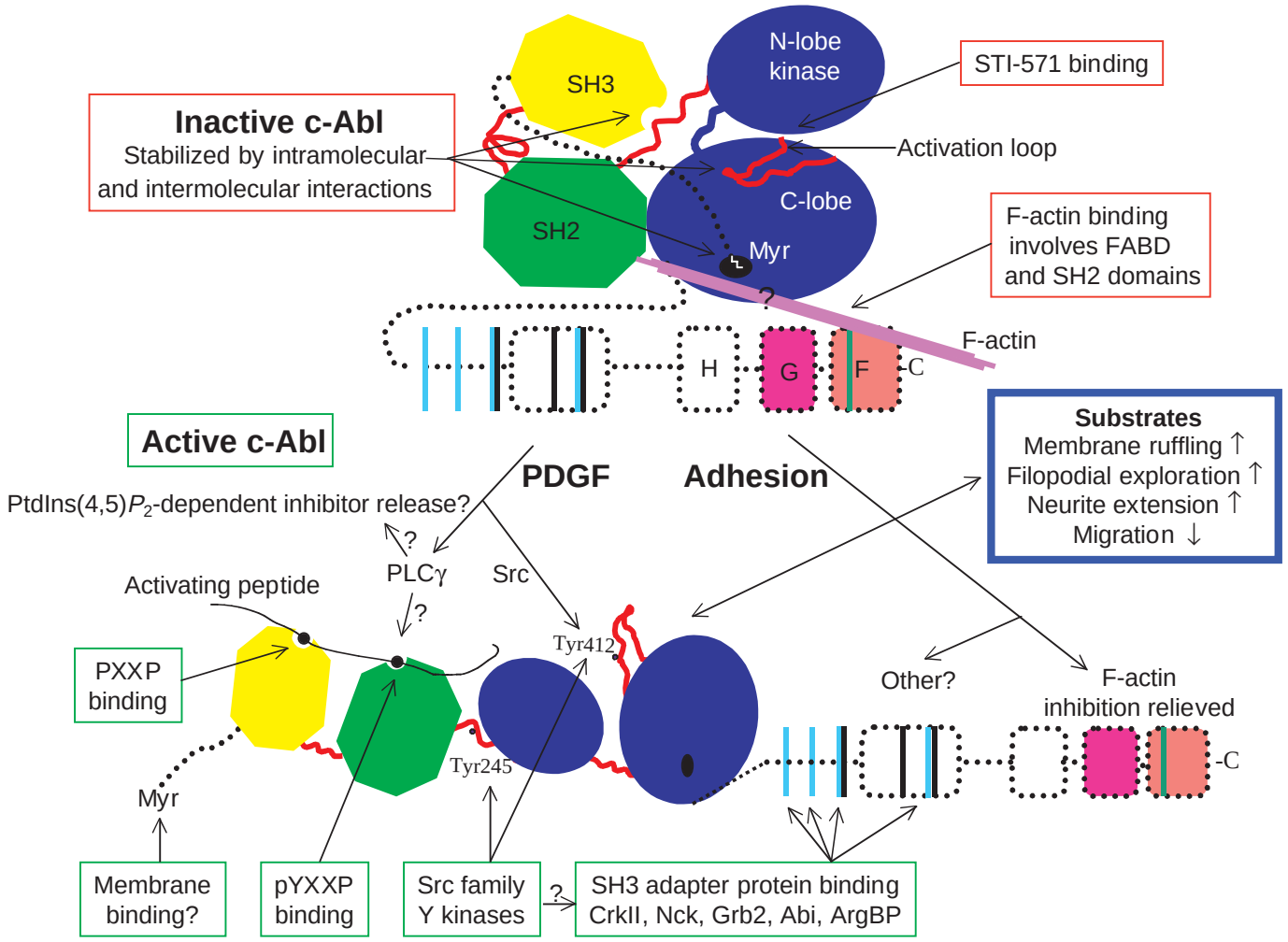

Fig. 2. Proposed mechanisms for the regulation of c-Abl tyrosine kinase. The crystal structure of the N-terminal region of c-Abl suggests it is folded into an inactive conformation through three intramolecular interactions: (1) Myr-CAP interaction with the C-lobe of the kinase domain; (2) SH3 interaction with the SH2-CAT linker; and (3) placement of the activation loop in such a way that hinders substrate entry. The C-terminus and the Cap are depicted with dotted lines, because the position of these regions was not elucidated in the current c-Abl crystal structure. The inactive conformation may involve the C-terminal region. For example, we have found F-actin to inhibit purified Abl protein, and this requires the FABD and an intact SH2 domain (P. J. Woodring, S. A. Johnson, K. Shah et al., unpublished). Binding of F-actin may further enforce the inactive conformation. Disruption of the inactive conformation can be achieved by several mechanisms (bottom). For example, the Myr-Cap may be unlatched through membrane binding. SH3 or SH2 ligands may unclamp the kinase domain from the SH3-SH2 regulatory domains. Proteins with SH3 domains that bind to the Abl PXXP motifs may also activate Abl (Table 1). Phosphorylation of Y245 in the SH2-CAT linker and Y412 in the activation loop may stabilize the active conformation. PDGF-dependent stimulation of c-Abl requires Src and PLC-γ. Src can phosphorylate Y412 whereas PLC-γ is proposed to reduce the level of PtdIns(4,5)P_2 to activate c-Abl. Cell-adhesion-mediated activation of c-Abl is dependent on mechanisms that override the negative effect of F-actin on the kinase. Importantly, the FABD is required to keep Abl in the inactive conformation in detached cells. Once activated, c-Abl phosphorylates substrate proteins to regulate various F-actin-based processes, such as membrane ruffling, filopodial exploration, neurite extension and cell migration.

al., 2002; Hantschel et al., 2003; Nagar et al., 2003). Inhibitor proteins that selectively bind to inactive c-Abl may further enforce the repressed kinase conformation. Several c-Abl inhibitors have been reported, including Pag/Msp23, AAP1, Abi, RB and F-actin (Dai and Pendergast, 1995; Pendergast et al., 1991; Prospéri et al., 1998; Shi et al., 1995; Welch and Wang, 1993; Woodring et al., 2001; Zhu and Shore, 1996). The inhibition of c-Abl kinase by Abi and Pag is accompanied by tyrosine phosphorylation of Abi and Pag, indicating that these proteins might also be substrates of c-Abl, and the inhibition may be the result of competition with the substrates used in the kinase reactions. By contrast, Rb binds directly to the ATP-binding lobe of c-Abl to inhibit catalytic activity without itself becoming phosphorylated (Welch and Wang, 1993; Woodring et al., 2001). F-actin can also inhibit the activity of purified c-Abl protein, and this depends on the direct interaction between F-actin and the F-actin-binding motif at the extreme C-terminus of c-Abl (Woodring et al., 2001).

The inactive conformation of c-Abl must be relieved to allow substrate phosphorylation. This may occur through several mechanisms, such as displacement of the SH3 domain from the SH2-CAT linker, phosphorylation of regulatory residues, displacement of the myristate group from the C-lobe of the kinase domain, loss of association to an inhibitor protein or gain of association of an activator protein (Fig. 2). Substrates may transiently disrupt the repressed c-Abl conformation as discussed above. Alternatively, 'trans-activators' of c-Abl that relieve the inactive conformation may exist. Several SH2/SH3 adapter proteins activate c-Abl tyrosine kinase when co-expressed (Table 1), although it is unclear whether this occurs through direct stable interaction (Juang and Hoffmann, 1999; Shishido et al., 2001; Smith et al., 1999). Taken together, the current evidence suggests that c-Abl activity is regulated by multiple different mechanisms. The complexity in c-Abl kinase regulation may allow it to function as an integrator of multiple signals (Wang, 2000b).

Reciprocal regulation of c-Abl and F-actin

c-Abl binds to filamentous actin through a conserved consensus F-actin-binding domain (FABD) at the extreme C-terminus (McWhirter and Wang, 1993). Studies using a mutant of c-Abl that lacks this domain and cannot bind F-actin (ΔFABD c-Abl) reveal that its deletion has no effect on c-Abl kinase activity in vitro (Woodring et al., 2001). However, unlike wild-type c-Abl, which has no detectable activity in cells detached from the ECM, ΔFABD c-Abl activity remains high in suspended cells (Woodring et al., 2001). This suggested that c-Abl is inhibited through its association with F-actin in suspended cells in vivo. Indeed, a fraction of the cellular Abl is associated with the F-actin cytoskeleton in suspended cells (Woodring et al., 2002). Cell attachment and spreading causes a dissociation of the c-Abl and F-actin, which correlates with increased c-Abl activity. Moreover, purified F-actin inhibits the activity of purified c-Abl (Woodring et al., 2001). Since the FABD is necessary for F-actin-mediated inhibition of c-Abl activity, this suggested that trans-inhibition is mediated through direct binding to F-actin, which may enforce the folding of c-Abl into the inactive conformation. Interestingly, inhibition requires the SH2 domain but not its phosphotyrosine-binding activity (P.J.W. and T.H., unpublished), which is consistent with the structural model for auto-inhibition of c-Abl IV (Nagar et al., 2003). Arg also directly associates with F-actin but Arg has a second FABD not found in c-Abl (Wang et al., 2001). Further structural analysis is necessary to determine precisely how F-actin trans-inhibits c-Abl and whether a similar mechanism applies to Arg.

Several reports indicate that c-Abl can regulate F-actin structures within the cellular cytoskeleton. For example, c-Abl is involved in membrane ruffling, filopodia formation, neurite extension and cell migration. The catalytic activity of c-Abl is required in order for c-Abl to modulate the F-actin cytoskeleton (Kain and Klemke, 2001; Plattner et al., 1999; Woodring et al., 2002; Zukerberg et al., 2000). In contrast, Arg may act locally by directly binding to F-actin since it can assemble F-actin into tight bundles in vitro (Wang et al., 2001). It is currently unknown whether Arg also contributes to F-actin dynamics through its kinase activity and whether c-Abl can also bundle F-actin in vivo. The c-Abl FABD can bundle F-actin in vitro (Van Etten et al., 1994). Loss of both c-Abl and Arg causes a more severe phenotype than does either alone (Koleske et al., 1998), suggesting that the disorganized F-actin structure found in *abl*$^{-/-}$ *arg*$^{-/-}$ neural epithelium results from the loss of the dual mechanisms by which c-Abl and Arg modify the F-actin cytoskeleton.

c-Abl increases the number of F-actin microspikes and filopodia on spreading fibroblasts and neurons. Treatment of cells with an Abl kinase small molecule inhibitor [STI571, also referred to as imatinib mesylate or Gleevec™ (Schindler et al., 2000)] largely blocks this effect (see below). ΔFABD c-Abl increases the number of F-actin microspikes in suspended cells; its effect is constitutive and independent of ECM stimulation (Woodring et al., 2002). These combined data suggest that there is a reciprocal relationship between c-Abl and F-actin. Although increased c-Abl activity can increase the number of F-actin microspikes/filopodia and contribute to membrane ruffling and neurite extension, the mechanism may be self-limiting because association of c-Abl with F-actin can decrease c-Abl activity and thus reduce the number of F-actin-rich protrusions.

Localization of c-Abl to dynamic F-actin structures

c-Abl localizes to various F-actin structures, including focal adhesions, pseudopodia, lamellipodia, filopodia, membrane ruffles, neuronal extensions and synapses (Table 2). Arg also localizes to dynamic F-actin structures, specifically to the pseudopods of migrating cells and neurons (Wang et al., 2001; Koleske et al., 1998). To localize active tyrosine kinases in live cells, Kurokawa et al. and Ting et al. have designed specific fluorescent resonance energy transfer (FRET)-based biosensors (Kurokawa et al., 2001; Ting et al., 2001). These biosensors detect the activity of specific tyrosine kinases on the basis of the preference of individual tyrosine kinases for substrates containing consensus phosphorylation sequences (Songyang et al., 1994). The optimal peptide sequence for phosphorylation by the c-Abl tyrosine kinase is AYXXP, where A is an apolar residue and X is any residue but tends to be one with a small side chain (e.g. $PY_{221}AQP$ in the CrkII adapter protein). Since pY221 of CrkII has high affinity for the CrkII SH2 domain (Songyang et al., 1993), the c-Abl kinase indicator is simply the CrkII protein sandwiched between cyan

Table 2. Localization of c-Abl protein or detection of c-Abl activity at cytoskeletal structures

F-actin structure	Abl effectors*	Cell type	c-Abl references
Focal adhesions	CrkII, Cas, Paxillin, PSTPIP1, Mena/VASP†	NIH3T3 fibroblasts; 10T1/2 fibroblasts	Lewis et al., 1996; Woodring et al., 2002
PDGF-stimulated plasma membrane ruffles	WAVE1, CrkII, Cas, PSTPIP1, Dok1	NIH3T3 fibroblasts; MEFs (Abl biosensor)	Westphal et al., 2000; Ting et al., 2001
Lamellipodia	WAVE1, Abi1/2, PSTPIP1, Mena/VASP?†	NIH3T3 fibroblasts; MEFs (Abl biosensor)	Woodring et al., 2002; P.J.W. et al., unpublished
Filopodia	Dok1, Abi1/2, PSTPIP1, Mena/VASP†	c-Abl-reconstituted *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs (Abl biosensor)	Greaves, 2002; P.J.W. et al., unpublished
Neurites, synapses	Cables, Cdk5, Abi1/2, TrkAR, EphB2R, NMDAR	Primary rat E18 cortical neurons; Mouse hippocampal sections	Zukerberg et al., 2000; Moresco et al., 2003
Pseudopods of migrating cells	HGFR, Crk, Cas, Paxillin	Ca18/3 thyroid cancer cells; Swiss 3T3 fibroblasts (Arg)	Frasca et al., 2001; Wang et al., 2001
F-actin itself (F-actin cytoskelton)		c-Abl-reconstituted *abl*$^{-/-}$ detached fibroblasts; Swiss 3T3 fibroblasts (Arg)	Woodring et al., 2002; Wang et al., 2001; Woodring et al., 2001; Van Etten et al., 1994

*Potential Abl effectors that also localize to the specified F-actin structure (Zamir and Geiger, 2001; Small et al., 2002; Cho and Klemke, 2002); see text for additional references.

†Ena/VASP in an Abl substrate in *Drosophila* but has not been reported to be a substrate in mammalian cells, although VASP has been reported to co-immunoprecipitate with c-Abl (Howe et al., 2002).

and yellow fluorescent proteins (CFP and YFP). When the N-terminal SH2 domain of CrkII binds to the C-terminal pY221 residue, the orientation of the CFP and YFP is altered such that a change in FRET efficiency occurs (Ting et al., 2001).

PDGF stimulation of NIH3T3 cells expressing the c-Abl biosensor shows a striking accumulation of c-Abl activity within membrane ruffles (Ting et al., 2001). This suggests that c-Abl is active in these highly dynamic actin structures and is consistent with the previously reported data indicating that the membrane-localized pool of c-Abl is activated upon PDGF stimulation (Plattner et al., 1999). FRET studies in live spreading cells show c-Abl activity is elevated within membrane protrusions, particularly at the tips of lamellipodial and filopodial structures (P. J. Woodring, S. A. Johnson, K. Shah et al., unpublished). Indeed, immunofluorescence experiments on fixed cells indicate that c-Abl protein is localized within membrane protrusions (Frasca et al., 2001; Greaves, 2002; Woodring et al., 2002). Use of the FRET biosensor to detect the localization of active c-Abl in live cells may thus provide important insights into the spatiotemporal localization of active c-Abl during dynamic F-actin-based processes.

c-Abl transduces extracellular signals to F-actin

The kinase activity of c-Abl is increased by signals that stimulate F-actin rearrangement. These include ECM proteins, such as fibronectin (Lewis et al., 1996; Renshaw et al., 2000; Woodring et al., 2001; Woodring et al., 2002), which promote cell spreading, and growth factors such as PDGF, which induce membrane ruffling (Cong et al., 2000; Dorey et al., 2001; Furstoss et al., 2002; Plattner et al., 1999). c-Abl can also affect neurite extension (Woodring et al., 2002; Zukerberg et al., 2000) and the more complex process of cell migration (Frasca et al., 2001; Kain and Klemke, 2001). During these F-actin based processes, c-Abl is localized to specific F-actin structures (Table 2). Since the underlying mechanisms for modulating the F-actin structures involve c-Abl kinase activity, specific c-Abl substrates are likely to be crucial for c-Abl-induced cytoskeletal effects (Table 3). The reciprocal regulation of F-actin and c-Abl may act as a molecular rheostat for the rapid and dynamic regulation of c-Abl-dependent actin-based processes.

Cell spreading and migration

The ECM activates c-Abl

Fibronectin engagement of integrins stimulates the tyrosine kinase activity of c-Abl three- to fivefold in cultured fibroblasts (Lewis et al., 1996; Renshaw et al., 2000; Woodring et al., 2001; Woodring et al., 2002). c-Abl is associated with F-actin at the cell perimeter of suspended cells (Woodring et al., 2002), which positions c-Abl to have immediate effect on F-actin once activated by cell adhesion. As c-Abl separates from F-actin during fibronectin-stimulated cell spreading, its activity increases. Since ΔFABD c-Abl can still be partially stimulated by cell adhesion, the mechanism of c-Abl activation by integrins must involve more than disassociation of c-Abl and F-actin (Fig. 2). For example, cell adhesion might stimulate post-translational modification of c-Abl, such as a transient phosphorylation event (Lewis and Schwartz, 1998). Another hypothesis is that the loss of association between c-Abl and F-actin allows c-Abl to bind to a trans-activator. Interestingly, c-Abl is activated by co-expression with SH3 adapter proteins reported to modulate the cytoskeleton, including CrkII, Abi and myr-Nck (Juang and Hoffmann, 1999; Shishido et al., 2001; Smith et al., 1999). Further investigation is required to determine precisely how cell adhesion activates c-Abl.

During the initial 20-30 minutes of fibronectin stimulation, when c-Abl activity is the highest, the nuclear pool of c-Abl in 10T1/2 fibroblasts re-localizes transiently to focal adhesions (Lewis et al., 1996). This transient re-localization also occurs in NIH3T3 cells, where a fraction of the cellular Abl associates with the focal adhesion proteins paxillin and Grb2 (Lewis and

Table 3. Cytoskeletal effectors of c-Abl tyrosine kinase

Effector	Interaction	Proposed cytoskeletal functions of Abl effector*	c-Abl references*
CrkII	AblPXXPs	Adapter protein associates with C3G, p130Cas and paxillin, involved in cell adhesion, migration, phagocytosis, neurite differentiation and B-cell response	Feller et al., 1994a; Feller et al., 1994b; Ren et al., 1994; Shishido et al., 2001; Escalante et al., 2000; Kain and Klemke, 2001
p130Cas	Indirectly through CrkII	Cell spreading and migration	Mayer et al., 1995
Paxillin	Indirectly through CrkII	Focal adhesion dynamics	Lewis and Schwartz, 1998; Escalante et al., 2000
PSTPIP1	AblSH3 AblSH2	Associates with WASp and PTP-PEST, localizes to F-actin structures including stress fibers, lamellipodia, filopodia, cortical actin and cleavage furrow	Cong et al., 2000
WAVE1	AblSH3	Stimulates Arp2/3-dependent actin polymerization	Westphal et al., 2000
Abi1/2	AblPXXPs AblSH3	Adapter proteins, stimulate Rac when complexed with Sos and Eps8, localize to lamellipodia and filopodia of metastatic cells and to developing neurons, co-localizes with Mena	Dai et al., 1998; Shi et al., 1995; Stradal et al., 2001; Courtney et al., 2000; Tani et al., 2003
Nck1/2	AblPXXPs	Adapter protein, stimulates WASp and WAVE1, involved in enteropathogenic *Escherichia coli* actin pedestal formation	Ren et al., 1994; Smith et al., 1999
Dok1	AblSH2	Adapter protein, associates with Nck, localizes to tips of filopodia and to plasma membrane ruffles, affects cell spreading and migration	P. J. Woodring, S. A. Johnson, K. Shah et al., unpublished
Cables	AblSH3	Simultaneously associates with c-Abl and Cdk5 and is involved in neurite outgrowth	Zukerberg et al., 2000
Cdk5	Indirect binding through cables	Neurite outgrowth and neuronal migration	Zukerberg et al., 2000
Receptor			
HGFR	n.d.	Cell:cell adhesion, migration	Frasca et al., 2001; Brown et al., 2000; Koch et al., 2000; Yano et al., 2000; Yu et al., 2001; Glover et al., 2000
TrkA	AblSH2	Neurite differentiation	
EphB2	AblSH2	Axon pathfinding	
NMDA	AblSH3	Synaptic transmission	
F-actin	AblFABD	Cell structure and architecture	Van Etten et al., 1994; Woodring et al., 2001; Woodring et al., 2002

*See text for specific references regarding the roles of the Abl effectors; n.d., not determined.

Schwartz, 1998; Renshaw et al., 2000). Although it was thought that this translocation could play a role in the reactivation of c-Abl, further experimentation showed that the nuclear pool of c-Abl could be reactivated independently of shuttling out of the nucleus (P.J.W. and J.Y.J.W., unpublished). Thus, there is likely to be another rationale for the recruitment of c-Abl to focal adhesions during cell attachment and spreading. Some cell types do not maintain a substantial pool of c-Abl in the nucleus. For example, when c-Abl is re-expressed in the *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs, it is present mostly in the cytoplasm. When these cells are replated onto fibronectin, c-Abl is localized throughout the cytoplasm to cytoskeletal structures, including focal adhesions, cell membranes and filopodia (Greaves, 2002; Woodring et al., 2002). Therefore, c-Abl is correctly positioned to regulate the reorganization of the cytoskeleton at sites of membrane protrusion and at focal adhesions when integrins are engaged.

c-Abl increases the number of F-actin microspikes and filopodia

During cell spreading over fibronectin-coated surfaces, c-Abl promotes an increased number of F-actin microspikes and filopodia, allowing them to persist for longer periods of time on the surface of cells relative to cells lacking c-Abl (Woodring et al., 2002). These structures are likely to serve a sensory function, being used by the cell to pick up spatial information about the nearby environment during the spreading process. This suggests that c-Abl prolongs the exploratory phase of cell spreading. The c-Abl-reconstituted fibroblasts not only have more filopodia but also are less polarized during the early phases of cell spreading (M. Sheetz, personal communication). MEFs treated with STI571 or lacking c-Abl and Arg (or c-Abl alone) have reduced numbers of microspikes and filopodia during cell spreading and appear to flatten out isotropically using lamellipodial structures. Reduced numbers of filopodia are also observed in primary cultured embryonic cortical neurons treated with STI571 or neurons isolated from mice lacking c-Abl (Woodring et al., 2002). Intriguingly, expression of kinase-deficient c-Abl or STI571 treatment of PC12 cells decreases the number of filopodia and increases formation of lamellipodia-like structures, which suggests that particular F-actin structures result from active versus inactive c-Abl (P.J.W. and T.H., unpublished). Together, these data suggest a role for c-Abl in cell exploration during cell spreading or navigation over the ECM.

Rate of cell migration is reduced by c-Abl

Random cell migration towards fibronectin is also affected by c-Abl activity, although it is unclear how c-Abl alters the F-actin cytoskeleton during cell movement (Frasca et al., 2001; Kain

and Klemke, 2001). Immortalized *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs are modestly impaired in their ability to move, whereas *abl*$^{-/-}$ *arg*$^{-/-}$ mouse embryos show significant defects in the actin latticework (Koleske et al., 1998). This is probably due to activation of compensatory mechanisms in the immortalized MEFs.

A fraction of the cellular Abl or Arg is concentrated at the tips of extending pseudopods in the stimulated metastatic thyroid cancer cell line Ca18/3 (Frasca et al., 2001) and in Swiss 3T3 fibroblasts (Wang et al., 2001), respectively. In stimulated Ca18/3 cells, MEFs or COS-7 cells overexpressing c-Abl, the rate of haptotaxis towards fibronectin is reduced by c-Abl. Wild-type MEFs treated with STI571 and MEFs lacking c-Abl and Arg fill in a wounded area more rapidly than do untreated wild-type MEFs (Kain and Klemke, 2001). These results were somewhat unexpected given the positive role that c-Abl plays in filopodia formation, membrane ruffling and neurite extension, but might reflect the activation of alternative or additional signaling pathways that occur during the longer time course of cell migration. Another interpretation is that c-Abl may extend the time it takes for a cell to migrate because c-Abl prolongs the exploratory phase of cell movement.

Prospective c-Abl effectors during cell spreading and migration

Although still in early stages of investigation, the targets of c-Abl downstream of integrins may include focal adhesion proteins (e.g. paxillin, p130Cas and CrkII) and/or proteins found in filopodial structures (e.g. Dok1, Abi and PSTPIP). c-Abl can phosphorylate paxillin (Lewis and Schwartz, 1998; Escalante et al., 2000), p130Cas (Mayer et al., 1995) and CrkII (Feller et al., 1994a; Feller et al., 1994b; Ren et al., 1994) in vitro, and all four proteins localize to focal adhesions (Zamir and Geiger, 2001). Although the integrin-induced tyrosine phosphorylation of paxillin (Nakamura et al., 2000; Turner, 2000; Schaller, 2001) and p130Cas (O'Neill et al., 2000; Vuori et al., 1996) is associated with enhanced rates of fibroblast spreading and migration, tyrosine phosphorylation of CrkII may negate these effects by disrupting Abl:Crk:Cas or Abl:Crk:paxillin complexes (Mayer et al., 1995; Escalante et al., 2000; Kain and Klemke, 2001). This raises the interesting possibility that c-Abl may both activate and inactivate cytoskeletal rearrangements at focal adhesions during cell spreading and migration.

Anti-pY221 CrkII antibodies reveal that CrkII is a specific substrate of Abl kinases (Kurokawa et al., 2001) and that the phosphorylation of Y221 in CrkII is reduced in fibroblasts lacking c-Abl and Arg (Kain and Klemke, 2001). The pY221-induced conformational change in CrkII is thought to inactivate the adapter by sterically blocking the accessibility of its N-terminal SH3 domain (Escalante et al., 2000; Feller et al., 1994a; Hashimoto et al., 1998; Rosen et al., 1995). A tyrosine phosphorylation-dephosphorylation cycle is critical for the function of CrkII in the activation of Rac, a GTPase that in its GTP-bound form stimulates lamellipodia formation and membrane ruffling (Abassi and Vuori, 2002). It is thought that CrkII affects the localization and activation of Rac through its SH3-domain-dependent interactions with Rac-specific GEFs, such as C3G or DOCK180 (Feller, 2001). Further investigation is necessary to determine what this means for the integrity of focal adhesions and the actin cytoskeleton.

Dok1 is also a substrate for c-Abl in spreading cells (P. J. Woodring, S. A. Johnson, K. Shah et al., unpublished). It localizes to the tips of filopodia during cell spreading. Furthermore, MEFs lacking Dok1 have fewer filopodia and cells co-expressing Dok1 and c-Abl have more filopodia. Fibronectin stimulation induces tyrosine phosphorylation of Dok1 (Noguchi et al., 1999). One residue that is specifically targeted by c-Abl is Y361 of Dok1 (P. J. Woodring, S. A. Johnson, K. Shah et al., unpublished), a site that has been implicated in the recruitment of Nck (Master et al., 2001; Murakami et al., 2002; Noguchi et al., 1999; Shah and Shokat, 2002; Tang et al., 1997). In addition to Dok1, three other c-Abl targets have been reported to be localized to filopodia: PSTPIP (Spencer et al., 1997), Mena/VASP (Lanier and Gertler, 2000) and the Abi proteins (Stradal et al., 2001). While PSTPIP and Abi appear to be substrates for c-Abl in cells, tyrosine phosphorylation of Mena and VASP has not been detected in mammalian systems under physiological conditions (Gertler et al., 1996). Nevertheless, since VASP does co-immunoprecipitate with c-Abl in an adhesion-dependent manner, VASP protein complexes may target c-Abl to F-actin structures (Howe et al., 2002). These data suggest that c-Abl uses multiple modulators to affect filopodia during cell spreading and migration.

Plasma membrane ruffling

PDGF activates c-Abl

PDGF and EGF can stimulate the kinase activity of a pool of c-Abl localized at/near the plasma membrane in fibroblasts (Plattner et al., 1999). Interestingly, c-Src, which is also modestly activated by treatment of cells with PDGF, may act upstream of c-Abl stimulation by PDGF (Fig. 2). Expression of a PDGFR lacking the c-Src-binding sites or expression of a kinase-deficient c-Src can reduce c-Abl activity by 35-50%. In addition, PDGF treatment of MEFs lacking Src, Yes and Fyn results in 50% less active c-Abl compared with the same cells reconstituted with c-Src (Plattner et al., 1999). It is intriguing that tyrosine kinases may activate each other through direct phosphorylation of the activation loop. However, the details of how PDGF stimulates c-Abl are not clear, since an increase in pY on c-Abl after PDGF treatment is not detected without pre-treatment of cells with the tyrosine phosphatase inhibitor pervanadate (Dorey et al., 2001; Furstoss et al., 2002), a condition that is not required to observe PDGF-induced increase in c-Abl activity in IP-kinase assays (Plattner et al., 1999). This raises the possibility that a downstream substrate of c-Src is responsible for c-Abl activation by PDGF. Consistently, c-Src and c-Abl substrates (such as Abi) are susceptible to dephosphorylation by PTP-PEST phosphatase, and the activation of c-Abl by PDGF is prolonged in PTP-PEST-deficient fibroblasts (Cong et al., 2000). The mechanism involves another component as well, since c-Src activity is necessary but not sufficient for c-Abl activation by PDGF. Recent studies have implicated phospholipase Cγ and decreased levels of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] in the activation of c-Abl kinase by PDGF. However, this mechanism is probably self-limited, since c-Abl downregulates phospholipase Cγ activity upon PDGF stimulation (Plattner et al., 2003). Expression of inositol polyphosphate 5-phosphatase can also increase c-Abl activity,

suggesting that a PtdIns(4,5)P_2-bound protein may be involved (Fig. 2). This may provide a link to F-actin inhibition of c-Abl (Woodring et al., 2001) since many F-actin-binding proteins have been reported to bind PtdIns(4,5)P_2.

PDGF-induced plasma membrane ruffling is increased by c-Abl

The activation of c-Abl contributes to the morphological response associated with treatment of cells with PDGF: plasma membrane ruffling. MEFs lacking c-Abl contain four- to fivefold fewer dorsal/circular membrane ruffles when stimulated by PDGF (Plattner et al., 1999). Expression of wild-type c-Abl or c-Abl lacking the nuclear localization sequences, but not kinase-deficient c-Abl, in *abl*$^{-/-}$ *arg*$^{-/-}$ MEFs rescues the effect of PDGF, indicating that c-Abl substrates are involved in membrane ruffling (Furstoss et al., 2002; Plattner et al., 1999).

Prospective c-Abl effectors during plasma membrane ruffling

Although downstream effectors of c-Abl in membrane ruffling remain to be fully characterized, several prospective candidates are worthy of mention: WAVE1, adapter proteins (CrkII, Nck and Abi1/2) and PSTPIP. Membrane ruffling activity involves the activation of the Rac GTPase (Hall, 1992; Nobes and Hall, 1995; Ridley et al., 1992), which is also activated at the leading edge of migrating cells (Kraynov et al., 2000). As mentioned above, CrkII might play a role in the recruitment of Rac to the membrane. Through relieving the trans-inhibition of WAVE1, activated Rac can stimulate Arp2/3-mediated actin polymerization. WAVE1 translocates to cell membranes upon PDGF stimulation and is found at the membrane in a complex containing c-Abl and other signaling proteins (Westphal et al., 2000). Furthermore, the complex of proteins that co-purifies with WAVE1 includes Abi2 and the Nck-binding proteins PIR121 and Nap125 (Eden et al., 2002). Although, it is unclear whether c-Abl signals to the WAVE1 complex, it is interesting to note that Abi proteins are substrates of c-Abl and are localized to the tips of lamellipodia in metastatic cells (Stradal et al., 2001). Also, Nck, which can associate with c-Abl (Feller et al., 1994a; Ren et al., 1994), relieves the trans-inhibition of WAVE1 (Eden et al., 2002) and activates WASp (Benesch et al., 2002; Rivero-Lezcano et al., 1995; Rohatgi et al., 2001). The importance of Nck1 and Nck2 in activating F-actin polymerization is supported by the observation that *nck1*$^{-/-}$ *nck2*$^{-/-}$ MEFs cannot form actin pedestals when infected with enteropathogenic *Escherichia Coli* (Gruenheid et al., 2001).

PSTPIP is a cytoskeletal protein that becomes tyrosine phosphorylated in an Abl-dependent manner upon PDGF stimulation (Cong et al., 2000). c-Abl appears to associate with PSTPIP through its SH2 and SH3 domains (Cong et al., 2000; Cote et al., 2002). PSTPIP can interact with WASp (Cote et al., 2002) and co-localizes with several types of F-actin structures including cortical actin and actin in lamellipodia, elongated filopodia, stress fibers and the cleavage furrow of cytokinetic cells (Spencer et al., 1997). PSTPIP may recruit PTP-PEST, a tyrosine phosphatase, to regulate F-actin dynamics by reversing the effects of tyrosine-phosphorylated proteins (Cong et al., 2000). Although PSTPIP is clearly a substrate of c-Abl, the physiological function of pY-PSTPIP is currently unknown. Further investigation is necessary to determine whether PSTPIP, Nck, WAVE1, Abi and/or CrkII are essential downstream targets of c-Abl in the PDGF-induced membrane ruffling response.

Neurite extension

Drosophila Abl functions in axonogenesis

One of the first indications that Abl functions in regulating the neuronal F-actin cytoskeleton was provided by the genetic studies of *Drosophila* Abl (D-Abl) (Lanier and Gertler, 2000; Gertler et al., 1989). D-Abl positively regulates growth cone motility through both catalytic and non-catalytic mechanisms (Henkemeyer et al., 1990; Lanier and Gertler, 2000; Wills et al., 1999a). Inactivating mutations in D-Abl result in disorganization of CNS commissural axon bundles, motoneurons that 'stop-short' of innervating targets and a disrupted neural epithelium (Bashaw et al., 2000; Baum and Perrimon, 2001; Grevengoed et al., 2001; Liebl et al., 2000; Wills et al., 1999a; Wills et al., 1999b). Deletion of a number of *Drosophila* genes can either enhance or suppress the D-Abl-knockout phenotypes, including *chicadee* (which encodes profilin*), disabled, enabled, Dtrio* (a GEF encoding gene), *prospero, Notch, fasciclin, fax, dLAR, slit, robo, capulet, armidillo, shotgun* (which encodes DE-cadherin) and *scab* (which encodes an integrin α-chain) (Bashaw et al., 2000; Baum and Perrimon, 2001; Gertler et al., 1989; Gertler et al., 1995; Gertler et al., 1993; Grevengoed et al., 2001; Lanier and Gertler, 2000; Liebl et al., 2000; Wills et al., 1999a; Wills et al., 1999b; Wills et al., 2002). In addition, biochemical studies support the idea that D-Abl regulates a signal transduction pathway involving the repulsive neuronal receptor roundabout (Robo), cadherins, capulet and/or enabled (Bashaw et al., 2000; Rhee et al., 2002; Wills et al., 2002). Other studies have suggested that D-Abl functions at the level of epithelial cell:cell adhesion junctions in the *Drosophila* follicular neuroepithelium (Baum and Perrimon, 2001; Grevengoed et al., 2001). Interestingly, c-Abl increases the number of filopodial extensions in MEFs (Woodring et al., 2002), and filopodia are important in neuronal dendritic spine formation and for the epithelial cell:cell adhesion necessary for dorsal closure of epithelial sheets during development (Wood and Martin, 2002). With some key players now identified, the neuronal D-Abl signaling pathways are likely to be more precisely characterized in the near future.

Murine c-Abl stimulates neurite outgrowth

Although growing evidence indicates that murine c-Abl also functions positively in neurite outgrowth, the mechanisms do not appear to be conserved between D-Abl and murine c-Abl. Expression of murine c-Abl in *Drosophila* D-Abl-null mutants does not restore proper neuronal navigation (Henkemeyer et al., 1990). This might be due to different substrate specificities of D-Abl and c-Abl, and/or structural differences, which include the absence of binding sites for Ena/VASP in murine c-Abl (Fig. 1). Although *Drosophila* Abl and the murine c-Abl are not strictly homologous and may use different mechanisms, both do appear to function in modulation of neuronal F-actin dynamics.

Key observations regarding c-Abl and Arg in neurulation were first made with *abl*$^{-/-}$ *arg*$^{-/-}$ mouse embryos (Koleske et al., 1998). Pathological and histological analysis of embryonic day 9-11 *abl*$^{-/-}$ *arg*$^{-/-}$ mice revealed significant deficiencies in neurulation. In these mice the neuroepithelium was buckled and highly disordered. The actin latticework at the apical surface is also disrupted and ectopic actin-rich deposits occur at the basolateral surface. This suggests a role for c-Abl and Arg in regulating the actin cytoskeleton in both neurons and neuroepithelial cells. It is not known whether it is the loss of c-Abl/Arg kinase activity, their adapter function or both that is responsible for these defects. A subsequent report has suggested that c-Abl activity is important for embryonic neuronal growth (Zukerberg et al., 2000).

c-Abl protein localizes to the cell body, neurite extensions, the growth cone and excitatory synapses of rodent embryonic cortical and hippocampal neurons (Zukerberg et al., 2000; Moresco et al., 2003). Arg is also found in neurons; it is especially abundant in brain regions containing high concentrations of synapses, which may explain the behavioral abnormalities observed in *arg*$^{-/-}$ mice (Koleske et al., 1998). Expression of active c-Abl in rodent embryonic neurons stimulates neurite outgrowth on laminin (Zukerberg et al., 2000; Woodring et al., 2002). Moreover, the overall length of neurons is decreased by inhibition of c-Abl, and these neurons contain fewer filopodial exploratory structures. The stimulus for c-Abl in neurite extension may be either the ECM protein laminin or an autocrine regulatory factor. A recent report also reveals another role for c-Abl and Arg activity in neurons: modulation of synaptic transmission induced by paired-pulse stimulation (Moresco et al., 2003). Further research is necessary to determine whether modulation of F-actin by Abl and Arg can contribute to regulating neuronal synapses.

Prospective c-Abl effectors during neurite extension

Cables is a protein that simultaneously interacts with c-Abl and Cdk5, a serine/threonine kinase essential for regulating neuronal migration and neurite outgrowth through the phosphorylation of specific substrates (Zukerberg et al., 2000). In the Cdk5-cables-c-Abl complex, c-Abl is proposed to phosphorylate Y15 on Cdk5 to stimulate its kinase activity. Antisense RNA to cables and expression of dominant negative Cdk5 each inhibit embryonic neurite outgrowth, while overexpression of activated c-Abl lengthens neurites. It will be interesting to determine whether cables and Cdk5 also play a role in the stimulatory effects of c-Abl on embryonic neuronal filopodia (Woodring et al., 2002). Additionally, c-Abl directly associates with neuronal receptors including TrkA receptors (Brown et al., 2000; Koch et al., 2000; Yano et al., 2000), EphB receptors (Yu et al., 2001) and the *N*-methyl-D-aspartic acid receptor (Glover et al., 2000). The Abl-interacting Abi proteins are also expressed in developing neurons (Courtney et al., 2000) and overexpression of Abi together with Mena can stimulate the tyrosine phosphorylation of Mena (Tani et al., 2003). However, the biological significance of these interactions remains elusive.

Future prospects

The current evidence suggests that c-Abl and Arg kinases are activated and recruited by different extracellular stimuli to regulate distinct F-actin structures. Progress has been made in understanding the mechanisms of c-Abl activation by growth factors and the ECM, and in identifying some of the substrates or collaborators of c-Abl in regulating the F-actin cytoskeleton. Because c-Abl is involved in several different F-actin-dependent processes, it is likely to collaborate with other F-actin regulators to determine the dynamic biological output. Multi-protein complexes containing c-Abl or c-Abl substrates may have specific subcellular localization to regulate distinct F-actin structures in various F-actin-dependent processes. Further investigation is now required to characterize the key upstream components in c-Abl cytoskeletal signaling pathways. Moreover, it is important to determine precisely when, where and why the crucial c-Abl substrates are phosphorylated and how this affects the architecture of the F-actin cytoskeleton during cell spreading, cell migration, wound healing, membrane ruffling, neurite extension and other F-actin-dependent processes.

Paradoxically, c-Abl seems to have a negative role in cell migration but positively contributes to filopodia formation, membrane ruffling and neurite extension. The Ena/VASP proteins exhibit a similar paradox: they decrease cell motility yet positively regulate actin polymerization (Bear et al., 2002; Krause et al., 2002). Although Ena/VASP proteins increase the lamellipodial protrusion rate, they also inhibit cell motility. Directed cell movement involves the coordination of several events. Individual cycles of protrusion and retraction are only one parameter. Thus, c-Abl may decrease overall cell migration by a mechanism independent of its positive effect on filopodia and membrane ruffling. Alternatively, c-Abl may decrease overall cell migration by inducing persistence of membrane protrusions that do not facilitate cell movement. For example, protrusions may not be stabilized for movement or may be stimulated globally instead of locally at the leading edge. Another possible explanation could relate to experimental design. At present, the effect of c-Abl on F-actin is determined in response to a single stimulus, such as PDGF or the ECM. However, processes such as cell migration are simultaneously regulated by a variety of diverse signals in vivo. Because c-Abl is responsive to a plethora of signals, its physiological effect on F-actin may not be represented by the results obtained thus far in isolated experimental systems.

In consideration of cellular signaling networks, regulation of F-actin-dependent processes is not the only function of c-Abl. Activated nuclear c-Abl kinase can inhibit differentiation or induce apoptosis in response to DNA damage (Puri et al., 2002; Wang, 2000a). The question, therefore, arises as to why a single tyrosine kinase is equipped to perform such a diverse array of biological functions? Two models can account for the multi-functionality of c-Abl (Wang, 2000b). The first proposes that c-Abl possesses cytoplasmic and nuclear functions, which are distinct and unrelated. This model considers the regulation of F-actin-dependent processes to be the function of cytoplasmic c-Abl, which has no relationship to the regulation of differentiation and apoptosis by the nuclear c-Abl. Recently, it was shown that c-Abl does not enter the nucleus of differentiated myocytes while it undergoes nuclear-cytoplasmic shuttling in undifferentiated myoblasts (Puri et al., 2002). This supports the idea that c-Abl in terminally differentiated myocytes may have a cytoplasmic function that

is independent of its nuclear localization. The second model proposes that the cytoplasmic and the nuclear functions of c-Abl are coordinated. In this model, the participation of c-Abl in F-actin-dependent processes is hypothesized to have a direct bearing on the nuclear c-Abl function. Results from three recent studies provide some evidence to support the second model. Activation of caspases by apoptotic stimuli leads to the cleavage of cytoplasmic c-Abl, producing a truncated protein that lacks the FABD and NES of c-Abl. The caspase-dependent cleavage of c-Abl allows the nuclear accumulation of c-Abl to promote cell death (Barilá et al., 2003). The FABD-truncated Abl may also induce cytoskeletal rearrangements associated with apoptosis. Consistent with this idea, is the finding that CrkII phosphorylation by c-Abl may contribute to the cytoskeletal alterations that occur during apoptosis (Kain et al., 2003). Tyrosine phosphorylation of CrkII-Y221 by c-Abl disrupts the Cas-Crk complex. The loss of Cas-Crk coupling induced by c-Abl inhibits cell migration and promotes apoptosis (Kain and Klemke, 2001; Klemke et al., 1998; Cho and Klemke, 2000). In addition, Truong and colleagues observed that DNA damage cannot activate nuclear c-Abl tyrosine kinase in cells that are deprived of the ECM signal (T. Truong, G. Sun, M. Doorly, J. Y. J. Wang and M. A. Schwartz, unpublished). They found that the stable adhesion of fibroblasts to fibronectin decreases migration and promotes DNA-damage-induced apoptosis by allowing the activation of c-Abl and p53. These cultured-cell-based results are intriguing but future work will be required to determine whether cell migration and DNA damage response are indeed coordinately regulated through c-Abl in animal models.

This work was supported by National Institute of Health grants HL57900 (to J.Y.J.W.) and CA82863 (to T.H.). J.Y.J.W. is the Herbert Stern Endowed Chair of Biology, UCSD and T.H. is a Frank and Else Schilling American Cancer Society Research Professor.

References

Abassi, Y. and Vuori, K. (2002). Tyrosine 221 in Crk regulates adhesion-dependent membrane localization of Crk and Rac and activation of Rac signaling. *EMBO J.* **21**, 4571-4582.

Alexandropoulos K. and Baltimore D. (1996). Coordinate activation of c-Src by SH3- and SH2-binding sites on a novel p130Cas-related protein, Sin. *Genes Dev.* **10**, 1341-1355.

Allen, P. B. and Wiedemann, L. M. (1996). An activating mutation in the ATP binding site of the Abl kinase domain. *J. Biol. Chem*. **271**, 19585-19591.

Barilá, D. and Superti-Furga, G. (1998). An intramolecular SH3-domain interaction regulates c-Abl activity. *Nat. Genet.* **18**, 280-282.

Barilá, D., Rufini, A., Condo, I., Ventura, N., Dorey, K., Superti-Furga, G. and Testi, R. (2003). Caspase-dependent cleavage of c-Abl contributes to apoptosis. *Mol. Cell. Biol*. **23**, 2790-2799.

Bashaw, G., Kidd, T., Murray, D., Pawson, T. and Goodman, C. (2000). Repulsive axon guidance: Abelson and Enabled play opposing roles downstream of the roundabout receptor. *Cell* **101**, 703-715.

Baum, B. and Perrimon, N. (2001). Spatial control of the actin cytoskeleton in Drosophila epithelial cells. *Nat. Cell Biol.* **3**, 883-890.

Bear, J., Svitkina, T., Krause, M., Schafer, D., Loureiro, J., Strasser, G., Maly, I., Chaga, O., Cooper, J., Borisy, G. and Gertler, F. (2002). Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* **109**, 509-521.

Benesch, S., Lommel, S., Steffen, A., Stradal, T., Scaplehorn, N., Way, M., Wehland, J. and Rottner, K. (2002). PIP2-induced vesicle movement depends on N-WASP and involves Nck, WIP and Grb2. *J. Biol. Chem.* **277**, 37771-37776.

Brasher, B. and Van Etten, R. (2000). c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the Src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. *J. Biol. Chem.* **275**, 35631-35637.

Brown, A., Browes, C., Mitchell, M. and Montano, X. (2000). c-Abl is involved in the association of p53 and trk A. *Oncogene* **19**, 3032-3040.

Cao, C., Ren, X., Kharbanda, S., Koleske, A., Prasad, K. V. and Kufe, D. (2001). The ARG tyrosine kinase interacts with Siva-1 in the apoptotic response to oxidative stress. *J. Biol. Chem.* **276**, 11465-11468.

Cho, S. Y. and Klemke, R. L. (2000). Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration and suppress apoptosis during invasion of the extracellular matrix. *J. Cell Biol.* **149**, 223-236.

Cho, S. Y. and Klemke, R. L. (2002). Purification of pseudopodia from polarized cells reveals redistribution and activation of Rac through assembly of a Cas/Crk scaffold. *J. Cell Biol.* **156**, 725-736.

Cong, F., Spencer, S., Cote, J., Wu, Y., Tremblay, M., Lasky, L. and Goff, S. (2000). Cytoskeletal protein PSTPIP1 directs the PEST-type protein tyrosine phosphatase to the c-Abl kinase to mediate Abl dephosphorylation. *Mol. Cell* **6**, 1413-1423.

Cote, J., Chung, P., Theberge, J., Halle, M., Spencer, S., Lasky, L. and Tremblay, M. (2002). PSTPIP is a substrate of PTP-PEST and serves as a scaffold guiding PTP-PEST toward a specific dephosphorylation of WASP. *J. Biol. Chem.* **277**, 2973-2986.

Courtney, K., Grove, M., Vandongen, H., Vandongen, A., LaMantia, A. and Pendergast, A. (2000). Localization and phosphorylation of Abl-interactor proteins, Abi-1 and Abi-2, in the developing nervous system. *Mol. Cell Neurosci.* **16**, 244-257.

Dai, Z. and Pendergast, A. M. (1995). Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. *Genes Dev.* **9**, 2569-2582.

Dai, Z., Quackenbush, R. C., Courtney, K. D., Grove, M., Cortez, D., Reuther, G. W. and Pendergast, A. M. (1998). Oncogenic Abl and Src tyrosine kinases elicit the ubiquitindependent degradation of target proteins through a Ras-independent pathway. *Genes Dev.* **15**, 1415-1424.

David-Cordonnier, M. H., Hamdane, M., Bailly, C. and D'Halluin, J.-C. (1998). The DNA binding domain of the human c-Abl tyrosine kinase preferentially binds to DNA sequences containing an AAC motif and to distorted DNA structures. *Biochemistry* **37**, 223-230.

Dorey, K., Engen, J., Kretzschmar, J., Wilm, M., Neubauer, G., Schindler, T. and Superti-Furga, G. (2001). Phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. *Oncogene* **20**, 8075-8084.

Echarri, A. and Pendergast, A. (2001). Activated c-Abl is degraded by the ubiquitin-dependent proteasome pathway. *Curr. Biol.* **11**, 1759-1765.

Eden, S., Rohatgi, R., Podtelejnikov, A., Mann, M. and Kirschner, M. (2002). Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* **418**, 790-793.

Escalante, M., Courtney, J., Chin, W., Teng, K., Kim, J., Fajardo, J., Mayer, B., Hempstead, B. and Birge, R. (2000). Phosphorylation of c-Crk II on the negative regulatory Tyr222 mediates nerve growth factor-induced cell spreading and morphogenesis. *J. Biol. Chem.* **275**, 24787-24797.

Feller, S. (2001). Crk family adaptors – signalling complex formation and biological roles. *Oncogene* **20**, 6348-6371.

Feller, S., Knudsen, B. and Hanafusa, H. (1994a). c-Abl kinase regulates the protein binding activity of c-Crk. *EMBO J.* **13**, 2341-2351.

Feller, S., Ren, R., Hanafusa, H. and Baltimore, D. (1994b). SH2 and SH3 domains as molecular adhesives: the interactions of Crk and Abl. *Trends Biochem. Sci.* **19**, 453-458.

Franz, W., Berger, P. and Wang, J. Y. J. (1989). Deletion of an N-terminal regulatory domain of the c-Abl tyrosine kinase activates its oncogenic potential. *EMBO J.* **8**, 137-147.

Frasca, F., Vigneri, P., Vella, V., Vigneri, R. and Wang, J. (2001). Tyrosine kinase inhibitor STI571 enhances thyroid cancer cell motile response to Hepatocyte Growth Factor. *Oncogene* **20**, 3845-3856.

Furstoss, O., Dorey, K., Simon, V., Barilá, D., Superti-Furga, G. and Roche, S. (2002). c-Abl is an effector of Src for growth factor-induced c-myc expression and DNA synthesis. *EMBO J.* **21**, 514-524.

Gertler, F., Bennett, R., Clark, M. and Hoffmann, F. (1989). Drosophila Abl tyrosine kinase in embryonic CNS axons: a role in axonogenesis is revealed through dosage-sensitive interactions with disabled. *Cell* **58**, 103-113.

Gertler, F., Hill, K., Clark, M. and Hoffmann, F. (1993). Dosage-sensitive modifiers of Drosophila Abl tyrosine kinase function: prospero, a regulator of axonal outgrowth, and disabled, a novel tyrosine kinase substrate. *Genes Dev.* **7**, 441-453.

Gertler, F., Comer, A., Juang, J., Ahern, S., Clark, M., Liebl, E. and Hoffmann, F. (1995). Enabled, a dosage-sensitive suppressor of mutations in the Drosophila Abl tyrosine kinase, encodes an Abl substrate with SH3 domain-binding properties. *Genes Dev.* **9**, 521-533.

Gertler, F. B., Niebuhr, K., Reinhard, M., Wehland, J. and Soriano, P. (1996). Mena, a relative of VASP and Drosophila Enabled, is implicated in the control of microfilament dynamics. *Cell* **87**, 227-239.

Glover, R., Angiolieri, M., Kelly, S., Monaghan, D., Wang, J., Smithgall, T. and Buller, A. (2000). Interaction of the N-methyl-D-aspartic acid receptor NR2D subunit with the c-Abl tyrosine kinase. *J. Biol. Chem.* **275**, 12725-12729.

Goddard, J., Weiland, J. and Capecchi, M. (1986). Isolation and characterization of Caenorhabditis elegans DNA sequences homologous to the v-abl oncogene. *Proc. Natl. Acad. Sci. USA* **83**, 2172-2176.

Greaves, S. (2002). Ready and Abl. *Nat. Cell Biol*. **4**, E74.

Grevengoed, E., Loureiro, J., Jesse, T. and Peifer, M. (2001). Abelson kinase regulates epithelial morphogenesis in Drosophila. *J. Cell Biol.* **155**, 1185-1198.

Gruenheid, S., DeVinney, R., Bladt, F., Goosney, D., Gelkop, S., Gish, G., Pawson, T. and Finlay, B. (2001). Enteropathogenic E. coli Tir binds Nck to initiate actin pedestal formation in host cells. *Nat. Cell Biol.* **3**, 856-859.

Hall, A. (1992). Ras related GTPases and the cytoskeleton. *Mol. Biol. Cell* **3**, 475-479.

Hanks, S. K. and Polte, T. R. (1997). Signaling through focal adhesion kinase. *Bioessays* **19**, 137-145.

Hantschel, O., Nagar, B., Guettler, S., Kretzschmar, J., Dorey, K., Kuriyan, J. and Superti-Furga, G. (2003). A myristate/phosphotyrosine switch regulates c-Abl. *Cell* **112**, 845-857.

Hashimoto, Y., Katayama, H., Kiyokawa, E., Ota, S., Kurata, T., Gotoh, N., Otsuka, N., Shibata, M. and Matsuda, M. (1998). Phosphorylation of CrkII adaptor protein at tyrosine 221 by epidermal growth factor receptor. *J. Biol. Chem.* **273**, 17186-17191.

Henkemeyer, M., West, S. R., Gertler, F. B. and Hoffmann, F. M. (1990). A novel tyrosine kinase-independent function of Drosophila Abl correlates with proper subcellular localization. *Cell* **63**, 949-960.

Hoffman-Falk, H., Einat, P., Shilo, B. and Hoffmann, F. (1983). Drosophila melanogaster DNA clones homologous to vertebrate oncogenes: evidence for a common ancestor to the src and abl cellular genes. *Cell* **32**, 589-598.

Howe, A. K., Hogan, B. P. and Juliano, R. L. (2002). Regulation of vasodilator-stimulated phosphoprotein phosphorylation and interaction with Abl by protein kinase A and cell adhesion. *J. Biol. Chem.* **277**, 38121-38126.

Hubbard, S. R. (1999). Src autoinhibition: let us count the way. *Nat. Struct. Biol*. **6**, 711-714.

Jackson, P. and Baltimore, D. (1989). N-terminal mutations activate the leukemogenic potential of the myristoylated form of *c-Abl*. *EMBO J.* **8**, 449-456.

Juang, J. L. and Hoffmann, F. M. (1999). Drosophila abelson interacting protein (dAbi) is a positive regulator of abelson tyrosine kinase activity. *Oncogene* **18**, 5138-5147.

Kain, K. and Klemke, R. (2001). Inhibition of cell migration by Abl family tyrosine kinases through uncoupling of Crk-CAS complexes. *J. Biol. Chem.* **276**, 16185-16192.

Kain, K., Gooch, S. and Klemke, R. (2003). Cytoplasmic c-Abl provides a molecular rheostat controlling carcinoma cell survival and invasion. *Oncogene* (in press).

Klemke, R. L., Leng, J., Molander, R., Brooks, P. C., Vuori, K. and Cheresh, D. A. (1998). CAS/Crk coupling serves as a 'molecular switch' for induction of cell migration. *J. Cell Biol.* **140**, 961-972.

Knight, B., Laukaitis, C., Akhtar, N., Hotchin, N., Edlund, M. and Horwitz, A. (2000). Visualizing muscle cell migration in situ. *Curr. Biol.* **10**, 576-585.

Koch, A., Mancini, A., Stefan, M., Niedenthal, R., Niemann, H. and Tamura, T. (2000). Direct interaction of nerve growth factor receptor, TrkA, with non-receptor tyrosine kinase, c-Abl, through the activation loop. *FEBS Lett.* **469**, 72-76.

Koleske, A., Gifford, A., Scott, M., Nee, M., Bronson, R., Miczek, K. and Baltimore, D. (1998). Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron* **21**, 1259-1272.

Kozma, R., Ahmed, S., Best, A. and Lim, L. (1996). The GTPase-activating protein *n*-chimaerin cooperates with Rac1 and Cdc42Hs to induce the formation of lamellipodia and filopodia. *Mol. Cell. Biol.* **16**, 5069-5080.

Krause, M., Bear, J. E., Loureiro, J. J. and Gertler, F. B. (2002). The Ena/Vasp enigma. *J. Cell Sci.* **115**, 4721-4726.

Kraynov, V., Chamberlain, C., Bokoch, G., Schwartz, M., Slabaugh, S. and Hahn, K. (2000). Localized Rac activation dynamics visualized in living cells. *Science* **290**, 333-337.

Kruh, G., King, C., Kraus, M., Popescu, N., Amsbaugh, S., McBride, W. and Aaronson, S. (1986). A novel human gene closely related to the abl proto-oncogene. *Science* **234**, 1545-1548.

Kurokawa, K., Mochizuki, N., Ohba, Y., Mizuno, H., Miyawaki, A. and Matsuda, M. (2001). A pair of fluorescent resonance energy transfer-based probes for tyrosine phosphorylation of the CrkII adaptor protein in vivo. *J. Biol. Chem.* **276**, 31305-31310.

Lanier, L. and Gertler, F. (2000). From Abl to actin: Abl tyrosine kinase and associated proteins in growth cone motility. *Curr. Opin. Neurobiol.* **10**, 80-87.

Lewis, J. and Schwartz, M. (1998). Integrins regulate the association and phosphorylation of paxillin by c-Abl. *J. Biol. Chem.* **273**, 14225-14230.

Lewis, J., Baskaran, R., Taagepera, S., Schwartz, M. and Wang, J. (1996). Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. *Proc. Natl. Acad. Sci. USA* **93**, 15174-15179.

Li, B., Boast, S., de los Santos, K., Schieren, I., Quiroz, M., Teitelbaum, S., Tondravi, M. and Goff, S. (2000). Mice deficient in Abl are osteoporotic and have defects in osteoblast maturation. *Nat. Genet.* **24**, 304-308.

Liebl, E., Forsthoefel, D., Franco, L., Sample, S., Hess, J., Cowger, J., Chandler, M., Shupert, A. and Seeger, M. (2000). Dosage-sensitive, reciprocal genetic interactions between the Abl tyrosine kinase and the putative GEF trio reveal trio's role in axon pathfinding. *Neuron* **26**, 107-118.

Loisel, T., Boujemaa, R., Pantaloni, D. and Carlier, M. (1999). Reconstitution of actin-based motility of Listeria and Shigella using pure proteins. *Nature* **401**, 613-616.

Master, Z., Jones, N., Tran, J., Jones, J., Kerbel, R. and Dumont, D. (2001). Dok-R plays a pivotal role in angiopoietin-1-dependent cell migration through recruitment and activation of Pak. *EMBO J.* **20**, 5919-5928.

Mayer, B. and Baltimore, D. (1994). Mutagenic analysis of the roles of SH2 and SH3 domains in regulation of the Abl tyrosine kinase. *Mol. Cell. Biol.* **14**, 2883-2894.

Mayer, B., Hirai, H. and Sakai, R. (1995). Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. *Curr. Biol.* **5**, 296-305.

McWhirter, J. and Wang, J. Y. J. (1991). Activation of tyrosine kinase and microfilament-binding functions of c-Abl by BCR sequences in BCR/ABL fusion proteins. *Mol. Cell. Biol.* **11**, 1553-1565.

McWhirter, J. and Wang, J. Y. J. (1993). An actin-binding function contributes to transformation by the Bcr-Abl onncoprotein of Philadelphia chromosome-positive human leukemias. *EMBO J.* **12**, 1533-1546.

McWhirter, J. R., Galasso, D. L. and Wang, J. Y. J. (1993). A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol. Cell. Biol.* **13**, 7587-7595.

Miao, Y. and Wang, J. Y. J. (1996). Binding of A/T-rich DNA by three high mobility group like domains in c-Abl tyrosine kinase. *J. Biol. Chem.* **271**, 22823-22830.

Moresco, E. M. Y., Scheetz, A. J., Bornmann, W. G., Koleske, A. J. and Fitzsimonds, R. M. (2003). Abl family nonreceptor tyrosine kinases modulate short-term synaptic plasticity. *J. Neurophysiol.* **89**, 1678-1687.

Murakami, H., Yamamura, Y., Shimono, Y., Kawai, K., Kurokawa, K. and Takahashi, M. (2002). Role of Dok1 in cell signaling mediated by RET tyrosine kinase. *J. Biol. Chem.* **277**, 32781-32790.

Nagar, B. Hantschel, O., Young, M., Scheffzek, K., Veach, D., Bornmann, W., Clarkson, B., Superti-Furga, G. and Kuriyan, J. (2003). Structural basis for the auto-inhibition of c-Abl tyrosine kinase. *Cell* **112**, 859-871.

Nakamura, K., Yano, H., Uchida, H., Hashimoto, S., Schaefer, E. and Sabe, H. (2000). Tyrosine phosphorylation of paxillin alpha is involved in temporospatial regulation of paxillin-containing focal adhesion formation and F-actin organization in motile cells. *J. Biol. Chem.* **275**, 27155-27164.

Nobes, C. D. and Hall, A. (1995). Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **81**, 53-62.

Noguchi, T., Matozaki, T., Inagaki, K., Tsuda, M., Fukunaga, K., Kitamura, Y., Kitamura, T., Shii, K., Yamanashi, Y. and Kasuga, M. (1999). Tyrosine phosphorylation of p62(Dok) induced by cell adhesion and insulin: possible role in cell migration. *EMBO J.* **18**, 1748-1760.

O'Neill, G., Fashena, S. and Golemis, E. (2000). Integrin signalling: a new Cas(t) of characters enters the stage. *Trends Cell Biol.* **10**, 111-119.

Pendergast, A. M., Muller, A. J., Havlik, M. H., Clark, R., McCormick, F. and Witte, O. N. (1991). Evidence for regulation of the human Abl tyrosine kinase by a cellular inhibitor. *Proc. Natl Acad. Sci. USA* **88**, 5927-5931.

Plattner, R., Kadlec, L., DeMali, K. A., Kazlauskas, A. and Pendergast, A. (1999). c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. *Genes Dev.* **13**, 2400-2411.

Plattner, R., Irvan, B. J., Guo, S., Blackburn, K., Kazlauskas, A., Abraham, R. T., York, J. D. and Pendergast, A. (2003). A new link between the c-Abl tyrosine kinase and phosphoinositide signaling through PLC-γ1. *Nat. Cell Biol.* **5**, 309-319.

Pluk, H., Dorey, K. and Superti-Furga, G. (2002). Autoinhibition of c-Abl. *Cell* **108**, 247-259.

Pollard, T., Blanchoin, L. and Mullins, R. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu. Rev. Biophys. Biomol. Struct.* **29**, 545-576.

Prospéri, M. T., Ferbus, D., Rouillard, D. and Goubin, G. (1998). The pag gene product, a physiological inhibitor of c-abl tyrosine kinase, is overexpressed in cells entering S phase and by contact with agents inducing oxidative stress. *FEBS Lett.* **423**, 39-44.

Puri, P., Bhakta, K., Wood, L., Costanzo, A., Zhu, J. and Wang, J. (2002). A myogenic differentiation checkpoint activated by genotoxic stress. *Nat. Genet.* **32**, 585-593.

Ren, R., Ye, Z.-S. and Baltimore, D. (1994). Abl protein-tyrosine kinase selects the Crk adapter as a substrate using SH3-binding sites. *Genes Dev.* **8**, 783-795.

Renshaw, M., Lewis, J. and Schwartz, M. (2000). The c-Abl tyrosine kinase contributes to the transient activation of MAP kinase in cells plated on fibronectin. *Oncogene* **19**, 3216-3219.

Rhee, J., Mahfooz, N., Arregui, C., Lilien, J., Balsamo, J. and VanBerkum, M. (2002). Activation of the repulsive receptor Roundabout inhibits N-cadherin-mediated cell adhesion. *Nat. Cell Biol.* **4**, 798-805.

Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401-410.

Rivero-Lezcano, O., Marcilla, A., Sameshima, J. and Robbins, K. (1995). Wiskott-Aldrich syndrome protein physically associates with Nck through Src homology 3 domains. *Mol. Cell. Biol.* **15**, 5725-5731.

Rohatgi, R., Nollau, P., Ho, H., Kirschner, M. and Mayer, B. (2001). Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. *J. Biol. Chem.* **276**, 26448-26452.

Rosen, M. K., Yamazaki, T., Gish, G. D., Kay, C. M., Pawson, T. and Kay, L. E. (1995). Direct demonstration of an intramolecular SH2-phosphotyrosine interaction in the Crk protein. *Nature* **374**, 477-479.

Schaller, M. (2001). Paxillin: a focal adhesion-associated adaptor protein. *Oncogene* **20**, 6459-6472.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W., Clarkson, B. and Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science*, **289**, 1938-1942.

Schwartzberg, P. L., Stall, A. M., Hardin, J. D., Bowdish, K. S., Humaran, T., Boast, S., Harbison, M. L., Robertson, E. J. and Goff, S. P. (1991). Mice homozygous for the abl^{m1} mutation show poor viability and depletion of selected B and T cell populations. *Cell* **65**, 1165-1175.

Shah, K. and Shokat, K. (2002). A chemical genetic screen for direct v-Src substrates reveals ordered assembly of a retrograde signaling pathway. *Chem. Biol.* **9**, 35-47.

Shaul, Y. (2000). c-Abl: activation and nuclear targets. *Cell Death Differ.* **7**, 10-16.

Shi, Y., Alin, K. and Goff, S. (1995). Abl-interactor-1, a novel SH3-protein binding to the carboxy-terminal portion of the v-Abl protein, suppresses v-*abl* transforming activity. *Genes Dev*. **9**, 2583-2597.

Shishido, T., Akagi, T., Chalmers, A., Maeda, M., Terada, T., Georgescu, M. and Hanafusa, H. (2001). Crk family adaptor proteins trans-activate c-Abl kinase. *Genes Cells* **6**, 431-440.

Small, J. V., Isenberg, G. and Celis, J. E. (1978). Polarity of actin at the leading edge of cultured cells. *Nature* **272**, 638-639.

Small, J., Stradal, T., Vignal, E. and Rottner, K. (2002). The lamellipodium: where motility begins. *Trends Cell Biol.* **12**, 112-120.

Smith, J. M. and Mayer, B. J. (2002). Abl: mechanisms of regulation and activation. *Front. Biosci.* **1**, d31-42.

Smith, J. M., Katz, S. and Mayer, B. J. (1999). Activation of the Abl tyrosine kinase in vivo by Src homology 3 domains from the Src homology 2/Src homology 3 adaptor Nck. *J. Biol. Chem.* **274**, 27956-27962.

Songyang, Z., Shoelson, S., Chaudhuri, M., Gish, G., Pawson, T., Haser, W., King, F., Roberts, T., Tatnofsky, S., Lechleider, R. et al. (1993). SH2 domains recognize specific phosphopeptide sequences. *Cell* **72**, 767-778.

Songyang, Z., Blechner, S., Hoagland, N., Hoekstra, M., Pniwica-Worms, H. and Cantley, L. (1994). Use of an oriented peptide library to determine the optimal substrates of protein kinases. *Curr. Biol.* **4**, 973-982.

Soubeyran, P., Barac, A., Szymkiewicz, I. and Dikic, I. (2003). Cbl-ArgBP2 complex mediates ubiquitination and degradation of c-Abl. *Biochem. J.* **370**, 29-34.

Spencer, S., Dowbenko, D., Cheng, J., Li, W., Brush, J., Utzig, S., Simanis, V. and Lasky, L. (1997). PSTPIP: a tyrosine phosphorylated cleavage furrow-associated protein that is a substrate for a PEST tyrosine phosphatase. *J. Cell Biol.* **138**, 845-860.

Stradal, T., Courtney, K., Rottner, K., Hahne, P., Small, J. and Pendergast, A. (2001). The Abl interactor proteins localize to sites of actin polymerization at the tips of lamellipodia and filopodia. *Curr. Biol.* **11**, 891-895.

Sun, X., Wu, F., Datta, R., Kharbanda, S. and Kufe, D. (2000a). Interaction between protein kinase C delta and the c-Abl tyrosine kinase in the cellular response to oxidative stress. *J. Biol. Chem.* **275**, 7470-7473

Sun, X., Majumder, P., Shioya, H., Wu, F., Kumar, S., Weichselbaum, R., Kharbanda, S. and Kufe, D. (2000b). Activation of the cytoplasmic c-Abl tyrosine kinase by reactive oxygen species. *J. Biol. Chem.* **275**, 17237-17244.

Svitkina, T., Verkhovsky, A., McQuade, K. and Borisy, G. (1997). Analysis of the actin-myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. *J. Cell Biol.* **139**, 397-415.

Taagepera, S., McDonald, D., Loeb, J. E., Whitaker, L. L. and Wang, J. Y. J. (1998). Nuclear-cytoplasmic shuttling of c-Abl tyrosine kinase. *Pro. Natl Acad. Sci. USA* **95**, 7457-7462.

Tang, J., Feng, G. and Li, W. (1997). Induced direct binding of the adapter protein Nck to the GTPase-activating protein-associated protein p62 by epidermal growth factor. *Oncogene* **15**, 1823-1832.

Tani, K., Sat, S., Sukezane, T., Kojima, H., Hirose, H., Hanafusa, H. and Shishido, T. (2003). Abl interactor 1 promotes tyosine phosphorylation of Mena (mammalian enabled) by c-Abl kinase. *J. Biol. Chem.* (in press).

Tanis, K. Q., Veach, D., Duewel, H. S., Bornmann, W. G. and Koleske, A. J. (2003). Two distinct phosphorylation pathways have additive effects on abl family kinase activation. *Mol. Cell. Biol.* **23**, 3884-3896.

Ting, A., Kain, K., Klemke, R. and Tsien, R. (2001). Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells. *Proc. Natl. Acad. Sci. USA* **98**, 15003-15008.

Turner, C. (2000). Paxillin interactions. *J. Cell Sci.* **113**, 4139-4140.

Tybulewicz, V. L. J., Crawford, C. E., Jackson, P. K., Bronson, R. T. and Mulligan, R. C. (1991). Neonatal lethality and lymphopenia in mice with a homozygous disruption of the *c-abl* proto-oncogene. *Cell* **65**, 1153-1163.

Van Etten, R. A., Jackson, P. and Baltimore, D. (1989). The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. *Cell* **58**, 669-678.

Van Etten, R. A., Jackson, P., Baltimore, D., Sanders, M., Matsudaira, P. and Janmey, P. (1994). The COOH terminus of the c-Abl tyrosine kinase contains distinct F- and G-actin binding domains with bundling activity. *J. Cell Biol.* **124**, 325-340.

Van Etten, R. A., Debnath, J., Zhou, H. and Casasnovas, J. M. (1995). Introduction of a loss-of-function point mutation from the SH3 region of the Caenorhabditis elegans sem-5 gene activates the transforming ability of c-abl in vivo and abolishes binding of proline-rich ligands in vitro. *Oncogene* **10**, 1977-1988.

Vigneri, P. and Wang, J. Y. J. (2001). Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat. Med.* **7**, 228-234.

Vuori, K., Hirai, H., Aizawa, S. and Ruoslahti, E. (1996). Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. *Mol. Cell. Biol.* **16**, 2606-2613.

Wang, J. Y. J. (2000a). Regulation of cell death by the Abl tyrosine kinase. *Oncogene* **19**, 5643-5650.

Wang, J. Y. J. (2000b). Integrative signaling through c-Abl: a tyrosine kinase with nuclear and cytoplasmic functions. In *Signal Transduction, Cell Cycle and their Inhibitors* (ed. J. S. Gutkind), pp. 303-324. Humana Press.

Wang, J. Y. J., Ledley, F., Goff, S., Lee, R., Groner, Y. and Baltimore, D. (1984). The mouse c-abl locus: molecular cloning and characterization. *Cell* **36**, 349-356.

Wang, Y., Miller, A., Mooseker, M. and Koleske, A. (2001). The Abl-related gene (Arg) nonreceptor tyrosine kinase uses two F-actin-binding domains to bundle F-actin. *Proc. Natl. Acad. Sci. USA* **98**, 14865-14870.

Webb, D., Parsons, J. and Horwitz, A. (2002). Adhesion assembly,

disassembly and turnover in migrating cells – over and over and over again. *Nat. Cell Biol.* **4**, E97-E100.

Welch, P. J. and Wang, J. Y. J. (1993). A C-terminal protein-binding domain in the retinoblastoma protein regulates nuclear c-Abl tyrosine kinase in the cell cycle. *Cell* **75**, 779-790.

Wen, S.-T., Jackson, P. K. and Van Etten, R. A. (1996). The cytostatic function of c-Abl is controlled by multiple nuclear localization signals and requires the *p53* and *Rb* tumor suppressor gene products. *EMBO J.* **15**, 1583-1595.

Westphal, R. S., Soderling, S. H., Alto, N. M., Langeberg, L. K. and Scott, J. (2000). Scar/WAVE-1, a wiskott-aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J.* **19**, 4589-4600.

Wills, Z., Bateman, J., Korey, C. A., Comer, A. and Van Vactor, D. (1999a). The tyrosine kinase Abl and its substrate enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance. *Neuron* **22**, 301-312.

Wills, Z., Marr, L., Zinn, K., Goodman, C. S. and Van Vactor, D. (1999b). Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the Drosophila embryo. *Neuron* **22**, 291-299.

Wills, Z., Emerson, M., Rusch, J., Bikoff, J., Baum, B., Perrimon, N. and Van Vactor, D. (2002). A Drosophila homolog of cyclase-associated proteins collaborates with the Abl tyrosine kinase to control midline axon pathfinding. *Neuron.* **36**, 611-622.

Wood, W. and Martin, P. (2002). Structures in focus: filopodia. *Int. J. Biochem. Cell Biol.* **34**, 726-730.

Woodring, P. J., Hunter, T. and Wang, J. Y. J. (2001). Inhibition of c-Abl tyrosine kinase by filamentous-actin. *J. Biol. Chem.* **276**, 27104-27110.

Woodring, P. J., Litwack, E., O'Leary, D., Lucero, G., Wang, J. Y. J. and Hunter, T. (2002). Modulation of the F-actin cytoskeleton by c-Abl tyrosine kinase in cell spreading and neurite extension. *J. Cell Biol.* **156**, 879-892.

Yano, H., Cong, F., Birge, R., Goff, S. and Chao, M. (2000). Association of the Abl tyrosine kinase with the Trk nerve growth factor receptor. *J. Neurosci. Res.* **59**, 356-364.

Yu, H., Zisch, A., Dodelet, V. and Pasquale, E. (2001). Multiple signaling interactions of Abl and Arg kinases with the EphB2 receptor. *Oncogene* **20**, 3995-4006.

Zamir, E. and Geiger, B. (2001). Components of cell-matrix adhesions. *J. Cell Sci.* **114**, 3577-3579.

Zhu, J. and Shore, S. K. (1996). c-ABL tyrosine kinase activity is regulated by association with a novel SH3-domain-binding protein. *Mol. Cell. Biol.* **16**, 7054-7062.

Zipfel, P. A., Grove, M, Blackburn, K., Fujimoto, M., Tedder, T. F. and Pendergast, A. M. (2000). The c-Abl tyrosine kinase is regulated downstream of the B-cell antigen receptor and interacts with CD19. *J. Immunol.* **165**, 6872-6879.

Zukerberg, L., Patrick, G., Nikolic, M., Humbert, S., Wu, C., Lanier, L., Gertler, F., Vidal, M., Van Etten, R. and Tsai, L. (2000). Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. *Neuron* **26**, 633-646.