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# Cycling, stressed-out and nervous: cellular functions of c-Abl

Richard A. Van Etten

*c-Abl, the product of the cellular homologue of the transforming gene of Abelson murine leukaemia virus, has been a protein in search of a purpose for over two decades. Because c-Abl is implicated in the pathogenesis of several human leukaemias, understanding the functions of Abl is an important goal. Recently, biochemical and genetic approaches have converged to shed new light on the mechanism of regulation of c-Abl kinase activity and the multiple roles of c-Abl in cellular physiology. This review summarizes our current understanding of the many facets of c-Abl biology, emphasizing recent studies on Drosophila and mammalian Abl.*

The c-*abl* gene was first identified as the cellular homologue of the transforming gene of Abelson murine leukaemia virus and was found subsequently to be involved in the t(9;22) Philadelphia chromosome translocation in human leukaemia and to encode a non-receptor tyrosine kinase (for review, see Ref. 1). The mammalian c-*abl* gene is expressed ubiquitously and has two alternative 5′ exons with separate promoters, generating distinct 5- and 6.5-kb mRNAs and proteins that differ only in their N-terminal sequences. The two c-Abl polypeptides are denoted type Ia and Ib for human c-Abl, and type I and type IV for murine c-Abl.

The functional domains of c-Abl have been characterized extensively (Fig. 1). The N-terminal 60 kDa is homologous to c-Src and other Src-family members, but c-Abl has a large unique C-terminal domain of ~90 kDa. There is one known homologue of c-*abl*, identified by low-stringency screening of genomic DNA, denoted *abl*-related gene (*arg*)[2]. The *arg* gene product shares considerable structural and sequence homology with c-Abl in the N-terminal portion, but the C-terminal domain of Arg is fairly divergent from Abl (Fig. 1). The c-*abl* gene has been conserved fairly well throughout metazoan evolution, and recognizable orthologues exist in the *Drosophila* (Fig. 1) and *C. elegans* genomes.

### Subcellular location of c-Abl

Early efforts at understanding the role of c-Abl focused on where the protein resided in the cell, with the hope that the location would give important clues about function. The subcellular location of c-Abl was first determined by overexpressing the murine type IV protein in fibroblasts and was unexpectedly found to be largely nuclear, but with a significant fraction in the cytoplasm that is associated mostly with filamentous actin and the plasma membrane[3]. This general pattern of localization of c-Abl has been confirmed in other cell types and for endogenous Abl, although it is apparent that, in some tissues, such as primary haematopoietic cells[4] and neurons[5], c-Abl is more cytoplasmic than nuclear. Overexpression of the non-myristoylated type Ia/I form of c-Abl is very difficult, and, although its localization is assumed to be similar to the myristoylated form, this has not been demonstrated directly.

Localization of c-Abl to these different cellular compartments is controlled by distinct signals


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PII: S0962-8924(99)01549-4

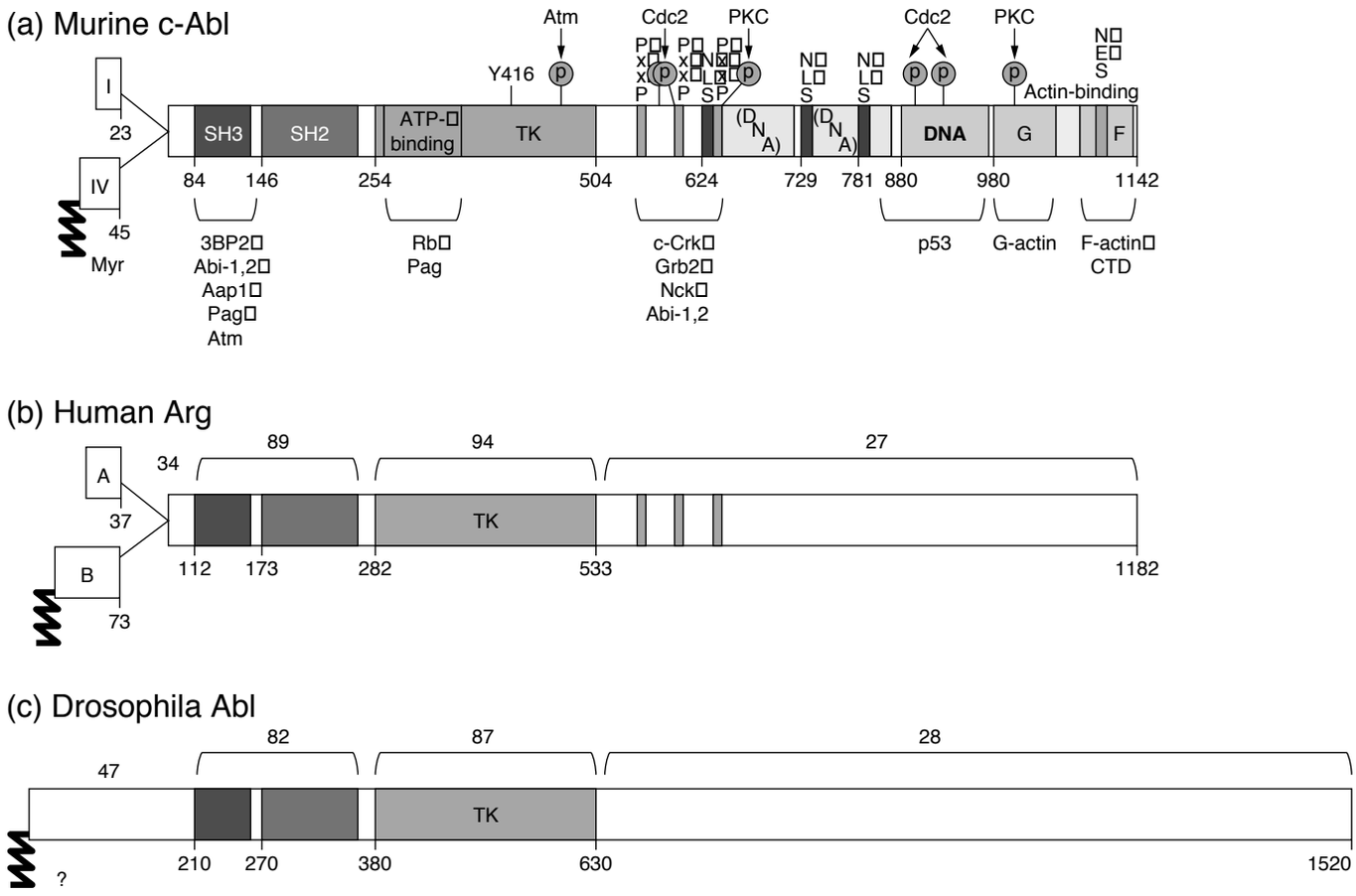


**FIGURE 1**

Schematic representation of the domain structure of mammalian c-Abl and the Abl-related Arg and *Drosophila* Abl proteins. (a) Structure of murine c-Abl. From N- to C-terminus, the defined domains include: alternative first-exon-encoded sequences, type Ia/I or type Ib/IV, with the type Ib/IV form bound to a myristoyl fatty acid (Myr); Src-homology 3 (SH3) and 2 (SH2) domains, modular domains that mediate binding to protein ligands with proline-containing and phosphotyrosine binding sites, respectively; a catalytic tyrosine kinase (TK) domain, with an N-terminal ATP-binding cleft; three proline-rich binding sites (PxxP) for adaptor proteins; three basic nuclear-localization signals (NLSs); three tandemly repeated DNA-binding domains, including one domain originally implicated in direct binding ('**DNA**') and two additional domains ('DNA'); G- and F-actin binding domains (G, F); and a nuclear-export signal (NES). Sites of autophosphorylation (Tyr416) and phosphorylation by ataxia-telangiectasia-mutated protein (Atm), Cdc2 and protein kinase C (PKC) are indicated. Only four of the nine putative Cdc2 phosphorylation sites are shown. Amino acid numbers (type Ib/IV numbering) are indicated. The known Abl-interacting proteins and their regions of interaction with Abl are shown below. (b) Structure of human Arg. The percentage amino acid identity and similarity to c-Abl is indicated above each region. The three PxxP-containing regions are conserved between Abl and Arg, but the NLSs are not. Although the C-terminus of Abl and Arg overall have little similarity, there are subregions with higher homology. (c) Structure of the *Drosophila* Abl homologue. Nomenclature is as in (b). It is unclear whether *Drosophila* Abl is myristoylated.

(Fig. 1) and is likely to be a regulated process. c-Abl has three nuclear-localization signals (NLSs), comprising short basic sequences in the C-terminal domain[6]. These three signals function differently in different cell types and exhibit overlapping and redundant function in fibroblasts, such that the presence of any one of them is sufficient to localize Abl to the nucleus. The accumulation of Abl in the nucleus is balanced by the presence of a nuclear-export signal (NES) at the C-terminus of the protein that mediates translocation of Abl to the cytoplasm in a pathway sensitive to leptomycin B[7]. In the cytoplasm, the majority of overexpressed c-Abl is associated with the F-actin cytoskeleton. F-actin localization requires the presence of a small C-terminal domain that overlaps with the NES[8,9]. Lastly, a portion of myristoylated c-Abl is associated with the inner surface of the plasma membrane, and the myristoyl group is required for membrane localization[10].

The presence of c-Abl in multiple cellular compartments suggests that the protein might move from one place to another within the cell, transducing signals in response to physiological stimuli. Alternatively, c-Abl might have distinctly different functions in different compartments. Several observations suggest that both models have relevance. When fibroblasts are trypsinized and replated onto fibronectin, there is a relocalization of c-Abl from the nucleus to F-actin-rich focal adhesions, with a subsequent return to the nucleus within an hour, suggesting that Abl responds to integrin-induced signals[11]. Also, the subcellular location of all transforming Abl proteins is exclusively cytoplasmic by immunofluorescence analysis[3], suggesting that the essential transforming activities of Abl occur in the cytoplasm, whereas transformation of fibroblasts is facilitated by the presence of the myristoyl group[10], implying a membrane function in transformation of adherent cells.

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### Insights from Abl-deficient mice

The mouse c-*abl* gene was one of the first genes targeted by homologous recombination, which generated a true null allele[12] and one encoding a truncated Abl protein with intact kinase activity[13]. Interestingly, both knockout alleles resulted in the same phenotype: $abl^{-/-}$ mice are born runted, have shortened survival and exhibit abnormal eyes, frequent rectal prolapse and defective spermatogenesis[14]. Some animals also have splenic and thymic atrophy, with a 10–30-fold decrease in the number of mature B- and T-lymphocytes. These observations suggest that Abl is required for multiple cellular functions and that the distal C-terminus of c-Abl is essential for these roles.

There is some uncertainty as to whether the lymphoid defect in these mice is autonomous to the haematopoietic system because foetal liver or bone marrow from $abl^{-/-}$ mice can reconstitute the haematolymphoid system of lethally irradiated syngeneic $abl^{+/+}$ recipients[12]. This suggests that the lymphopoenia observed in some $abl^{-/-}$ mice is a secondary phenomenon, perhaps due to increased stress and corticosteroid levels. However, the complementary experiment of rescue of $abl^{-/-}$ mice with normal marrow is not feasible owing to the survival defect, so it is possible that a combination of stromal and haematopoietic defects accounts for the decreased lymphoid cell number. The survival and lymphopoenia defects can be rescued by a c-*abl* transgene under the control of a β-actin promoter, with both type I and type IV isoforms capable of rescue[15].

### Regulation of c-Abl kinase activity

Like c-Src, wild-type c-Abl protein does not transform fibroblasts or haematopoietic cells, even when overexpressed[3], suggesting that Abl kinase activity is regulated tightly in cells. However, biochemical and mutational studies suggest that the mechanism of regulation of Abl kinase activity is different from that of Src-family kinases. c-Src is regulated negatively by phosphorylation of the C-terminal Tyr527 by Csk and other cellular kinases and assumes an inactive state where the phosphotyrosine is complexed with the Src SH2 domain in an intramolecular fashion[16]. In this inactive conformation, the Src SH3 domain binds to the linker region between the SH2 and kinase domains in an atypical interaction with a single proline residue at position 253. Activation of c-Src by dephosphorylation, mutation or deletion of Tyr527, or conversely by mutation of the SH2 or SH3 domains, results in increased tyrosine kinase activity *in vitro*, increased tyrosine phosphorylation of cellular proteins and, in most cases, cellular transformation.

By contrast, c-Abl is not tyrosine phosphorylated in its inactive state, lacks a homologue of Tyr527, and truncation of the Abl C-terminus or mutation of the SH2 domain does not activate Abl *in vivo*[17]. Deletions and point mutations in the Abl SH3 domain that prevent binding of proline-rich SH3 ligands *in vitro* activate c-Abl kinase activity *in vivo*[18], resulting in elevated tyrosine phosphorylation of c-Abl and other proteins and cellular transformation. Mutation of a proline equivalent to Src Pro253 in the linker region between the Abl SH2 and kinase domains activates transformation by c-Abl[19], suggesting that the Abl SH3 domain might repress Abl kinase activity in an intramolecular fashion similar to c-Src. However, unlike Src, c-Abl and SH3-mutated Abl have identical *in vitro* tyrosine kinase activity[17,18], suggesting that the *in vivo* effect of SH3 mutation might be due to loss of binding of a cellular inhibitor[20]. It is possible that such an inhibitor might function to stabilize binding between the Abl SH3 domain and the linker proline site because the corresponding interaction in Src is of low affinity and is disrupted upon loss of binding of Src SH2 to Tyr527.

The Abl SH3 domain was the first to be used to identify specific SH3 ligands[21], and several of the known Abl SH3-binding proteins (Table 1) are candidate inhibitors. Abi-1[22] and Abi-2[23] are related SH3-containing proteins with homology to homeodomain transcription factors. Expression of a truncated form of Abi-1 blocks transformation by v-Abl, while coexpression of truncated Abi-2 with c-Abl induces cellular transformation. Therefore, these proteins appear to function more as effectors of Abl than as inhibitors. Aap1 is a novel protein that

**TABLE 1 – ABL SH3-BINDING PROTEINS**

| Name | Identification and functional significance to Abl | Refs |
|---|---|---|
| 3BP1 | Rho-GAP homology; significance to Abl unknown | 21 |
| 3BP2 | SH2 domain; significance to Abl unknown | 58 |
| Abi-1 | SH3 and homeo-like domains; truncated form inhibits v-Abl transformation | 22 |
| Abi-2 | SH3 and homeo-like domains; truncated form induces transformation by c-Abl | 23 |
| Aap1 | Abl SH2–SH3 binding protein; inhibits c-Abl kinase activity *in vitro* | 24 |
| Mena | Murine Ena, VASP-related, regulates cytoskeleton; significance to Abl unknown | 59 |
| Pag/MSP23 | Peroxiredoxin family member; inhibits c-Abl kinase and cytostatic activity *in vivo* | 25 |
| Atm | Nuclear serine kinase; phosphorylates and activates Abl kinase activity after IR | 34, 35 |
| e3B1/hssh3bp1 | SH3 domain, Abi-1 homologue, binds to eps8 and spectrin; significance to Abl unknown | 60, 61 |

Abbreviations: GAP, GTPase-activating protein; IR, ionizing radiation; SH2, Src-homology 2; SH3, Src-homology 3; VASP, vasodilator-associated phosphoprotein.

inhibits Abl kinase activity *in vitro*, but no *in vivo* role has been described[24]. Pag/MSP23 is a member of the peroxiredoxin family of antioxidant enzymes, induced by serum stimulation and oxidative stress, that complexes with and inhibits c-Abl when coexpressed *in vivo*[25]. More work is needed to determine whether any of these candidates are major physiological regulators of c-Abl kinase activity *in vivo*.

**Nuclear functions of c-Abl**

Several lines of evidence suggest a role for nuclear c-Abl in regulation of the cell cycle[26]. A portion of the nuclear pool of c-Abl in cells in G1 phase of the cell cycle is complexed with the retinoblastoma protein, Rb. In this complex, the C-terminal pocket of Rb binds to the ATP-binding lobe of the Abl kinase domain, resulting in inhibition of Abl kinase activity. Phosphorylation of Rb by cyclin-D–cdk4/6 kinases at the G1–S boundary results in release of c-Abl and activation of Abl kinase activity during S phase. In S phase, c-Abl can contribute to phosphorylation of the C-terminal domain (CTD) of RNA polymerase II, possibly stimulating the transcription of S-phase genes. These observations suggest that c-Abl might have a growth-promoting action during S phase, and indeed transfection with c-*abl* abrogates Rb-dependent growth arrest in Rb-deficient Saos-2 cells[27]. However, some observations are not consistent with this model. Fibroblasts derived from *abl*$^{-/-}$ mice do not have defined defects in S-phase progression, and there are no known examples of genes whose transcription is Abl-dependent. Rb functions as a stoichiometric inhibitor of nuclear Abl, but, because the Abl SH3 domain is not required for Rb binding, Rb is unlikely to be the inhibitor suggested by mutational studies of Abl.

In different circumstances, Abl inhibits growth in G1 phase[6,28]. When overexpressed by transfection or conditional expression, c-Abl induces cell-cycle arrest in G1, with apoptosis of a significant fraction of the population. The cytostatic and cytotoxic effects of c-Abl are nuclear functions that require the Abl SH2 domain and kinase activity and the p53 and Rb tumour-suppressor gene products[6,29]. The experiments that led to these conclusions involve overexpression of c-Abl, raising the concern that the growth inhibition might be artifactual. However, conditional expression of kinase-inactive c-Abl (which might act in a dominant–negative fashion)[28] or treatment of cells with antisense oligodeoxynucleotides directed against *abl*[30] both accelerate the onset of S phase and shorten G1, suggesting that inhibition of the G1–S transition is a physiological function of endogenous c-Abl. The biochemical mechanism of growth inhibition by c-Abl is unknown. The requirement for Abl kinase activity and the SH2 domain implicates one or more nuclear substrates of Abl in the process, but these are unlikely to be either p53 or Rb because neither of these proteins is detectably tyrosine phosphorylated. c-Abl might interact directly with p53 and weakly stimulate p53 transactivation activity[29], but this effect does not require Abl kinase activity, and its relevance to growth arrest is unclear. Interestingly, the cytostatic and cytotoxic effects of c-Abl, but not of SH3-mutated Abl, are blocked by coexpression of the Pag/MSP23 SH3-binding protein[25], suggesting that Pag/MSP23 might regulate directly the cell-cycle effects of nuclear c-Abl.

To add to the complexity, c-Abl itself has DNA-binding activity mediated by three tandemly repeated DNA-binding domains with homology to HMG proteins[31]. The three DNA-binding domains are roughly coincident with the three Abl nuclear-localization signals, suggesting duplication of a functional unit (Fig. 1). The second and third domains facilitate DNA binding in the presence of the original domain but have not been shown to bind to DNA in isolation. Although the homology to HMG-like proteins is provocative, the degree of similarity is low, and the helix–turn–helix structure of HMG proteins is unlikely to be preserved in Abl because there are numerous proline residues in this region. Although Abl was reported initially to have sequence-specific DNA-binding activity, subsequent studies showed only a weak preference for AT-rich oligonucleotides[31]. The functional significance of Abl DNA binding is not known. Although c-Abl lacks intrinsic transcriptional activity, v-Abl and c-Abl can complex with the transcription factors CREB and E2F-1[32] and modulate their transcriptional activity, including induction of c-*myc* transcription. However, it is not known whether this coactivating function of Abl requires the Abl DNA-binding domain. An alternative possibility is that Abl DNA binding is involved in the response to DNA damage.

Ionizing radiation (IR) and radiomimetic chemicals such as mitomycin C appear to activate the kinase activity of nuclear c-Abl by 3–5 fold (assessed by immunoprecipitation and *in vitro* kinase assay)[33]. Because the activation is observed after immunoprecipitation, which eliminates the effect of an inhibitor, it is likely to be mediated by direct modification of Abl through phosphorylation. Indeed, subsequent reports demonstrated that, in response to IR, c-Abl interacts with and is phosphorylated by the ataxia-telangiectasia-mutated (ATM) gene product[34,35] as well as the severe combined immunodeficiency (*scid*) gene product[36], the catalytic subunit of DNA-dependent protein kinase (DNA-PK). Genetic and mutational studies provide further evidence for the functional connection between these large nuclear serine kinases and c-Abl: IR-induced activation of Abl kinase activity is absent in *atm*$^{-/-}$ cells and reduced in *scid* fibroblasts, while mutation of a candidate ATM phosphorylation site (Ser465) in the Abl kinase domain (Fig. 1) also abrogates IR-induced activation of Abl[34]. In addition, the antioxidant activity of a candidate inhibitor of c-Abl, Pag/MSP23[25], suggests that an additional level of regulation of c-Abl might exist, where IR-generated free radicals induce oxidation and dissociation of Pag/MSP23 from c-Abl, contributing to activation of Abl kinase activity (Fig. 2).

Upon activation, c-Abl phosphorylates several nuclear substrates, including DNA-PK[36], Rad51[37], SHPTP1[38] and the p85 subunit of phosphoinositide

3-kinase[39], negatively regulating their respective activities. In addition, c-Abl has been reported to be required for activation of stress-activated kinase/*jun* N-terminal kinase (SAPK/JNK)[33] and the related p38 kinase in response to IR but not to tumour-necrosis factor alpha (TNF-α). However, careful examination of the physiological properties of *abl*$^{-/-}$ primary murine embryo fibroblasts suggests that c-Abl is not actually required for many of these responses. The facts that Abl can arrest cells in G1 and that Abl kinase activity is stimulated by IR suggest that Abl might play a role in the G1 arrest response to IR. A defect in the G1–S checkpoint response to IR in *abl*$^{-/-}$ fibroblasts has been reported[40], but subsequent studies from several groups failed to detect such a defect[5,41]. Similarly, IR is a relatively weak inducer of JNK/SAPK activity, and there appears to be no defect in the modest level of JNK/SAPK activation in *abl*$^{-/-}$ fibroblasts in response to high doses of IR[41]. The interactions of Abl with DNA-PK and Rad51 are provocative, but *abl*$^{-/-}$ cells do not have defined defects in double-strand DNA break repair and in fact appear to be somewhat radioresistant relative to *abl*$^{+/+}$ cells[42]. Therefore, the available biochemical and genetic evidence suggests that c-Abl is involved in multiple pathways activated by genotoxic and possibly oxidative stress[43], regulated at several levels by protein–protein interactions and by phosphorylation (Fig. 2). Because cells lacking Abl or both Abl and the related Arg protein[5] do not seem to have obvious defects in DNA repair or cell-cycle progression, the role of c-Abl in these processes must be redundant, subtle, or both. Obviously, the precise role played by Abl in these complex cellular responses will require much more investigation.

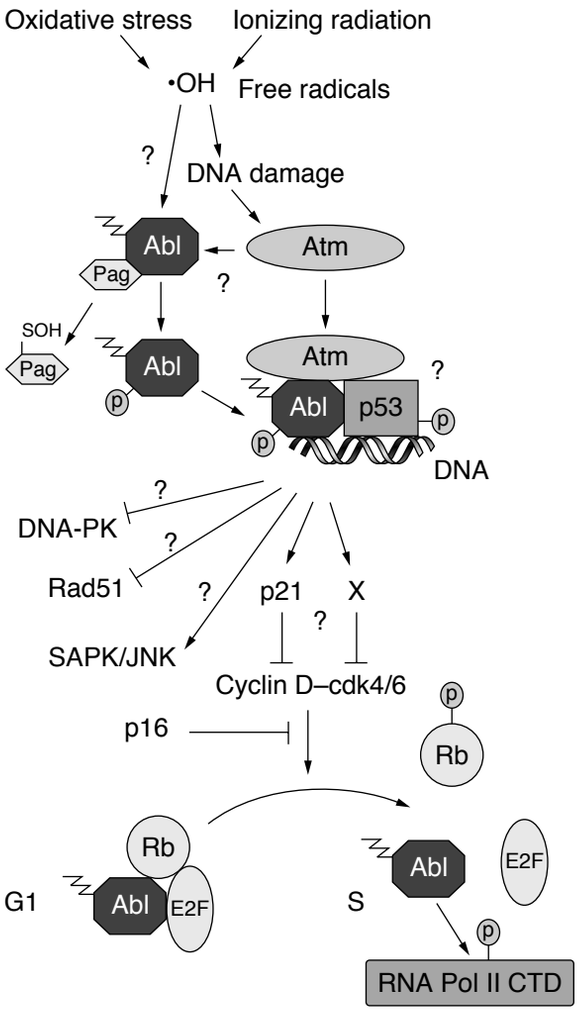


**FIGURE 2**

Hypothetical pathway of nuclear functions of mammalian c-Abl. In response to DNA damage and/or oxidative stress, c-Abl is released from the inhibitor Pag, which undergoes oxidation, while Abl and p53 are phosphorylated by ataxia-telangiectasia-mutated protein (Atm), forming an active DNA-bound complex. Activated Abl phosphorylates and inhibits DNA-dependent protein kinase (DNA-PK) and Rad51 and activates stress-activated kinase and *jun* N-terminal kinase (SAPK/JNK), while p53 mediates growth arrest and perhaps apoptosis through p21-dependent and -independent (X) pathways. Abl is also activated in S phase by dissociation of the retinoblastoma protein Rb, leading to phosphorylation of the C-terminal domain (CTD) of RNA polymerase II. Relationships that are hypothesized but not fully established biochemically are indicated by question marks. There are some conflicting observations (see text). c-Abl has been reported to be required for ionizing radiation (IR)-induced G1 arrest and to be independent of p21[40], while other observations suggest that it is not required[5,41]. A fraction of Atm might bind to Abl constitutively[35], and c-Abl in S phase might be activated preferentially by IR[41].

**Cytoplasmic functions of c-Abl**

Compared with its nuclear functions, less is known about the function of c-Abl in the cytoplasm. A large proportion of cytoplasmic Abl is associated with the F-actin cytoskeleton through the C-terminal actin-binding domain. This domain has distinct binding activity for both filamentous (F) and monomeric (G) actin *in vitro*[9], and together the two domains can mediate bundling of F-actin filaments. *In vivo*, the low concentration of c-Abl makes it unlikely that Abl is a major modifier of the cellular F- and G-actin pools. However, it is possible that Abl influences the cytoskeleton locally, and in turn Abl kinase activity might be modified by cytoskeletal signals. When detached fibroblasts are replated onto fibronectin, c-Abl is recruited to focal adhesions and Abl kinase activity increases transiently[11]. The mechanism of activation is not clear, but might be mediated by cytoskeletal binding or signalling. Integrin-mediated activation of Abl is accompanied by binding and phosphorylation of paxillin[44], a focal adhesion protein of unknown function. Another cytoplasmic substrate of c-Abl is c-Crk. Crk is negatively regulated by tyrosine phosphorylation, in which the Crk SH2 domain binds to the tyrosine-phosphorylation site in an intramolecular fashion reminiscent of c-Src. Extracts from *abl*$^{-/-}$ fibroblasts have significantly reduced c-Crk kinase activity, implicating c-Abl as the major regulator of Crk *in vivo*[45]. In its activated state, Crk binds to Cas and C3G, two signalling molecules that also influence actin microfilaments, strengthening the connection between Abl and the cytoskeleton.

Genetic studies in *Drosophila* suggest a cytoplasmic role for the Abl orthologue DAbl in neuronal development. During *Drosophila* embryogenesis,

DAbl is expressed predominantly in neuronal axons in the central nervous system (CNS). *abl*$^{-/-}$ flies exhibit pupal lethality with no gross structural abnormalities of the nervous system, but heterozygotes with mutations in several genes, such as *disabled* (*dab*)[46], die as embryos with complete disruption of CNS axon bundles. Dab is a tyrosine-phosphorylated adaptor protein with several potential Abl phosphorylation sites and an Shc-like phosphotyrosine-binding domain. It colocalizes with Abl in axons and associates with Notch[47], a receptor present in neuronal growth cones that also demonstrates synergistic genetic interactions with DAbl in axonogenesis. By contrast, flies that lack DAbl but have only one copy of the *enabled* (*ena*) gene survive to adulthood with no obvious defects, and heterozygous *ena* mutations also substantially restore normal axonal development in *abl*$^{-/-}$ *dab*$^{+/-}$ embryos[48]. Ena is a proline-rich, tyrosine-phosphorylated axonal protein that binds to the Abl SH3 domain *in vitro* and has similarity to the profilin-binding protein VASP. Collectively, these observations suggest that Dab and Ena are DAbl substrates that play opposing positive and negative roles in axonogenesis through partially redundant Abl-dependent and Abl-independent pathways (Fig. 3a).

Additional genetic studies suggest that Dabl functions in axonal pathfinding and target recognition, possibly by regulating cell adhesion through transmembrane adhesion molecules and motility through the actin cytoskeleton. Embryos doubly mutant for *abl* and the neural cell-adhesion molecule fasciclin I (*fas I*) display major defects in commissural axon pathways and growth cone guidance not observed in single mutants[49], implying that DAbl and Fas I function in parallel, redundant

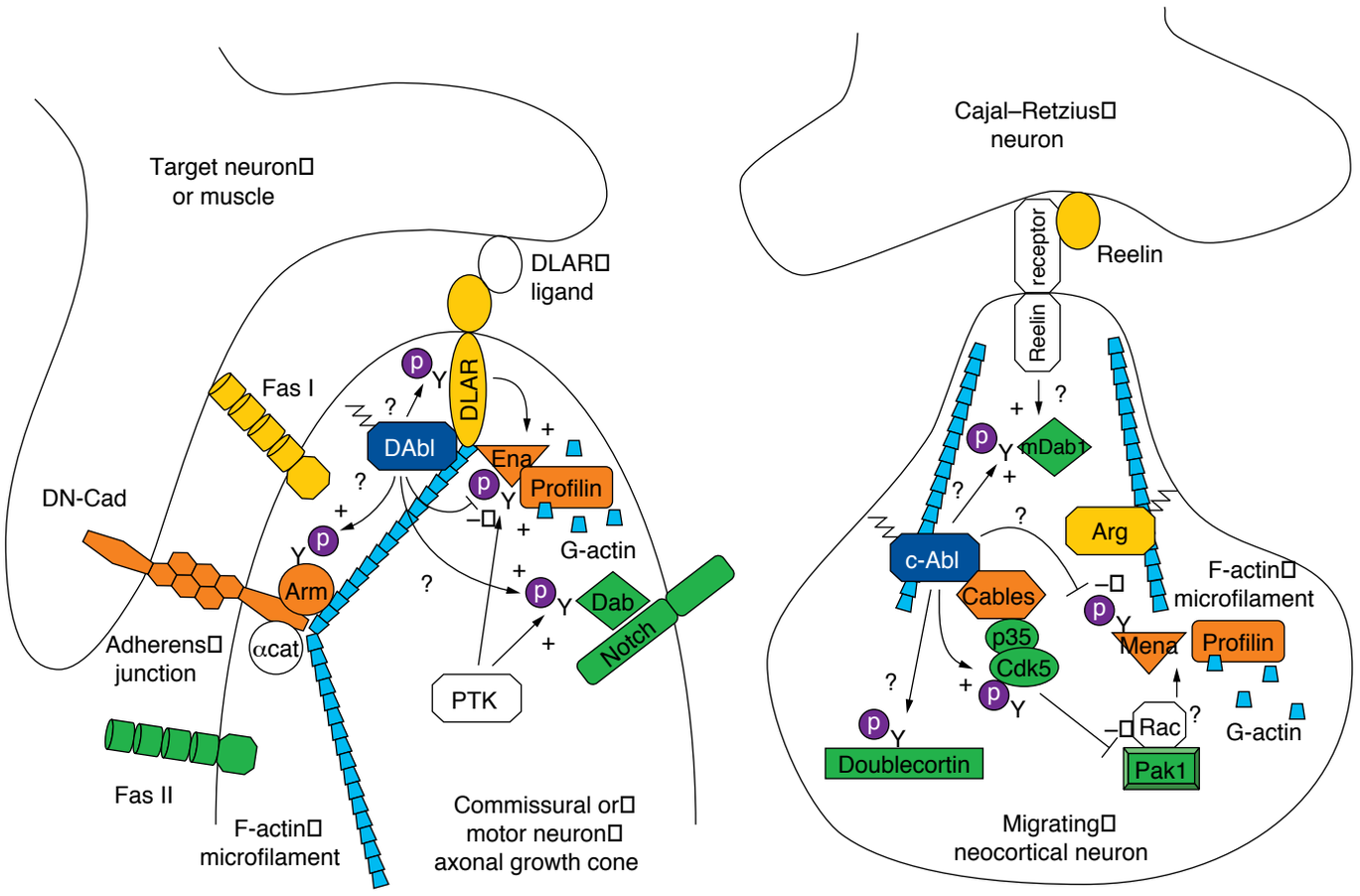


**FIGURE 3**

Hypothetical model of Abl in neural development. (a) Role of *Drosophila* Abl in pathfinding by a commissural or motor neuronal axon. Proteins known to be tyrosine phosphorylated in growth cones are indicated; arrows suggest enzyme–substrate relationships, while the effect of phosphorylation or dephosphorylation on the function of an individual substrate in axonogenesis is given by the +/– sign. In many cases, these relationships are not yet established biochemically; hypothetical interactions are indicated by a question mark. The effect of Ena on profilin function is not clear but is presumed to be negative based on genetic considerations. PTK is an unknown tyrosine kinase whose function is partially redundant with DAbl[46]. (b) Conjectural model of mammalian c-Abl function in cortical development; nomenclature is as in (a). Here, the majority of the suggested interactions have not been established biochemically. Abl and Arg are required together for neural tube closure[5]; the event depicted is the subsequent migration of neocortical neurons. Mutations in Reelin, a putative ligand, cause a phenotype identical to Scrambler and alter mDab1 levels, suggesting that mDab1 is downstream of a Reelin signalling pathway[62]. The connection between Abl and Doublecortin is solely through a potential Abl kinase phosphorylation site in Doublecortin; neuronal substrates of Arg are unknown.

signalling pathways controlling axon guidance across the commissures. Similarly, simultaneous mutation of DAbl and *Drosophila* Armadillo (Arm) induces severe disruption of the CNS, with enhancement of subtle defects in the ventral nerve cord observed in *arm* single mutants[50]. This suggests that DAbl might act with Arm, which is also tyrosine phosphorylated in neurons, to promote assembly or maintenance of adherens junctions. Recent work demonstrates a balance between DAbl-mediated tyrosine phosphorylation and dephosphorylation by tyrosine phosphatases in axon guidance of motor neurons[51]. A genetic screen for 'bypass' mutants in which the intersegmental nerve b (ISNb) neuron exits the CNS but fails to turn and connect with its nearby ventral muscle target yielded several genes, including the transmembrane tyrosine phosphatases *Dlar* and *DPTP69D* and the DAbl suppressor *ena*. Heterozygous loss of function of DAbl suppresses the *Dlar* bypass phenotype, suggesting that Abl tyrosine kinase activity opposes Dlar tyrosine phosphatase activity in controlling ISNb axon pathfinding[52]. Consistent with this, neuronal overexpression of DAbl also induces a bypass phenotype in ISNb. Dlar is phosphorylated by DAbl *in vitro* and binds to both Dabl and Ena through its cytoplasmic domain[52]. In addition, mutations in DAbl and in the *Drosophila* gene encoding profilin (*chickadee*) induce identical growth-cone arrest of the ISNb axon[53]. Although further work will be necessary to define the biochemical pathways involved, these observations suggest a model in which DAbl acts in the axonal growth cone of CNS and peripheral nervous system (PNS) neurons in concert with other tyrosine kinases and tyrosine phosphatases to influence axonal pathfinding and connectivity (Fig. 3a), perhaps by direct modulation of the actin cytoskeleton through the activities of Ena and profilin.

Several recent observations suggest that mammalian c-Abl might also play a role in neuronal development. A direct requirement for c-Abl in neurulation is uncovered by mutation of the related gene *arg*. *arg*$^{-/-}$ mice develop normally but display several behavioural abnormalities, suggesting defects in neuronal function. However, embryos lacking both Abl and Arg die before embryonic day 11 with gross defects in the neural tube and disorganization of the neuroepithelial actin cytoskeleton[5]. Because the Arg protein is exclusively cytoplasmic, this suggests that Abl and Arg have redundant cytoplasmic functions in the embryonic CNS, and indeed the two kinases colocalize with each other and with actin microfilaments at the apical surface of the neuroepithelium. Studies of recessive mouse mutants and human pedigrees with abnormalities of the cerebral cortex suggest that c-Abl might also function during later cortical development. The cerebral cortex of mammals has six distinct layers of neurons, generated from embryonic precursors adjacent to the lateral ventricle, followed by inside-out migration past previously formed neurons. Scrambler is a recessive mouse mutation in which homozygotes exhibit ataxia, cerebellar malformation and complete absence of cortical neuron layers. The *scrambler* gene encodes the murine homologue of Disabled (mDab1)[54] that, like its *Drosophila* counterpart, is a tyrosine-phosphorylated PTB-containing adaptor protein expressed in developing cortical neurons. Human patients with recessive X-linked lissencephaly/double cortex syndrome, characterized by defective migration of a population of cortical neurons leading to migrational arrest in the subcortical white matter, have mutations in Doublecortin, a novel brain-specific signalling protein with potential Abl phosphorylation sites[55].

Other results point to biochemical pathways downstream of c-Abl in brain development. p35 is a neuronal-specific regulatory subunit of the ubiquitously expressed Cdk5 kinase. p35-deficient mice are viable and fertile but display increased sensitivity to seizures and nearly complete inversion of the normal lamination pattern of the cerebral cortex[56]. In addition to binding and activating Cdk5 in postmitotic neurons, p35 also interacts with a novel adaptor protein called Cables (L-H. Tsai, pers. commun.) that binds to c-Abl and facilitates tyrosine phosphorylation of Cdk5 by Abl, a stimulatory event for p35/cdk5 kinase activity. Neural substrates of p35/Cdk5 include the p21$^{rac}$-activated kinase PAK1 – where phosphorylation of PAK1 by cdk5 inhibits PAK1 kinase activity[57] and might modulate the actin cytoskeleton. Mammalian cortical neuron migration is clearly a very complex process, and, for the most part, biochemical connections between these various gene products have not been established. However, the observations to date suggest a signalling model (Fig. 3b) with similarity to that proposed for *Drosophila*, where the biochemistry is on firmer ground.

## Conclusions and future prospects

Very rapid progress has been made in the past several years in understanding the complex and multifaceted biology of c-Abl. Roles for c-Abl in cell-cycle regulation, stress responses, integrin signalling and neural development are likely. Despite this progress, a single comprehensive model of Abl function is not possible at this time. In the near future, we can expect advances on several fronts. A crystal structure of mammalian c-Abl, perhaps both in an active form and complexed with an inhibitor, should allow great insight into the regulation of Abl. Careful biochemical and genetic analysis of *abl*-deficient primary cells, including cells derived from more sophisticated conditional mutants, should clarify the role of Abl in cell-cycle control and stress responses. Continued traditional and reverse-genetic approaches in flies, mice and human together with biochemical analysis of *in vitro* models of neural development will increase our understanding of the role of Abl in axonogenesis and neural migration. Finally, an additional challenge will be to apply our new knowledge of Abl function to understanding the molecular abnormalities of human Philadelphia-positive leukaemias, with the long-term goal of improving treatments for these diseases.

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Acknowledgements

I thank Li-Huei Tsai, Christopher A. Walsh, Bruce Mayer and Tony Koleske for helpful discussions, and Peter Jackson and David Van Vactor for their critical review of the manuscript. This work was supported by NIH grants CA74625 and CA77691. R. A. V. is a Scholar of the Leukemia Society of America and the Carl and Margaret Walter Scholar in Blood Research at Harvard Medical School.


# Translocation series


This year, we are featuring a short series on translocation of proteins across membranes. The series started last month with a perspective entitled 'Regulation of protein biogenesis at the endoplasmic reticulum membrane' by Ramanujan Hegde and Vishwanath Lingappa. The rest of the series will include the following articles:

Protein import into chloroplasts *by* Xuejun Chen and Danny Schnell

Type II secretion in bacteria *by* David Nunn

Getting across the nuclear pore complex *by* Mary Shannon Moore

Protein import into mitochondria *by* Michael Brunner

Transport of folded proteins across membranes *by* Daniel Klionsky

Proteasomes and ER dislocation *by* Hidde Ploegh

Targeting to peroxisomes *by* Henk Tabak