Title

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Journal

Current biology : CB, 13(15)

ISSN

0960-9822

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Publication Date

2003-08-05

Peer reviewed

c-Abl Regulation: A Tail of Two Lipids

Dispatch

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c-Abl is a non-receptor tyrosine kinase whose activity is tightly controlled *in vivo* through unknown mechanisms. Recent studies suggest that c-Abl may be regulated in different cellular contexts by distinct lipids.

c-Abl is a clinically important tyrosine kinase whose activity is dysregulated by the t(9;22) Philadelphia chromosome translocation in chronic myeloid leukemia (CML), a translocation that results in the fusion of the *BCR* gene to the *ABL* gene. The critical role of Bcr-Abl in CML was demonstrated by reproduction of the disease in mice upon expression of Bcr–Abl in bone marrow stem cells and by the therapeutic efficacy of imatinib, a small molecule inhibitor of the Abl kinase, in CML patients. Obviously, the tyrosine kinase activity of c-Abl needs to be precisely controlled within the cell. However, the mechanism of regulation of c-Abl catalytic activity has remained elusive [1].

The ubiquitously expressed 140 kDa c-Abl protein localizes to the cell nucleus, plasma membrane, and actin cytoskeleton [2]. There are two different c-Abl isoforms (types Ia and Ib) that result from the expression of two small alternative first exons. Type Ib c-Abl contains a C_{14} myristoyl fatty acid moiety covalently linked to the amino terminus and is expressed at higher levels than type Ia, which is not myristoylated. The amino-terminal half of Abl resembles Src family kinases and contains Src homology 3 (SH3), SH2 and catalytic domains, but Abl differs from Src by possessing a large (60 kDa) carboxy-terminal domain. c-Abl has been implicated in signaling pathways initiated by growth factors, ionizing radiation, oxidative stress, and integrin stimulation, and in signaling during neuronal development [2] but the mechanisms of activation of Abl by these diverse stimuli have been obscure. Recent studies now suggest that membrane-associated c-Abl may be negatively regulated by phosphatidylinositol lipids [3], while in other contexts Abl may be autoinhibited through intramolecular association of the amino-terminal myristoyl group with the catalytic domain [4,5].

Negative Regulation of c-Abl by PtdIns(4,5)P_2
A previous study [6] demonstrated that the kinase activity of the membrane-associated c-Abl pool was transiently increased upon treatment of fibroblasts with certain growth factors, including platelet-derived growth factor (PDGF), and that Abl was required for PDGF-induced membrane ruffling. Activation of Abl by PDGF was greatly impaired in fibroblasts deficient in c-Src, Yes, and Fyn or in wild-type fibroblasts expressing a chimeric PDGF receptor lacking the Src-binding sites at

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tyrosines 579 and 581. Since the catalytic activity of purified c-Abl is stimulated by tyrosine phosphorylation [7], these observations suggested that Src kinases are directly activated by the PDGF receptor, and then phosphorylate and activate c-Abl in a secondary reaction. In a follow-up study in *Nature Cell Biology*, Ann Marie Pendergast and co-workers [3] have now shown that PDGF receptor signaling also overcomes negative regulation of c-Abl mediated by membrane phospholipids.

The key observation was that the binding site for phospholipase C-γ1 (PLC-γ1) at Tyr1021 on the PDGF receptor is required in addition to the Src-binding sites for robust PDGF-induced activation of c-Abl (Figure 1). Activation of c-Abl by PDGF is impaired by pre-treatment of cells with a PLC-γ inhibitor, diminished in fibroblasts lacking PLC-γ1, and restored by expression of wild-type but not phospholipase-inactive PLC-γ1. This finding suggests that either the substrate of PLC-γ, phosphatdylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), inhibits Abl or the products of the reaction, inositol-3,4,5-triphosphate (InsP$_3$) and diacylglycerol, stimulate Abl. Pendergast and colleagues [3] found that InsP$_3$ does not affect Abl kinase activity *in vitro,* but addition of liposomes containing PtdIns(4,5)P_2 inhibits c-Abl and this is significantly enhanced by pharmacological levels of calcium (400–800 μM). Conversely, depletion of cellular PtdIns(4,5)P_2 by ectopic expression of a yeast inositol phosphatase (Inp54) results in marked activation of endogenous c-Abl even in the absence of PDGF stimulation. Adding a final regulatory twist to the story, the Pendergast group went on to demonstrate reciprocal SH2-dependent complex formation between activated c-Abl and PLC-γ1, leading to phosphorylation of PLC-γ1 predominantly at tyrosines 771 and 1003 and inhibition of PLC-γ1 phospholipase activity. This regulation probably represents a negative feedback loop that serves to limit the activation of PLC-γ1 and c-Abl following growth factor stimulation (Figure 1).

A Structural Role for the Amino-Terminal Myristoyl Moiety in Autoinhibition of Type Ib c-Abl
Src kinases are maintained in the inactive state through intramolecular binding of the SH2 domain to a phosphorylated tyrosine (Tyr527 in c-Src) in the carboxy-terminal tail. In this structure, the SH3 domain makes an atypical interaction with a single proline (Pro250) in the linker region between the SH2 and catalytic domains (SH2-CD linker), forcing the catalytic domain into a closed conformation [8]. c-Abl is dysregulated by mutations in the SH3 domain that interfere with ligand binding [9], and a seminal 1998 paper from the laboratory of Giulio Superti-Furga demonstrated that mutation of the homologue of Pro250 in c-Abl also activates Abl [10], suggesting that Abl shares this part of the regulatory mechanism with Src. Point mutations in the connector region between SH3 and SH2 also dysregulate both Src and Abl [11,12], implying that dynamic coupling between the two domains is necessary for proper regulation of both kinases. However, Abl has no detectable tyrosine phosphorylation when expressed at
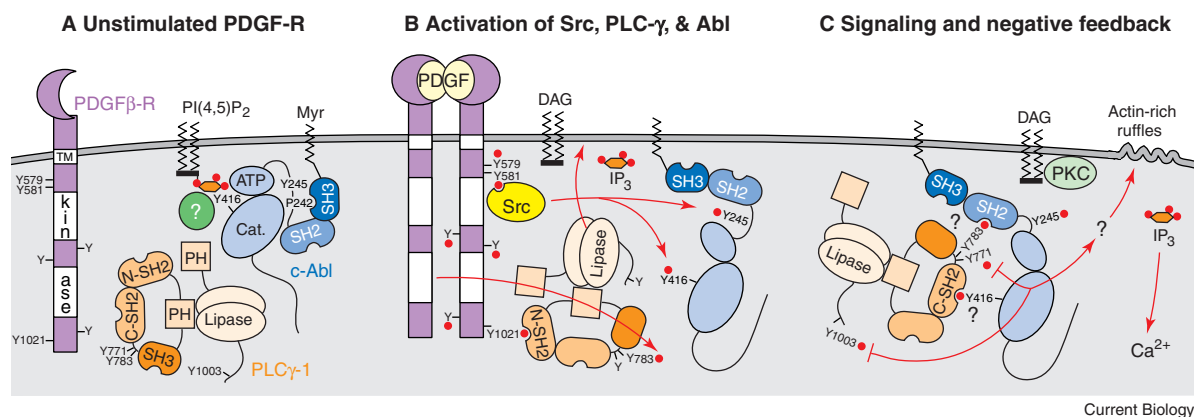
A Unstimulated PDGF-R **B Activation of Src, PLC-γ, & Abl** **C Signaling and negative feedback**

Figure 1. Regulation of c-Abl by PDGF.

(A) Unstimulated cells depicting PDGFβ receptor in purple with split kinase domain and tyrosine autophosphorylation sites (Y); PLC-γ1 in orange with two pleckstrin homology domains (PH), tandem amino- and carboxy-terminal SH2 domains and SH3 domain, and split catalytic domain (Lipase); and type Ib c-Abl in blue with amino-terminal myristoyl group (Myr) engaged in the membrane, SH3 bound intramolecularly to Pro242, and the ATP-binding and catalytic lobes of the kinase domain in closed conformation, bound to membrane PI(4,5)P$_2$ possibly via an accessory inhibitor protein (?, in green). (B) PDGF stimulation results in dimerization and autophosphorylation of PDGFβ receptor, then recruitment and activation of Src kinases (yellow) and PLC-γ1. Src phosphorylates Abl at regulatory sites Y416 and perhaps Y245, while PLC-γ1 hydrolyzes PI(4,5)P$_2$ to DAG and IP$_3$, resulting in full activation of Abl. (C) Activated Abl and PLC-γ1 form a complex mediated in part by reciprocal SH2 binding (the specific SH2-phosphotyrosine interactions depicted here (?) are hypothetical), resulting in phosphorylation of PLC-γ1 at Y771 and Y1003 and inhibition of lipase activity. Abl induces actin-rich membrane ruffles through an unknown pathway (?) while the phospholipase reaction products DAG and IP$_3$ activate PKC and calcium signaling.

physiological levels [9] and is not dysregulated by deletion of the entire carboxyl terminus or by point mutations in the SH2 domain that block phosphotyrosine binding [13]. Thus, it has been an enigma how an inhibited state of Abl could be maintained when Abl lacks the SH2–Tyr527 interaction that is essential for regulation of Src.

In a pair of recent papers in *Cell*, the laboratories of Superti-Furga and John Kuriyan have collaborated to give us the first structural glimpse of Abl in the inactive state. An amino-terminal fragment of Abl that lacks the first exon and ends with the catalytic domain would crystallize only when myristate fatty acid was added in *trans* [5]. The resulting structure reveals the myristate group bound in a deep hydrophobic pocket within the carboxy-terminal lobe (C-lobe) of the catalytic domain, inducing a kink in a carboxy-terminal αI-helix that permits close docking of the SH2 domain to the back face of the C-lobe via a series of hydrogen bonds. The overall structure bears a striking similarity to inactive Src, with the SH3 domain engaged with the Pro250 homologue in the SH2-CD linker. The accompanying paper [4] provides biochemical and genetic support for the structure by demonstrating that Abl sequences amino-terminal to the SH3 domain (the amino-terminal 'cap') and the myristoyl group are required for proper regulation of type Ib Abl kinase activity upon overexpression *in vivo*. The catalytic activity of Abl is also increased by mutations at the predicted SH2–kinase domain interface and by phosphotyrosine ligands that may disrupt this interaction.

Multilevel Regulation of c-Abl

These recent studies add to an emerging picture of c-Abl as a multifunctional protein localized to at least three different cellular compartments and whose

catalytic activity is regulated on several levels by different mechanisms (Figure 2). In the membrane-associated pool of Abl, the myristoyl group is presumably inserted in the lipid bilayer and unavailable for binding to the C-lobe. In this context, inhibition of Abl may be mediated by PtdIns(4,5)P$_2$, possibly acting through an accessory protein. One could speculate that PtdIns(4,5)P$_2$, which binds directly to the catalytic domain of Abl [3], might cause a conformational change in the C-lobe similar to that induced by myristate. In contrast, the cytoplasmic and cytoskeletal pools of Abl may assume an autoinhibited state suggested by the crystal structure [5], with the myristoyl group buried in the C-lobe. This inactive conformation could be disrupted by several routes, including auto- or transphosphorylation at regulatory tyrosines in the activation loop of the catalytic domain and the SH2-CD linker [7], or displacement of the SH2 or SH3 domains, or the myristoyl group [4].

However, there are several aspects of Abl biology that must still be explained. c-Abl gains significant catalytic activity when overexpressed [14] or upon purification [7] and its enzymatic activity in solution is unaffected by loss of the myristoyl group [15]. Furthermore, when expressed at physiological levels, type Ia c-Abl is regulated despite the lack of a myristoyl group, as is a c-Abl Ib mutant lacking the myristoylation site and first 45 residues of the cap [16]. These observations suggest that Abl may also be regulated *in vivo* by cellular factors. In the studies of Kuriyan and colleagues [4], the entire amino terminus of type Ib c-Abl (including the myristoyl group) crystallized poorly and the amino-terminal cap was disordered in both structures, hinting that an additional protein(s) might act in *trans* to stabilize this conformation. Candidate Abl inhibitory proteins include the
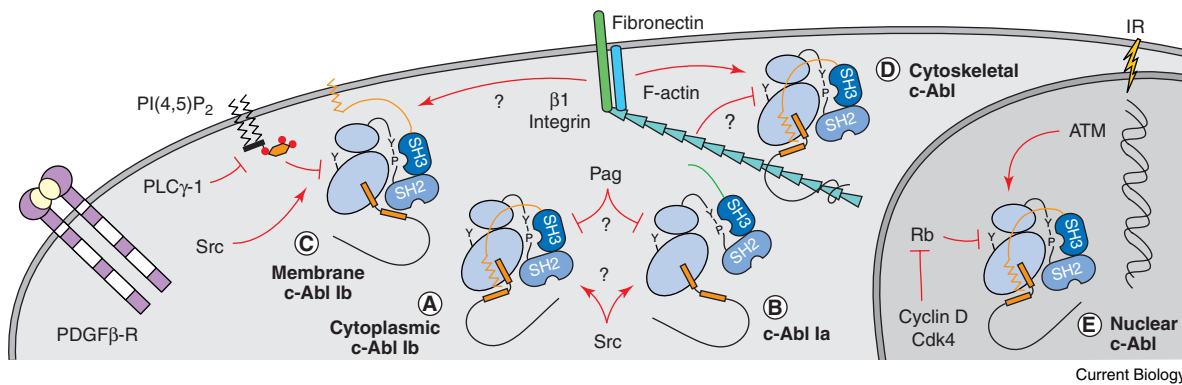
Figure 2. Multilevel regulation of c-Abl.

Different cellular pools of Abl are depicted, with established and postulated (?) regulatory mechanisms shown by red lines. (A) Cytoplasmic c-Abl Ib shown in the autoinhibited state [5], with the myristoyl group (orange) buried in a pocket in the carboxy-terminal lobe of the kinase domain, inducing a kink in the carboxy-terminal αI helix of this domain (orange rectangles) that permits close association of the SH2 domain. (B) Type Ia c-Abl with a distinct amino-terminal sequence (green) that lacks myristate. The structure of this isoform is unknown but is depicted with an extended αI helix and the SH2 domain disengaged. (C) Membrane-associated c-Abl Ib with the myristoyl group inserted in the membrane, also depicted with an extended αI helix. The position of the SH2 domain in this pool of Abl is not known. (D) Cytoskeletal c-Abl interacting with F-actin through a carboxy-terminal actin-binding domain [2]. An inhibitory role for F-actin on Abl has also been proposed [1]. (E) Nuclear c-Abl, inhibited in G1 phase by Rb and activated through ATM phosphorylation upon DNA damage.

retinoblastoma protein for nuclear c-Abl [17] and the peroxiredoxin Pag for cytoplasmic Abl [18]. We also do not know what effect the Abl carboxyl terminus may have on the inhibited form of Abl, as this entire domain was missing from the crystal structure but internal deletions in the carboxyl terminus can dysregulate Abl [19]. Lastly, there is a role for serine phosphorylation in regulation of Abl in some contexts, as the ATM serine kinase phosphorylates and activates nuclear c-Abl upon DNA damage [20]. Clearly, we have more to learn about the intricacies of the regulation of this fascinating kinase.

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