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Regulation of Abl kinases by adaptor proteins

Review Article

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Abbreviations: amino, (N); B cell adaptor for phosphoinositide 3-kinase, (BCAP); carboxyl, (C); Crk-associated substrate, (Cas); Mammalian Enabled, (Mena); tyrosine, (Y); phenylalanine, (F); proline-rich, (PR); Src homology, (SH)

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Summary

The kinase activity of c-Abl is known to be tightly regulated. Mechanisms of c-Abl kinase regulation can be classified as intramolecular or intermolecular interactions. Crystallization of the amino (N)-terminal region of c-Abl has revealed that intramolecular folding of the N terminus onto the kinase domain represses intrinsic kinase activity. With regard to intermolecular interactions, recent studies suggested that *trans*-acting molecules bind to a proline-rich (PR) regions in the carboxyl (C)-terminal portion of c-Abl and activate the kinase. In this review, we focus on c-Abl kinase activation and the mechanisms of substrate phosphorylation mediated by adaptor molecules which bind to the C-terminal PR regions of c-Abl kinase.

I. Introduction

c-abl, the proto-oncogene of the Abelson leukemia virus oncogene *v-abl*, encodes a non-receptor tyrosine kinase (Goff et al, 1980; Wang et al, 1984). Although the oncogenic forms of c-Abl, v-Abl and Bcr-Abl, possess increased and unregulated kinase activity, the activity of c-Abl is tightly regulated (Witte et al, 1980; Jackson and Baltimore, 1984). v-Abl transforms pre-B cells in mice (reviewed in Rosenberg, 1994) and Bcr-Abl causes chronic myelogenous leukemia in humans (reviewed in Wong and Witte, 2004). These observations indicate that elucidation of the mechanism underlying Abl kinase regulation is critical to understanding not only the c-Abl signal transduction pathway, but also how v-Abl and Bcr-Abl induce leukemia.

It is known that two isoforms of c-Abl (1b and 1a in human; IV and I in mouse) are expressed from the *c-abl* gene as alternative splicing variants. c-Abl 1b and IV contain a consensus signal for myristoylation. All of these isoforms contain an SH3 domain, an SH2 domain, and a kinase domain, and has a long carboxyl (C)-terminal region which consists of proline-rich (PR) regions, F- and G- actin-binding regions, and a DNA-binding region. The C-terminal region of c-Abl is not conserved in other members of the Src family. In terms of the regulation of kinase activity of c-Abl, the role of the amino (N) terminus has been extensively investigated. Shore et al, 1990 showed that deletion of the SH3 domain of c-Abl, which is replaced with Gag in v-Abl, activates c-Abl kinase, suggesting that the SH3 domain suppresses kinase activity. In addition, *trans*-acting molecules which inhibit c-Abl kinase activity do so through the SH3 domain (Pendergast et al, 1991; Mayer and Baltimore, 1994; Wen and Van Etten, 1997). Recent structural studies indicate that intramolecular folding of the N terminus onto the kinase domain inhibits the kinase activity of c-Abl (Hantschel et al, 2003; Nagar et al, 2003). Binding of myristoylated residues to the kinase domain induces a conformational change that allows accession of SH2 domain to the kinase domain, which facilitates the kinase in a closed structure. The SH3 domain contacts the SH2-kinase linker region, suggesting that the SH3 domain keeps the kinase in a closed conformation.

In terms of kinase function, an important role for the C terminus has also been suggested. *c-abl* mutant mice (Schwartzberg et al, 1991) lacking C-terminal proportion

but retaining the SH3, the SH2, and the kinase domains exhibit the same phenotype as *c-abl* knockout mice (Tybulewicz et al, 1991). In fact, adaptor molecules such as c-Crk (Feller et al, 1994; Ren et al, 1994), Nck (Ren et al, 1994; Smith et al, 1999), Abi-1 (Shi et al, 1995), and Cbl (Shishido et al, 2000) have been found to interact with the PR regions of the C terminus, resulting in activation of the kinase. Woodring et al, 2001 showed that F-actin binding to the F-actin-binding region of c-Abl inhibits kinase activity, but expression of c-Crk activates the inhibited kinase (Shishido et al, 2001; Woodring et al, 2005). The mechanisms of c-Abl kinase activation by adaptor molecules remain unclear, but their binding to the PR regions is most likely to facilitate the structural changes in c-Abl which lead to efficient phosphorylation of its substrates.

II. c-Crk adaptor protein activates c-Abl

The structural requirements of an adaptor molecule for c-Abl kinase activation have been studied in the case of c-Crk and v-Crk (Shishido et al, 2001). c-Crk consists of an SH2 domain, an N-terminal SH3 (SH3(N)) domain, and a C-terminal SH3 (SH3(C)) domain; a linker between SH3(N) and SH3(C) domains contains tyrosine Y221, which is phosphorylated by c-Abl (Feller et al, 1994). v-Crk consists of the viral Gag protein, an SH2 domain and an SH3(N) domain; the SH3(C) domain and Y221 of c-Crk are deleted in v-Crk (Reichman et al, 1992). While the expression of v-Crk induces activation of c-Abl kinase, with a dramatic increase in cellular tyrosine phosphorylation, expression of c-Crk results in phosphorylation of c-Crk itself, with only a low level of cellular tyrosine phosphorylation by c-Abl kinase (Shishido et al, 2001). c-Crk has been shown to form a closed structure as a result of intramolecular interaction between the SH2 domain and the phosphorylated tyrosine Y221 (Rosen et al, 1995). Since it lacks Y221, it is possible that v-Crk has a constitutively open structure which is responsible for the activation of c-Abl. These structural differences between c-Crk and v-Crk may explain the differences in their ability to activate c-Abl kinase. Indeed, a tyrosine phosphorylation-defective mutant of c-Crk (Y221F) activates c-Abl and induces cellular tyrosine phosphorylation to the same extent as does v-Crk, indicating that the phosphorylation of Y221 suppresses the ability of c-Crk to activate c-Abl kinase (Shishido et al, 2001). Thus, a structural transition in c-Crk appears to regulate c-Abl kinase.

The activation of c-Abl kinase by c-Crk occurs in discrete steps (Shishido et al, 2001). The initial and essential event is the interaction between the SH3(N) domain of c-Crk and one of the PR motifs of c-Abl. The SH3(N) domain of c-Crk is also essential for the phosphorylation of c-Crk by c-Abl. The SH2 and SH3(C) domains are not essential for phosphorylation of c-Crk, but they are required for phosphorylation of other cellular proteins by c-Abl. One of the suggested mechanisms for substrate phosphorylation is a processive phosphorylation caused by a feedback loop (Mayer et al, 1995). Crk-associated substrate (Cas) is an example of the substrates: c-Crk binds via its SH2 domain to phospho-tyrosine-containing Cas, thereby presenting Cas as a better substrate for c-Abl (Mayer et al, 1995). c-Crk is also phosphorylated by c-Abl, causing c-Crk to form a closed structure (Rosen et al, 1995) as a result of which c-Crk dissociates from Cas (Kain et al, 2003) and probably from c-Abl. It is unknown how the activated c-Abl kinase is turned off.

III. Abi-1 adaptor protein bridges the kinase and its substrates

Abi-1 was first identified as a c-Abl-binding protein that suppresses *v-abl* transforming activity (Shi et al, 1995). Abi-1 contains a coiled-coil region, PR sequences, an SH3 domain and a polyproline structure. *Drosophila* Abi has been shown to activate Abl, and promotes phosphorylation of Enabled (Juang and Hoffmann, 1999), which genetically interacts with Abl in *Drosophila* (Gertler et al, 1990, 1995). Our group showed that Abi-1 interacts with Mena (Mammalian Enabled) and promotes its phosphorylation by c-Abl (Tani et al, 2003), suggesting that Abi-1 presents Mena as a better substrate for c-Abl by bridging kinase and substrate. Our binding studies revealed that the polyproline structure of Abi-1 is critical for binding to Mena (Tani et al, 2003). Shi et al, 1995 reported that the SH3 domain of Abi-1 is essential for binding to c-Abl and moreover that the polyproline structure enhances the binding. The SH3 domain of c-Abl binds to the PR sequences of another c-Abl interactor, Abi-2 (Dai and Pendergast, 1995). A similar interaction between c-Abl and Abi-1 may also be important in the regulation of c-Abl kinase activation by Abi-1. Phosphorylation of Mena by c-Abl is promoted to some extent in the presence of Abi-1 mutants lacking either the SH3 domain or the polyproline structure (Tani et al, 2003). One explanation for this observation is that Abi-1 may induce conformational changes in c-Abl and/or Mena upon binding to these proteins: binding to c-Abl may activate c-Abl kinase, and binding to Mena may affect the accessibility of the phosphorylation site of Mena. In this sense, Abi-1 may not be just a scaffold for Mena and c-Abl, but also a regulator that mediates the presentation of Mena to c-Abl by binding to both substrate and kinase.

To examine whether this scenario for Abi-1 is applicable to other c-Abl substrates, we sought to identify Abi-1 interacting proteins by yeast two-hybrid screening. B cell adaptor for phosphoinositide 3-kinase (BCAP) was found to be an Abi-1-binding protein (Maruoka et al, 2005), and, as observed for Mena, Abi-1 promotes the phosphorylation of BCAP by c-Abl in cultured cells. The results show that the SH3 domain, but not the polyproline structure, of Abi-1 is critical for the interaction with BCAP (Maruoka et al, 2005). Conversely, as previously described, the polyproline structure of Abi-1 is critical for the interaction with Mena (Tani et al, 2003). A recent study by Leng et al, 2005, showed that WAVE2 is also an Abi-1-regulated substrate of c-Abl, the coiled-coil region of Abi-1 being important for the interaction with WAVE2. Furthermore, in *Drosophila*, Lin et al, 2004 showed that the N-terminal residues 7-174 of Abi-1 are essential for

binding of Abi-1 to cdc2, a novel Abi-1-mediated substrate of c-Abl. Therefore, the binding surfaces of Abi-1 for these four substrates must be different. The SH3 domain of Abi-1 interacts with c-Abl (Shi et al, 1995), and Fan et al, 2003, showed that Abi-1 forms oligomers for this function. Oligomerization of Abi-1 may generate multiple binding sites and permit its interactions with substrates such as BCAP, Mena and WAVE2, and different Abi-1 molecules within a single oligomer could bind to c-Abl kinase and substrate.

Although the precise mechanism of adaptor-regulated substrate phosphorylation remains unclear, both the adaptor-kinase and the adaptor-substrate interactions seem to be important. This idea is supported by the finding that Abi-1 also promotes v-Abl-mediated phosphorylation of BCAP (Maruoka et al, 2005). v-Abl possesses unregulated kinase activity, but this is not sufficient to phosphorylate BCAP. Abi-1 may therefore be required for v-Abl to recognize BCAP as a substrate. Alternatively, interactions between Abi-1 and BCAP may result in a structural change in BCAP, leading in turn to its effective phosphorylation by Abl kinases. The significance of phosphorylation of BCAP and Abi-1 is still unclear.

IV. Conclusion

Our current understanding of the mechanisms by which c-Abl kinase is regulated by adaptor proteins raises two questions. One is how binding of an adaptor to the PR regions of c-Abl activates the kinase. It is possible that adaptor binding affects the N-terminal structure of c-Abl, opening a previously closed structure. In this respect, the C-terminal region of c-Abl would act as a regulatory region for the kinase activity. Elucidation of the crystal structure of c-Abl will shed light on relationships between the PR regions and the kinase domain.

The other question is how binding of adaptor molecules to a substrate affects the substrate's structure. Adaptor proteins not only activate the kinase but are also involved in substrate specificity. In the cases of Mena, BCAP, WAVE2, and cdc2, Abi-1 seems to stimulate the phosphorylation of substrates, although the mechanisms underlying these events are still unclear. However, one plausible explanation is that structural alterations occur in the substrate on binding of an adaptor protein.

Clarifying the precise mechanisms of adaptor-mediated regulation of c-Abl kinase must await structural analyses of purified full-length c-Abl, adaptor, and substrate molecules.

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