v-Abl, encoded by the Abelson murine leukemia virus, is a non-receptor tyrosine kinase with potent oncoenic activity in mice (for review, see Refs. 1 and 2). A similar human oncoprotein, BCR-ABL, is critical in the pathogenesis of 95% of chronic myelogenous leukemia (CML) and 10% of acute lymphocytic leukemia (for review, see Refs. 3 and 4). The c-Abl proto-oncoprotein was identified as the normal cellular homolog of v-Abl (for review, see Ref. 2). The increased tyrosine kinase activities of v-Abl and BCR-ABL, compared with the cellular c-Abl, correlate with their transforming activities. The molecular mechanisms by which these activated tyrosine kinases cause malignant transformation have remained obscure until the last few years when there have been reports that multiple signaling pathways are activated by v-Abl and/or BCR-ABL. The intent of this review is to: 1) synthesize the current understanding of v-Abl signaling, 2) identify those signaling pathways that are critical for transformation, and 3) compare v-Abl signaling to BCR-ABL signaling, which has been reviewed elsewhere (for review, see Ref. 14).

There are several reasons for studying v-Abl. First, it is a potent transforming oncoprotein, and understanding its immediate substrates and final targets will help us understand the processes required for malignant transformation. Second, although in vitro v-Abl can transform many cell types, in vivo it only transforms pro or preB cells, the early B-lineage cells that have partially or completely rearranged their heavy chain genes (for review, see Ref. 12). The striking pro/preB cell tropism for transformation, in the absence of any evidence of pro/preB-specific viral infection, is likely to reveal regulatory pathways that are unique to the early B-lymphocyte lineage. Finally, studying v-Abl in mice provides a convenient approach to identify activities that may be common to (or shared by) v-Abl and the human oncoprotein BCR-ABL.

The v-abl oncogene in Abelson murine leukemia virus encodes a fusion protein in which a portion of retroviral Gag protein replaces the SH3 domain of c-Abl (Fig. 1) (for review, see Ref. 1) (7, 8). Removal of the SH3 domain constitutively activates the tyrosine kinase, and a myristoylation site in the Gag moiety confers localization to the inner plasma membrane; both modifications are important in the transforming activity of v-Abl (9). Infection of neonatal mice by Abelson murine leukemia virus results in rapid, 100% fatality because of pro/preB cell tumors. In vitro, v-Abl transforms pro/preB cells as well as myeloid cells and a subset of 3T3 fibroblasts (10) (for review, see Refs. 11 and 12). The human oncogene, BCR-ABL, is the result of a reciprocal chromosomal translocation in which the breakpoint cluster region (BCR) gene on chromosome 22 becomes fused to the c-abl proto-oncogene on chromosome 9. It encodes a fusion protein in which part of the SH3 domain of c-ABL is replaced by portions of the BCR protein (for review, see Refs. 3 and 4). Different forms of BCR-ABL result when different portions of BCR are included; however, all BCR-ABL proteins have tyrosine kinase activities intermediate between the weaker c-ABL and the stronger v-Abl (13). In vitro BCR-ABL expression confers growth factor independence but is fully transforming only for certain cells (for review, see Ref. 14). v-Abl and BCR-ABL share a C terminus that is unique among non-receptor kinases. It contains a nuclear localization signal, a proline-rich region capable of associating with SH3-containing proteins, a sequence-independent DNA-binding domain and an actin-binding domain (for review, see Refs. 15 and 16).

Ras-dependent Pathways Are Critical for v-Abl Activity

Data from a variety of experiments show that signaling through the GTP-binding protein p21Ras is essential for transformation by both v-Abl and BCR-ABL. Inhibition of p21Ras activity by antisense oligonucleotides to p21Ras (17), microinjection of a blocking monoclonal antibody to p21Ras (18) or expression of the catalytic domain of Ras GAP (19) all block transformation by v-Abl and BCR-ABL. Dominant negative forms of p21Ras inhibit v-Abl-dependent induction of c-Myc transcription (20), fibroblast and bone marrow cell transformation by v-Abl and BCR-ABL (19), and the anti-apoptotic effect of BCR-ABL (21). Although other Ras family proteins have been described, their role in v-Abl or BCR-ABL signaling has not been explored (for review, see Ref. 22).

How Does v-Abl Activate p21Ras?—There are multiple links from v-Abl to p21Ras. Although critical tests have not been done, it seems likely that more than one connection to p21Ras may be required to mediate full oncoenic activity of v-Abl. One clear link to p21Ras is direct binding of Shc to the SH2 domain of v-Abl, which may allow tyrosine phosphorylation of Shc by v-Abl and subsequent activation of the Ras pathway through assembly of a signaling complex with Grb2-mos (23). Another connection is binding of p21Ras to v-Abl. Binding to v-Abl causes phosphorylation of p62(dok), which then binds RasGAP, a negative regulator of p21Ras (24, 25). It is not known how phosphorylation may alter p62(dok) activity or whether a ternary complex may form in which v-Abl phosphorylates RasGAP directly, possibly inactivating RasGAP (24). p62(dok) is also phosphorylated in cells expressing BCR-ABL (25), another Dok-like protein, which interacts with v-Abl, has recently been identified and may play a similar role (2).

There is also evidence for a link between the C terminus of v-Abl and p21Ras because p21Ras complements C-terminal mutants of v-Abl for bone marrow cell transformation (26). The C termini of v-Abl and BCR-ABL are the same (Fig. 1); thus it is likely that both proteins make similar connections to p21Ras. A variety of proteins bind this region, particularly SH3-containing proteins that associate with the proline-rich region in the C terminus. Adaptor proteins Crk, Crkl, Nck, and Grb2 can bind this region (27). Of these, the Crk adapter protein may be a functionally important interactor because it is hyperphosphorylated in CML cells containing BCR-ABL (28). However, the role of Crk in BCR-ABL signaling is controversial because deletion of the CrkI-binding site in the C terminus of BCR-ABL impairs fibroblast transformation (29) but not myeloid cell growth factor independence (30) (for review, see Ref. 31).

v-Abl and BCR-ABL are proteins of the same family, with similar catalytic and regulatory domains. v-Abl and BCR-ABL may both have tyrosine kinase activities intermediate between the parental enzymes. v-Abl and BCR-ABL share a C terminus that is unique among non-receptor kinases. It contains a nuclear localization signal, a proline-rich region capable of associating with SH3-containing proteins, a sequence-independent DNA-binding domain and an actin-binding domain.
substrate of the epidermal growth factor and platelet-derived growth factor receptors, that also associates with Shc (36) and inhibits ERK activation in response to epidermal growth factor signaling.2

BCR-ABL also binds directly to Grb2 (37). Because this association involves the BCR portion of the protein, which is absent in v-Abl, this connection is unique to the human oncogene. However, BCR-ABL also connects to p21ras, the ATP-binding site in the kinase domain; Y, the main site of autophosphorylation; GAG, retroviral Gag domain; zip-zag line, myristoyl fatty acid; BCR, breakpoint cluster region sequence; PTK, protein tyrosine kinase activity; Onc, transformation and tumorigenesis ability.

The domains of v-Abl that associate with Shc, Jak, and Abi were mapped by direct studies and are indicated by red bars. The region where Crk, Crkl, Nck, and Grb2 bind to v-Abl is inferred from studies on c-Abl and BCR-ABL and is indicated by a blue bar. The region of BCR-ABL that associates with Grb2 is also shown by a blue bar.

In this system, ERK and JNK activation was also inhibited by dominant negative Rac, suggesting that these effectors are downstream of v-Abl/Rac (52). Rac is not required for v-Abl-dependent induction of c-myc transcription, which depends on p21ras and Raf (20), but Rac is necessary for activation transcription dependent on serum or 12-O-tetradecanoylphorbol-13-acetate response elements (52). Studies with dominant negative Rac also show that Rac is required for BCR-ABL-induced leukemogenesis (53). It is currently not clear whether activation of Rac by v-Abl or BCR-ABL proceeds directly or by activation of p21ras or PI3K, as has been observed in other systems (for review, see Ref. 22).

JNK is activated by both v-Abl and BCR-ABL but by different pathways. v-Abl-dependent JNK activation requires Rac (52), whereas BCR-ABL-dependent JNK activation requires p21ras (41). Phosphatases may provide another pathway to JNK activation because BCR-ABL associates with SHPT1, which regulates JNK activity (54). Dominant negative c-Jun inhibits BCR-ABL transformation, demonstrating the importance of JNK activation for BCR-ABL transformation (41).

**PI3K Is Activated by v-Abl**

v-Abl and BCR-ABL, but not c-Abl, associate with and activate PI3K (55). Interestingly, the accumulation of PI3K products phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate correlates better with v-Abl or BCR-ABL transformation than does association of Abl kinases with PI3K (55, 56). v-Abl and BCR-ABL may activate PI3K by more than one pathway because activated p21ras activates PI3K in other systems (57), and recent work suggests activation of Jak1 by v-Abl might contribute to PI3K activation (58). Inhibition of PI3K blocks proliferation of BCR-ABL-dependent cells, establishing the importance of PI3K for BCR-ABL activity (59). Akt kinase is an important effector of BCR-ABL-activated PI3K because a dominant negative mutant of Akt inhibits BCR-ABL-dependent transformation of murine bone marrow cells (60).

**v-Abl Activates Protein Kinase C**

IL-3-dependent mast cells transformed with a temperature-sensitive mutant of v-Abl revealed that v-Abl activates phospholipase C-mediated breakdown of phosphatidylinositol, generating diacylglycerol, which then activates PKC (61). The anti-apoptotic effect of v-Abl in this system was blocked by inhibiting PKC activity, indicating a functional role for PKC. Subsequent studies in the same cells show that the v-Abl/PKC pathway causes an increase in bc-X 
 mRNA, which may be responsible for the anti-apoptotic effect (62).

**v-Abl Activates Jak/STAT Pathways**

The recent discovery that STAT1, -3, -5, and -6 are constitutively activated in v-Abl-transformed proB cells (58, 63) led to the attractive model that constitutive activation of STATs by v-Abl confers cytokine independence and is critical for transformation. In normal cells, nuclear translocation of STATs occurs only in response to cytokine binding to receptor and activation of receptor-associating Janus kinases (Jaks) (for review, see Ref. 64). Evidence is accumulating to support the constitutive STAT activation model.

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3 M. Coutts, X. Zou, and K. Calame, unpublished data.
4 X. Zou and K. Calame, unpublished data.
Jak1 and Jak3 associate directly with v-Abl (58). Deletion of 200 amino acids in the DNA-binding portion of the v-Abl C terminus (Fig. 1) that are required for association with Jak1 results in a mutant v-Abl, which cannot provide cytokine-independent survival of Baf/3 pro-B lymphoblastoid cells. This provides evidence that Jak binding and STAT activation are important for v-Abl-dependent transformation (58). Furthermore, in murine tumors resulting from abl/myc retroviruses, constitutive activation of STAT3 makes the cells IL-6-independent (65). However, cytokine independence is not sufficient to cause transformation because a combination of IL-4 and IL-7 signaling cannot substitute for an active v-Abl kinase in transformed pre-B cells (66). STAT-independent paths from activated Jak5 may also be important for v-Abl activity because cytokine-dependent suppression of apoptosis (67) and induction of the anti-apoptotic gene bcl-X<sub>I</sub> (68) result from a Jak5-dependent, STAT-independent path in myeloid cells.

Contrary to v-Abl, BCR-ABL does not activate STATs by a Jak-dependent pathway. Jak kinases are not consistently activated in BCR-ABL-positive cells (69, 70), and activation of STAT5 by BCR-ABL is not blocked by dominant-negative JAK2 mutants (71). BCR-ABL does not associate with Jak5 (71) even though its C-terminal region is identical to that of v-Abl (Fig. 1). Subcellular localization and tyrosine kinase activity differ significantly between the two proteins; the inner plasma membrane localization and/or high tyrosine kinase activity of v-Abl may be critical for Jak association and activation. Nevertheless, STAT1 and STAT5 are constitutively activated in BCR-ABL lines from CML patients (72), and primary peripheral blood cells from patients with CML have constitutive activation of STATs (70). Direct association of STAT SH2 domains with phosphorylated tyrosines on BCR-ABL could mediate Jak-independent activation, but no data are available to prove this (69, 71).

**Signaling Paths Responsible for Other Effects of v-Abl and BCR-ABL Are Partially Understood**

Other downstream consequences are known to result from the action of either v-Abl or BCR-ABL, but the signaling paths leading to them are poorly understood. v-Abl has been reported to stabilize IxB, thereby blocking activation of NF-κB in preB cells (73). However, the role of NF-κB appears to be different for BCR-ABL. Inhibition of NF-κB by a non-degradable form of IxB showed that NF-κB is required for BCR-ABL-mediated tumorigenicity in nude mice and transformation of primary bone marrow cells (74). Activation of NF-κB in this system is Ras-dependent.

Both v-Abl and BCR-ABL activate proteasome-dependent degradation of specific proteins. In 3T3 fibroblasts, proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27<sup>Kip</sup> occurs when mitogen-starved or density-arrested cells enter S following v-Abl activation. In CML cells expressing BCR-ABL, proteasome-dependent degradation of the inhibitory protein Abi-2 occurs through a Ras-independent pathway (34). Degradation of p27 and Abi-2 may be induced by a common pathway, but this is not yet proven.

v-Abl and BCR-ABL affect the expression of genes that regulate apoptosis. v-Abl induces bcl-X<sub>I</sub> mRNA in pre-mast cells by a PKC-dependent pathway (62) but causes up-regulation of Bax in myeloid progenitor cells (75). v-Abl induces both Bcl-2 and Bcl-X<sub>I</sub> in preB cells (66), but it remains to be shown that this is important for transformation. BCR-ABL induces Bcl-2 mRNA in a Ras-dependent pathway, and Bcl-2 has been shown to be essential for BCR-ABL-mediated transformation (76, 77).

**Perspectives**

The numerous signaling pathways activated by v-Abl are summarized in Fig. 2. The ultimate effect, transformation or apoptosis, is likely to be determined by the relative strength of these signals in different cells. There is much left to learn. With the exception of E2F-dependent genes, few genes have been identified as functionally important final targets of the signaling pathways activated by v-Abl and BCR-ABL. Furthermore, no pathway has been completely characterized and few have been compared in different cell types. Thus, there are likely to be many more connections and many more examples of cross-talk and feedback than we currently understand. There may also be connections that vary in different types of cells. In addition, it is clear that we have little understanding of how a single protein, such as p21<sup>WAF1</sup> or E2F-1, may signal multiple downstream effectors and how the relative strength of signaling to different effectors may be determined.

v-Abl and BCR-ABL activate a remarkably similar set of signaling pathways including p21<sup>WAF1</sup>, Rac, and STATs and induction of c-myc mRNA. However, there are several significant differences. BCR-ABL does not activate ERK but v-Abl does, BCR-ABL does not associate with or activate Jak5 but v-Abl does, BCR-ABL associates directly with Grb2 but v-Abl does not, and BCR-ABL activates JNK through Ras whereas v-Abl activates JNK through Rac.

It seems likely that the transforming *versus* apoptotic activities of v-Abl and BCR-ABL result from a delicate balance between many signaling pathways. Many of these, such as induction of c-myc and other E2F-dependent genes, degradation of p27, and activation of JNK, Rac, and PI3K, lead to cell cycle progression. Other signals such as induction of Bcl-X<sub>I</sub> and Bcl-2 provide anti-apoptotic signals. Finally, it is possible that others, by inducing p19(ARF) may lead to p53-dependent cell cycle arrest or apoptosis.

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