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

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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 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 relatively divergent from Abl (Fig. 1). The c-abl gene has been conserved fairly well throughout metazoan evolution, and recognizable orthologues exist in 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.2 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 and neurons, 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.
c-Abl has three nuclear-localization signals (NLSs), comprising short basic sequences in the C-terminal domain. 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. 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. 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.

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. Also, the subcellular location of all transforming Abl proteins is exclusively cytoplasmic by immunofluorescence analysis, 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, implying a membrane function in transformation of adherent cells.
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 and one encoding a truncated Abl protein with intact kinase activity. Interestingly, both knockout alleles resulted in the same phenotype: abl−/− mice are born runt, have shortened survival and exhibit abnormal eyes, frequent rectal prolapse and defective spermatogenesis. 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 haematopoietic system of lethally irradiated syngeneic haematolymphoid system of lethally irradiated lymphopoenia observed in some cases. The survival and lymphopoietic or haematopoietic defects accounts for the decreased lymphoid cell number. The survival and lymphopoietic 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.

Regulation of c-Abl kinase activity

Like c-Src, wild-type c-abl protein does not transform fibroblasts or haematopoietic cells, even when overexpressed, 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 and is disrupted upon loss of binding of Src SH2 to the SH3 domain and the linker proline site because the amino-terminal Abl SH3 domain binds to the linker region between the SH2 and kinase domains in an atypical interaction similar to c-Src. However, unlike Src, c-Abl and SH3-mutated Abl have identical in vitro tyrosine kinase activity, suggesting that the SH2 domain might repress Abl kinase activity in an intramolecular fashion. 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 vivo, 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, resulting in elevated tyrosine phosphorylation of c-Abl and other proteins and cellular transformation. Mutation of a proline equivalent to Src Pro2533 in the linker region between the Abl SH2 and kinase domains activates transformation by c-Abl, 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, suggesting that the in vivo effect of SH3 mutation might be due to loss of binding of a cellular inhibitor. 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 and several of the known Abl SH3-binding proteins (Table 1) are candidate inhibitors. Abi-122 and Abi-223 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Identification and functional significance to Abl</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3BP1</td>
<td>Rho-GAP homology; significance to Abi unknown</td>
<td>21</td>
</tr>
<tr>
<td>3BP2</td>
<td>SH2 domain; significance to Abi unknown</td>
<td>58</td>
</tr>
<tr>
<td>Abi-1</td>
<td>SH3 and homeo-like domains; truncated form inhibits v-Abl transformation</td>
<td>22</td>
</tr>
<tr>
<td>Abi-2</td>
<td>SH3 and homeo-like domains; truncated form induces transformation by c-Abl</td>
<td>23</td>
</tr>
<tr>
<td>Aap1</td>
<td>Abl SH2–SH3 binding protein; inhibits c-Abl kinase activity in vitro</td>
<td>24</td>
</tr>
<tr>
<td>Mena</td>
<td>Murine Ena, VASP-related, regulates cytoskeleton; significance to Abi unknown</td>
<td>59</td>
</tr>
<tr>
<td>Pag/MSF23</td>
<td>Periarterial family member, inhibits c-Abl kinase and cystostatic activity in vivo</td>
<td>25</td>
</tr>
<tr>
<td>Abt</td>
<td>Nuclear sensor kinase; phosphorylates and activates Abl kinase activity after IR</td>
<td>34, 35</td>
</tr>
<tr>
<td>e381.hsh3bp1</td>
<td>SH3 domain, Abi-1 homologue, binds to epil and spectrin; significance to Abl unknown</td>
<td>60, 61</td>
</tr>
</tbody>
</table>

Abbreviations: GAP, GTase-activating protein; IR, ionizing radiation; SH2, Src-homology 2; SH3, Src-homology 3; VASP, vasodilator-associated phosphoprotein.
inhibits Abl kinase activity in vitro, but not in vivo role has been described. 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. 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\(^\text{16}\). 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 abolizes Rb-dependent growth arrest in Rb-deficient Saos-2 cells\(^\text{17}\). 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 SH1 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\(^\text{18,19}\). 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\(^\text{20-29}\). 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)\(^\text{28}\) or treatment of cells with antisense oligodeoxynucleotides directed against abl\(^\text{20}\) 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 detected by tyrosine phosphorylated. c-Abl might interact directly with p53 and weakly stimulate p53 transcription activity\(^\text{20}\), 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\(^\text{30}\), 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\(^\text{31}\). 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, whereas the first domain is dispensable. This appears to be the case because ablation of the first domain in ablation of the second domain results in DNA-binding activity of the remaining domains. However, the functional significance of this DNA-binding activity is unclear. Interestingly, the DNA-binding activity of c-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\(^\text{32}\)). 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\(^\text{33}\), 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 sis fibroblasts, while mutation of a candidate ATM phosphorylation site (Ser465) in the Abl kinase domain (Fig. 1) also abrogates IR-induced activation of Abl kinase activity\(^\text{34}\). In addition, the antioxidant activity of a candidate inhibitor of c-Abl, Pag/MSP23\(^\text{35}\), 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 Abl, contributing to activation of Abl kinase activity (Fig. 2).

Upon activation, c-Abl phosphorylates several nuclear substrates, including DNA-binding protein 1 (DBP1), Rb, SH3 domain-containing tyrosine phosphatase (SHPTP1)\(^\text{36}\), and the p85 subunit of phosphoinositide

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3-kinase<sup>40</sup>, 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)<sup>33</sup> 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<sup>+/−</sup> 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 candidate in the G1–S checkpoint response to IR in abl<sup>−/−</sup> fibroblasts has been reported<sup>48</sup>, but subsequent studies from several groups failed to detect such a defect<sup>44</sup>. 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<sup>−/−</sup> fibroblasts in response to high doses of IR<sup>40</sup>. The interactions of Abl with DNA-PK and Rad51 are provocative, but abl<sup>−/−</sup> cells do not have defined defects in double-strand DNA break repair and in fact appear to be somewhat radioresistant relative to abl<sup>+/−</sup> cells<sup>45</sup>. Therefore, the available biochemical and genetic evidence suggests that c-Abl is involved in multiple pathways activated by genotoxic and possibly oxidative stress<sup>43</sup>, regulated at several levels by protein–protein interactions and by phosphorylation (Fig. 2). Cells lacking Abl or both Abl and the related c-Src<sup>1</sup> 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.

### 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<sup>9</sup>, 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 reattached onto fibronectin, c-Abl is recruited to focal adhesions and Abl kinase activity increases transiently<sup>11</sup>. 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<sup>44</sup>, 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 Src<sup>10</sup>. Extracts from abl<sup>−/−</sup> fibroblasts have significantly reduced c-Crk kinase activity, implicating c-Abl as the major regulator of Crk in vitro<sup>47</sup>. 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 DAbi 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\(^{1-/-}\) 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 axons not observed in single mutants\(^{49}\), implying that DAbl and Fas I function in parallel, redundant...
with other tyrosine kinases and tyrosine phosphatases.

Profilin (t) has been shown to interact with Drosophila Abl and Arg in axonal growth cone activity, perhaps by direct modulation of the actin cytoskeleton through the activities of Ena and nectins. In addition, mammalian c-Abl might also play a role in neuronal development. Recent work demonstrates a balance between DAbl-mediated tyrosine phosphorylation and dephosphorylation by tyrosine phosphatases in axon guidance, perhaps by direct modulation of the actin cytoskeleton. Mammalian cortical neuron dendrites in vitro assume a normal lamina pattern of the cerebral cortex. 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 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) that, like its Drosophila counterpart, is a cytoskeleton-associated 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.

Other results point to biochemical pathways downstream of c-Abl in brain development. Act5 is a neuronal-specific regulatory subunit of the ubiquitin-specific protease activity. Neural substrates of Act5 include the p21-activated kinase (PAK1 – where phosphorylation of PAK1 by cdk5 inactivates PAK1 kinase activity 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 neuronal 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 its 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 genome- 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 c-Abl function to understanding the molecular abnormalities of human Philadelphia-positive leukemias, with the long-term goal of improving treatments for these diseases.
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