Oncogene (2002) 21, 8629–8642

www.nature.com/onc

The molecular mechanism of chronic myelogenous leukemia and its therapeutic implications: studies in a murine model

Ruibao Ren*,[1]

[1]*Rosenstiel Basic Medical Sciences Research Center, Department of Biology, Brandeis University, Waltham, Massachusetts, USA*

Chronic myelogenous leukemia (CML) is a malignant disease resulting from the neoplastic transformation of a hematopoietic stem cell. Generation of the *BCR–ABL* fusion gene plays an essential role in causing the vast majority of CML. Clinical and laboratory studies have indicated that development of CML involves both the effects of BCR–ABL within its correct target cells and interactions of BCR–ABL target cells with the rest of the *in vivo* environment, and that the progression of the disease to blast crisis involves multiple genetic alterations. An efficient mouse bone marrow transduction and transplantation model for CML has recently been developed. This review summarizes the analysis of the roles of functional domains and downstream signaling pathways of BCR–ABL, of altered cytokine production, of interferon signaling pathways and of oncogene cooperation in the pathogenesis of CML using this murine model. The *in vivo* studies of leukemogenesis will help to advance mechanism-based therapies for CML, as well as to understand fundamental rules of leukemogenesis and hematopoiesis.
Oncogene (2002) **21,** 8629–8642. doi:10.1038/sj.onc.1206090

Keywords: CML; BCR–ABL; mouse model; signalling; oncogene cooperation

Treatment of chronic myelogenous leukemia – the rise of mechanism-based therapy

As introduced in preceding reviews in this issue, chronic myelogenous leukemia (CML) is a malignant disease resulting from the neoplastic transformation of an hematopoietic stem cell (HSC). CML develops in distinct clinical phases. The initial chronic phase is characterized by a massive expansion of the granulocytic cell lineage in all maturation stages, even though the CML stem cell can give rise to multiple blood cell lineages. Progression of the disease after 3–5 years to terminal blast phase, usually through an accelerated phase, is characterized by a block in cell differentiation, resulting in accumulation of 30% or more myeloid or lymphoid blast cells in bone marrow, peripheral blood, and/or extramedullary tissues (reviewed in Faderl *et al*., 1999).

The granulocytosis in the chronic phase of CML can be suppressed by hydroxyurea, a ribonucleotide reductase inhibitor. This conventional chemotherapy is considered to be only palliative (reviewed in Sawyers, 2001). Treatment with interferon-α (IFN-α) induces not only hematological but also cytogenetic remissions (reviewed in Talpaz, 2001). However, although this treatment can significantly prolong life in CML patients, it is not curative and has significant adverse side effects. Allogeneic bone marrow and stem cell transplantation is the only known curative therapy for CML. However, a majority of patients are not eligible for this morbid therapy due to advanced age or lack of a suitable donor (reviewed in Goldman and Druker, 2001). In all of these therapies the molecular root of CML is not specifically targeted.

Studies of the molecular mechanism of CML in past decades have led to the development of a more specific therapy for CML. CML has been found in association with chromosome translocations that generate the *BCR–ABL* fusion oncogene, which has also been found in approximately 20% of adult and 2–5% of pediatric *de novo* acute lymphoblastic leukemia (ALL) (reviewed in Melo, 1997). The discoveries that ABL is a protein tyrosine kinase, and that the ABL tyrosine kinase is constitutively activated in BCR–ABL and is essential for the transforming activity of BCR–ABL, revealed that the ABL kinase is a critical target for therapeutic intervention for BCR–ABL-positive leukemias (reviewed in O'Dwyer and Druker, 2001). Meanwhile, the discovery that pharmacological inhibitors can inhibit a specific tyrosine kinase made it possible to develop small molecule drugs for cancers in which tyrosine kinase activity plays an important role (Yaish *et al*., 1988). Recently, a tyrosine kinase inhibitor – imatinib mesylate (Gleevec, also known as STI571) – that is specific for the ABL, platelet-derived growth factor (PDGF) receptor and KIT tyrosine kinases was developed (Buchdunger *et al*., 1996; Druker *et al*., 1996; Heinrich *et al*., 2000). Preclinical experiments and clinical trials have shown that imatinib was very effective in treating CML (reviewed in Druker *et al*., 2001). As an example, a recent multi-institutional phase 2 study showed that

*Correspondence: R Ren, Rosenstiel Basic Medical Sciences Research Center, MS029, Brandeis University, 415 South Street, Waltham, MA 02454-9110 USA; E-mail: ren@brandeis.edu

greater than 90% of imatinib-treated IFN-α-resistant CML patients in chronic phase had a complete hematologic response, and more than 40% had a complete cytogenetic response (defined as 0% Philadelphia-chromosome (Ph)-positive cells in metaphase in bone marrow (Kantarjian *et al*., 2002). Like a 'smart bomb', this mechanism-based drug also causes much less 'collateral damage' (side effects) in patients. As a result, imatinib has rapidly become a new standard treatment for CML that is refractory to interferon therapy.

The long-term benefits of the imatinib-treatment for CML are not yet known. Clinical trials have shown that drug resistance developed quickly in imatinib-treated CML patients in blast phase, largely due to gene amplification or mutations of the *BCR–ABL* fusion gene (Gorre *et al*., 2001). In addition, cytogenetic relapse (defined as at least 65% Ph-positive cells in metaphase or an increase of at least 30% from the previous study) has also been seen in a small fraction of imatinib-treated IFN-α-resistant chronic phase CML patients around 12 months after the start of therapy (Kantarjian *et al*., 2002). Further study of the functions of BCR–ABL, BCR–ABL-activated signaling pathways, the pathophysiology of CML stem cells and the molecular mechanism of blast transformation of CML will help to advance mechanism-based therapies for BCR–ABL-positive leukemias.

BCR–ABL and model systems for its oncogenic activity

Different forms of BCR–ABL proteins with different molecular weights (BCR–ABL/p185, BCR–ABL/p210, and BCR–ABL/p230) can be generated in patients, depending on the precise break points and RNA splicing (Figure 1) (reviewed in Melo, 1997). The most common form, BCR–ABL/p210, is primarily associated with CML and infrequently with ALL. BCR–ABL/p185, on the other hand, is usually associated with ALL and rarely with CML. BCR–ABL/p230 appears to be a weaker oncogene, and is often associated with a less aggressive form of CML. The molecular mechanism of the preferential association of different forms of BCR–ABL with different types of leukemia is not known.

There are numerous published data that have defined functional domains and properties of BCR–ABL, and have revealed many signaling pathways and cellular functions being affected by BCR–ABL (reviewed in Deininger *et al*., 2000). The ABL-derived portion of the fusion protein contains the Src-homology-3 (SH3), SH2, and tyrosine kinase domains in its N-terminal half, and nuclear localization signals, a DNA binding domain, G- and F-actin binding domains and SH3 binding sites in its COOH-terminal region (Figure 1). The *abl* oncogene was first identified as the oncogenic element in the Abelson murine leukemia virus, which induces an acute B-lymphoblastic leukemia/lymphoma in mice (reviewed in Rosenberg and Witte, 1988). The v-*abl* oncogene was created by a recombination event that fused viral *gag* sequences to a truncated c-*abl* gene with deletion of the sequences encoding the NH_2-terminal region that plays a role in auto-regulation of the Abl kinase and the SH3 domain of c-Abl (Figure 1). It was found that deletion of the SH3 domain could activate the kinase activity and the oncogenic potential of c-Abl (Franz *et al*., 1989; Jackson and Baltimore, 1989). Deletion of the NH_2-terminal region also activates, to a much lesser extent, the kinase activity and the oncogenic potential of c-Abl (Pluk *et al*., 2002). In addition, the fusion of *gag* sequences to *abl* and accumulation of additional mutations within *abl*

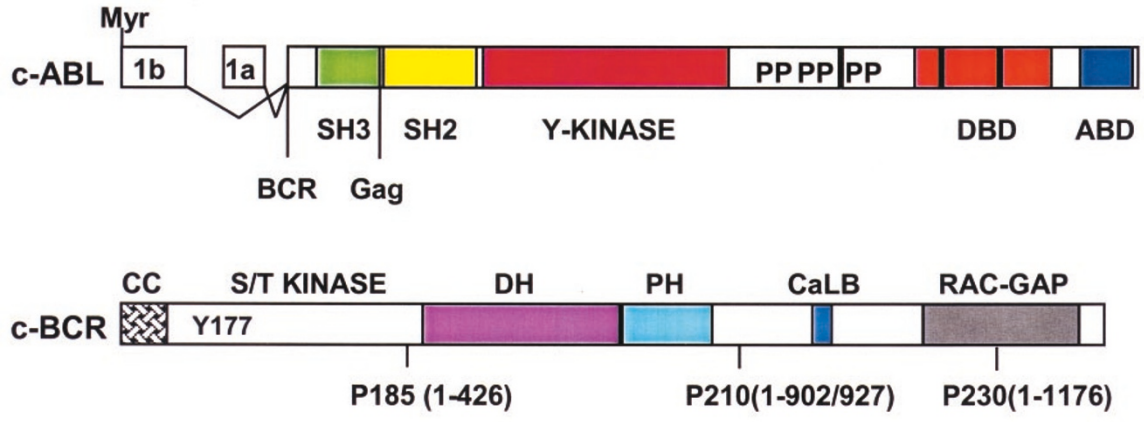


Figure 1 Schematic representation of the c-ABL and c-BCR proteins. Two isoforms of c-ABL (human types 1a and 1b) are generated by alternative splicing of the first exon. The domains of c-ABL and c-BCR are shown. The break point in c-ABL that fuses with BCR and Gag (for v-Abl) as well as different fusion points within BCR that produce the p185, p210 and p230 forms of BCR–ABL are indicated. Myr: myristoylation site; SH3 and SH2: Src-homology 3 and 2 domains; Y-kinase: protein tyrosine kinase; PP; PXXP-containing SH3 binding sites; DBD: DNA binding domain; ABD: actin binding domain; CC: Coiled-coil domain; Y177: a phosphorylation site required for GRB2–SH2 binding; S/T kinase: serine and threonine kinase; DH: Dbl-homology domain; PH: pleckstrin homology domain; CaLB: putative calcium-dependent lipid binding site; RAC–GAP: RAC guanosine triphosphatase-activating protein domain. Black bars in the COOH-terminal region of c-ABL represent nuclear localization signals

also contribute to the strong oncogenic potential of v-*abl* (Rosenberg and Witte, 1988). Studies of v-Abl and c-Abl have contributed greatly to the understanding of BCR–ABL biology and biochemistry (reviewed in Zou and Calame, 1999).

BCR is also a signaling protein (Figure 1). The BCR-derived part of BCR–ABL/p185 contains a coiled-coil (CC) oligomerization domain, a serine/threonine kinase domain, as well as binding sites for GRB2 (tyrosine 177), GRB10, 14-3-3 and the ABL SH2 domain. BCR–ABL/p210 contains in addition a Dbl/CDC24 guanine-nucleotide exchange factor homology (DH) domain and a pleckstrin homology (PH) domain, while the BCR-derived part of BCR–ABL/p230 extends further into the COOH-terminal region of BCR, including a putative calcium-dependent lipid binding site (CaLB) and part of a RAC guanosine triphosphatase-activating protein (GAP) domain. Many signaling proteins have been shown to interact with BCR–ABL through its functional domains/motifs and/or to be phosphorylated in BCR–ABL-expressing cells (reviewed in Deininger *et al*., 2000). Meanwhile, many signaling pathways have been shown to be activated by BCR–ABL. These pathways involve RAS, RAF, RAC, phosphatidylinositol 3-kinase (PI3-Kinase), AKT, JUN kinase, signal transducers and activators of transcription (STATs), c-MYC, and BCL-2 or $BCLX_L$ (reviewed in Deininger *et al*., 2000; Sawyers, 1997). In addition, BCR–ABL also disrupts the adhesion/migration pathway in CML cells and induces expression of cytokines, such as interleukin-3 (IL-3), granulocyte–macrophage colony stimulating factor (GM–CSF) and granulocyte colony stimulating factor (G-CSF) (Deininger *et al*., 2000; Jiang *et al*., 1999; Verfaillie *et al*., 1997; Zhang and Ren, 1998).

To elucidate the molecular pathogenesis of CML and to identify targets for therapeutic interventions for CML, the roles and relative importance of the domains of BCR–ABL, of BCR–ABL-activated signaling pathways and of the micro-environment of BCR–ABL targeted cells in neoplastic transformation by BCR–ABL need to be examined in biological model systems. A variety of model systems for BCR–ABL transformation with different advantages and disadvantages have been developed and used. One or more model system(s) can be chosen as relevant for a particular study, based on their advantages in addressing the experimental questions. One of the model systems measures the transforming potential of BCR–ABL in established cell lines. It has been shown that BCR–ABL is able to confer factor-independent survival and growth of established factor-dependent hematopoietic cell lines and to transform certain established fibroblast cell lines (reviewed in Deininger *et al*., 2000). The advantage of using cell lines is the relative ease of obtaining a large number of clonally derived cells for biochemical analysis, genetic manipulations and biological examinations. It is particularly important to use the same cellular context for studies that compare cellular and molecular events affected by various *ABL* oncogenes or their mutants where the oncogenes may have different oncogenic potentials in different blood lineages *in vivo*. Moreover, since the tyrosine kinase activity of BCR–ABL and many oncoproteins with an intrinsic tyrosine kinase activity is essential for their oncogenic potential, the factor-dependent hematopoietic cell lines are very useful for searching/testing specific pharmacological inhibitors of the kinases, which should restore the factor-dependence of these cell lines.

However, development of leukemia is a complex process that involves both the effects of BCR–ABL within its target cells and interactions of BCR–ABL target cells with the rest of the *in vivo* environment. Cell lines are limited in representing the physiologically relevant target cells of BCR–ABL *in vivo*. The existence of unknown genetic abnormalities in established cell lines might also obscure the function of the gene under study. Indeed, equivocal results have been obtained by using cell lines in assessing the role of certain functional domains or motifs, such as the GRB2–SH2 binding site at Y177 in BCR and the ABL SH2 domain, in transformation by BCR–ABL (reviewed in Ghaffari *et al*., 1999). To overcome the limitations of established cell lines, primary cells isolated from mouse or human have also been used to study BCR–ABL transformation. It has been shown that BCR–ABL is able to stimulate cytokine-independent growth of primary bone marrow cells and to stimulate growth of hematopoietic cells differentiated from embryonic stem (ES) cells (Era and Witte, 2000; Kelliher *et al*., 1993; McLaughlin *et al*., 1987). These systems can overcome some disadvantages of the established cell lines but they are still limited in representing *in vivo* biology. Effective laboratory animal models of CML are eventually needed to study the *in vivo* pathogenesis of CML and to identify targets critical for BCR–ABL leukemogenesis.

Several approaches have been used to develop *in vivo* models for CML, including methods that introduce the *BCR–ABL oncogene* into the laboratory mouse genome and methods that engraft human CML cells in immunodeficient mice (reviewed in Ren, 2002; Van Etten, 2001; Wong and Witte, 2001). Generating mouse models for CML by targeting the *BCR–ABL* oncogene into mouse cells is important for assessing roles of both oncogenic target cells and their environments in leukemogenesis. Since CML is a stem cell disease, it is critical to model the disease by targeting the *BCR–ABL* oncogene into multipotential hematopoietic stem/progenitor cells and expressing it in these cells and their progenies, similar to the way *BCR–ABL* is generated and expressed in CML patients. Three methods are generally used for targeting oncogenes into mice – transgenic, knock-in and retroviral transduction. All these approaches have been used for investigating BCR–ABL leukemogenesis (reviewed in Ren, 2002; Van Etten, 2001; Wong and Witte, 2001).

Most transgenic and knock-in mice generated thus far model BCR–ABL-positive hematopoietic neoplasms other than CML. It has been shown that

conventional transgenic and knock-in mice with expression of BCR–ABL driven by the c-*bcr* promoter caused embryonic lethality due to the toxicity of this activated tyrosine kinase during embryonic development (Castellanos *et al*., 1997; Heisterkamp *et al*., 1991). Transgenic mice with expression of BCR–ABL driven by inducible promoters, such as metallothionein promoter and tetracycline-off inducible promoter, primarily developed B- or T-ALL (Heisterkamp *et al*., 1990; Honda *et al*., 1995; Huettner *et al*., 2000; Voncken *et al*., 1992, 1995). Transgenic founder mice with expression of BCR–ABL driven by a *tec* hematopoietic specific promoter also developed T-ALL promoter (Honda *et al*., 1998). It is interesting that the progeny of one of the *tec/BCR–ABL* founders developed a mild granulocyte hyperplasia with a thrombocytosis after a greatly extended latency (about a year), but it is not clear whether this system effectively models CML. Although these mouse models help to understand the molecular mechanisms of BCR–ABL-positive neoplasms in general, models closely resembling CML are needed for understanding the specific molecular pathogenesis of myeloid neoplasm.

Expression of BCR–ABL in mouse bone marrow cells by retroviral transduction and bone marrow transplantation methods, on the other hand, was shown to induce a myeloproliferative disorder (MPD) closely resembling CML (Daley *et al*., 1990; Elefanty *et al*., 1990; Kelliher *et al*., 1990). However, in these earlier bone marrow transduction and transplantation models, mice that received *BCR–ABL/p210*-transduced bone marrow cells frequently developed other hematopoietic neoplasms, such as B-ALL and macrophage tumors. More importantly, the low efficiency of induction of MPD and poor reproducibility in these earlier models hindered the usefulness of this method to study the biology of BCR–ABL in CML.

Recently, we and others have shown that expression of BCR–ABL in mouse bone marrow cells rapidly induced an MPD resembling the chronic phase of human CML with 100% efficiency (Zhang and Ren, 1998; Pear *et al*., 1998; Li *et al*., 1999). Common features of the disease include high peripheral blood counts (typically several hundred thousand per microliter) with granulocyte predominance, splenomegaly, extramedullary hematopoiesis in liver and pulmonary hemorrhages owing to extensive granulocyte infiltration in the lung. In addition, the disease can be readily transplanted to secondary recipient mice. These results demonstrate that expression of BCR–ABL can induce a CML-like MPD in mice much more efficiently and reproducibly than in previous models, probably due to efficient expression of BCR–ABL in the correct target cell(s). Improvements in retroviral production methods and bone marrow transduction protocols played important roles in the successful establishment of the efficient mouse model for CML (Pear *et al*., 1993; Zhang and Ren, 1998). In addition, the ability of MSCV to target gene expression in hematopoietic stem/progenitor cells makes the induction of CML-like disease more effective (Hawley, 1994).

The key features of the MPD in this bone marrow transduction and transplantation model – targeting the multipotential hematopoietic stem/progenitor cells and selectively expanding the granulocyte lineage – resemble closely the chronic phase of human CML. Also consistent with the clinical observations of human CML, the model demonstrates that BCR–ABL alone is not sufficient to induce the myeloblastic leukemia seen in a majority of CML cases in blast phase. However, differences exist between this model and human CML. MPD in the mouse model develops very fast (usually in three weeks) and is fatal. Pulmonary hemorrhages, which rarely occur in human CML, are consistently observed in the mouse model and appear to be the main cause of death in diseased mice. The aggressiveness of the disease could be due to overexpression of BCR–ABL from the retroviral promoter. Modification of the retroviral vector to lower the expression of BCR–ABL may improve the model system.

The bone marrow transduction and transplantation system has its advantages as well as limitations, as discussed elsewhere (reviewed in Ren, 2002; Van Etten, 2001). In summary this system has the following advantages. (1) It is an efficient *in vivo* assay for structure-function analysis of an oncogene. (2) It can also be a more efficient approach for assessing the oncogenic potential of an oncogene in different genetic backgrounds, including in different transgenic and knockout strains that affect targets of an oncoprotein. Although in theory it is easy to cross-mate a transgenic and knock-in mouse disease model to other strains of mice for investigating the role of other genes in the oncogenic pathway, it often involves lengthy breeding to match the genetic backgrounds between the strains prior to the cross-mating experiments. (3) It mimics the somatic mutation that occurs in oncogenesis and affects only a limited number of hematopoietic cells. This more precise introduction of an oncogene into target cells can avoid the potential toxicity of an oncogene during development and should model clonal oncogenesis more closely, compared to the wider expression of an oncogene in most transgenic and knock-in mice. (4) It can assess the function of an oncogene in various sorted hematopoietic cell types, which should facilitate the identification of the target cell(s) of an oncogene. (5) The unique proviral integration site in individual targeted cells facilitates analysis of the clonality and transplantability of the neoplastic disease.

The bone marrow transduction and transplantation model also has its disadvantages. The first is the variability of the system. BCR–ABL has been shown capable of transforming many types of hematopoietic cells. The types of cells that BCR–ABL is targeted into can naturally influence the disease outcomes. Results may vary among different laboratories, because each diseased mouse needs to be generated *de novo* and the types and quantities of cells targeted by *BCR–ABL*

are likely influenced by virus titers, transduction protocols and retroviral constructs. Indeed, in early bone marrow transduction and transplantation systems, BCR–ABL was found to induce a wide variety of hematological neoplasms, including B-ALL, pre-B and T-cell lymphoma, reticulum cell sarcoma, erythroid tumors, myelomonocytic leukemia and MPD (Daley *et al*., 1990; Elefanty and Cory, 1992; Elefanty *et al*., 1990; Gishizky *et al*., 1993; Kelliher *et al*., 1990). As described below, for some mutants of BCR–ABL, different phenotypes were reported from different laboratories, possibly due to different viral titers and transduction protocols used. Standardization of the experimental procedures should help to avoid this problem. More importantly, studying the mechanism underlying various disease phenotypes caused by using various experimental protocols will help to understand the mechanism of leukemogenesis and to improve the model system. The second disadvantage is the immunosuppressive nature of the system. Since the recipient mice have to be irradiated, the influence of the host's immunity on the development of leukemia and potential immuno-therapy cannot be fully assessed.

Despite its limitations, the mouse bone marrow transduction and transplantation system is by far the most efficient, reproducible, and accurate laboratory animal model for BCR–ABL-induced CML. This *in vivo* model system for measuring BCR–ABL oncogenic potential has been, and will continue to be, useful for delineating the molecular mechanisms by which the *BCR–ABL* oncogene acts in the pathogenesis of CML and for testing CML therapies. Related studies using this model system are summarized below.

Roles of the functional domains of BCR–ABL

The success of imatinib in treating CML has proved that the BCR–ABL fusion protein itself is an effective target for developing therapeutic interventions for CML. Besides the ABL kinase domain, there are other domains/motifs in BCR–ABL that play important roles in regulating the ABL kinase and/or connecting downstream signaling pathways. Defining the roles and relative importance of various domains of BCR–ABL in leukemogenesis *in vivo* should help to understand the molecular mechanisms underlying the phenotypes of CML, and thus to identify targets both within BCR–ABL and downstream of BCR–ABL for developing therapeutic interventions for CML complementary to imatinib. This can be accomplished by a comprehensive structure-function analysis of BCR–ABL using the mouse model for CML described above.

Activation of the ABL tyrosine kinase activity is essential but not sufficient to induce MPD in mice

After the discovery that wild type (wt) BCR–ABL efficiently induced a CML-like MPD in mice, we examined the leukemogenicity of a BCR–ABL mutant with a point mutation (changing the lysine residue at the ATP binding site of the ABL kinase to arginine) that inactivates the ABL kinase. We found that the ABL kinase-deficient BCR–ABL mutant was incapable of inducing any disease in mice despite being targeted into the long-term repopulating hematopoietic cells, demonstrating that the ABL kinase activity is absolutely essential for BCR–ABL leukemogenesis *in vivo* (Zhang and Ren, 1998). This result reconfirmed that the ABL kinase is a crucial target for therapeutic interventions for CML.

In a test of CML therapy using the mouse bone marrow transduction and transplantation model, it was found that imatinib induces hematological response and prolongs life of mice receiving *BCR–ABL*-infected bone marrow cells (Wolff and Ilaria, 2001). However, none of the imatinib-treated mice were cured of the CML-like MPD. Overall, imatinib-treatment was less effective in the mouse model for CML compared to its treatment of CML patients in chronic phase. This might be attributed to overexpression of BCR–ABL in the current model system. In addition, it is not clear that the concentration of imatinib in mice could reach the same level as in patients.

Although the ABL kinase activity plays an essential role in BCR–ABL leukemogenesis, we found that c-Abl activated by an SH3 deletion, which has an elevated tyrosine kinase activity and is capable of transforming both fibroblast and hematopoietic cell lines *in vitro*, could only induce lymphoid leukemia/lymphomas with a greatly extended latency (Gross *et al*., 1999). Even v-Abl, which has a stronger oncogenic potential than the SH3-deleted c-Abl, was shown to have a much lower capacity for stimulating myeloid cell growth both *in vitro* and *in vivo* and to induce primarily a B-ALL in mice under the same conditions in which BCR–ABL induced a CML-like MPD (Gross and Ren, 2000). These results indicate that activation of the ABL kinase alone is not sufficient to induce the CML-like MPD and that other functional domains/motifs, particularly those in BCR, may play important roles in the induction of MPD by BCR–ABL. These functional domains/motifs and the signaling pathways that they link to might also be effective pharmacological targets for CML. The results also demonstrated that the type of hematopoietic neoplasm induced by an *ABL* oncogene is not only influenced by what types of hematopoietic cells the oncogene is targeted into, but also by the intrinsic oncogenic properties of the particular *ABL* oncogene. Further identifying genetic determinant(s) in BCR–ABL and v-Abl that control the lineage specific transformation using the mouse model should help to understand the mechanisms in the pathogenesis of myeloid *vs* lymphoid neoplasms.

The inability of the SH3-deleted c-Abl and v-Abl to induce MPD is not simply due to a loss of the SH3 domain function, since BCR–ABL with a deletion of the SH3 domain still effectively induced a fatal MPD (Gross *et al*., 1999). The latter experiment indicates that the function of the ABL SH3 domain is either redundant or unnecessary for BCR–ABL leukemogenesis. This conclusion is consistent with the fact that

BCR–ABL/p210 lacking *ABL* exon 2, which encodes a large part of the ABL SH3 domain, has been found in some CML and ALL patients (reviewed in Melo, 1997).

The NH_2-terminal coiled-coil domain of BCR is necessary for induction of MPD by BCR–ABL in mice

It has been shown that the NH_2-terminal coiled-coil (CC) domain of BCR plays an important role in activating the ABL kinase and enhancing the association of BCR–ABL with actin fibers and that deletion of the BCR–CC domain abolished the transforming ability of BCR–ABL in cultured cells (McWhirter *et al*., 1993). We further examined the role of the CC domain of BCR–ABL in the mouse CML model. We found that BCR–ABL without the BCR–CC domain (ΔCC–BCR–ABL) had a greatly reduced tyrosine kinase activity and failed to induce MPD in mice. However, ΔCC–BCR–ABL still induced a T-cell leukemia/lymphoma with a greatly extended latency (Zhang *et al*., 2001a; He *et al*., 2002). The long incubation time for the development of T-cell tumors suggests that secondary mutations may be involved. Reactivation of the ABL kinase by deleting the ABL SH3 domain rescued the ability of ΔCC–BCR–ABL to induce the CML-like MPD in mice, albeit with a lesser efficiency (Zhang *et al*., 2001a). These results indicate that the BCR–CC domain is essential for the induction of MPD by BCR–ABL in mice, mainly due to its ability to activate the ABL kinase. The *in vivo* analysis reconfirmed that BCR–CC domain is another target for developing pharmacological inhibitors of BCR–ABL. Inhibitors that interfere with the oligomerization of BCR–ABL may cooperate with imatinib in treating CML. The three-dimensional structure of the BCR–CC domain has recently been solved (Zhao *et al*., 2002). It showed that two N-shaped CC domain monomers dimerize by swapping N-terminal helices and by forming an antiparallel coiled-coil between C-terminal helices, and two dimers then stack onto each other to form a tetramer. This structural information should help to design new pharmacological inhibitors for BCR–ABL.

It is interesting that ΔCC–BCR–ABL is still capable of inducing T-cell malignancies in mice. The low oncogenic activity may be attributed to a low level of tyrosine kinase activity retained in ΔCC–BCR–ABL. Besides oligomerization mediated by the BCR–CC domain, other mechanism(s) must exist in BCR–ABL to activate, to a lesser extent, the ABL kinase. Deletion of the first exon of *c-ABL* in the process of the chromosome translocation may account for at least part of this weak kinase activation (Pluk *et al*., 2002).

The Grb2 SH2 binding site, Y177, is required for an efficient induction of MPD by BCR–ABL

Since the main function of the CC domain of BCR was to activate the ABL kinase, BCR may contain other functional domains/motifs that are important for specific induction of MPD by BCR–ABL. Another important motif in the BCR region is the GRB2 SH2 binding site, which contains the phosphorylated tyrosine 177 (Pendergast *et al*., 1993). It is believed that GRB2 binding leads to activation of the RAS pathway. The importance of Y177 in BCR–ABL transformation assayed *in vitro* was shown to be cell context- and assay-dependent (Cortez *et al*., 1995; Goga *et al*., 1995; Pendergast *et al*., 1993). We and others have investigated the role of Y177 in BCR–ABL leukemogenesis in mice (Zhang *et al*., 2001a; Million and Van Etten, 2000; He *et al*., 2002). We found that BCR–ABL/p210 with a point mutation at Y177 (changing the tyrosine residue to phenylalanine, Y177F) initially induced a mild and non-fatal MPD in mice after a long latency. These mice eventually developed T-cell leukemia/abdominal lymphoma. These results suggest that Y177 plays an important role in efficient induction of MPD by BCR–ABL.

Consistent with an important, yet non-essential, role of Y177 in induction of an MPD by BCR–ABL, a fusion of the NH_2-terminal CC domain of BCR directly to the ABL part of BCR–ABL (designated as CC–ABL) was found capable of inducing a non-fatal MPD in mice with a long latency (Zhang *et al*., 2001a). Like BCR–ABL/Y177 mice, most CC–ABL mice eventually developed a fatal T-cell leukemia/lymphoma. Interestingly, although CC–ABL and BCR–ABL/Y177F induced T-cell malignancies, the anatomical distribution of BCR–ABL/Y177F- and CC–ABL-induced T-cell tumors was drastically different. While CC–ABL-tumor cells spread mostly into peripheral blood and thymus, BCR–ABL/Y177F-tumor cells had a strong preference to locate in abdominal mesenteric lymph nodes. These results suggest that signaling by BCR sequences other than the CC domain and Y177 affect the homing of the neoplastic T-lymphoid cells.

Dr Pear's and Dr Van Etten's groups also showed that BCR–ABL/Y177F induced a massive abdominal T-cell malignancy with a long disease latency (He *et al*., 2002; Million and Van Etten, 2000). However, a transient MPD was virtually not seen by these groups. Differences in phenotypes observed by our laboratories may be due to different viral titers and transduction protocols. Standardizing the experimental conditions should improve the comparability of experiments using the bone marrow transduction and transplantation model. Nevertheless, all results demonstrate that mutation of Y177 greatly attenuates the ability of BCR–ABL to induce CML-like MPD.

BCR sequences play a role in determining the specificity of disease induced by BCR–ABL

In an experiment assessing the role of a region in BCR that binds GRB10, 14-3-3, the ABL–SH2 domain and potentially other proteins in induction of MPD in mice by BCR–ABL, we found that a BCR–ABL mutant that contains the NH_2-terminal 222 amino acids of BCR still induced a fatal disease with a similar latency

as the disease caused by wt BCR–ABL (Perera and Ren, unpublished). However, hematopathologic analysis revealed that BCR^{1-222}-ABL mice succumb to a mixed MPD and B-ALL. A similar finding was also made by Dr Pear's group (He *et al*., 2002). Southern blot analysis showed that many lymphoid and myeloid cells had the same cell origin (Perera and Ren, unpublished). These results suggest that the first 222 amino acids of BCR, which include both the CC domain and the GRB2–SH2 binding site, is sufficient to activate ABL to induce MPD and that the amino acids 222–426 region of BCR may negatively regulate BCR–ABL's ability to promote B-lymphoproliferation when it is targeted into hematopoietic stem/progenitor cells. Taken together, these data suggest that BCR sequences play a role in determining the lineage specificity of BCR–ABL-induced neoplasia. Further identifying the sequences in BCR that affect the lineage specificity of BCR–ABL-induced disease and the signaling pathway(s) linked to such determinant(s) will provide insights into the mechanism underlying the BCR–ABL-positive leukemias, as well as insights into the regulation of lineage determination/selection in hematopoiesis.

Change of disease phenotype by the fusion partner of ABL was also shown in a comparison of leukemogenesis by BCR–ABL *vs* TEL–ABL, the product of t(9;12)(q34;p13) translocation found in rare cases of human leukemias including atypical CML (Golub *et al*., 1996; Janssen *et al*., 1995). TEL is a member of the Ets-family transcription factors. A common property found between BCR and TEL, two unrelated proteins, is the formation of oligomers. We found that a TEL–ABL fusion gene (containing the first four exons of TEL) induced a MPD characterized by much less accumulation of granulocytes in peripheral blood and spleen and by a severe bowel disease, owing to a massive infiltration of myeloid cells in intestine (Perera and Ren, unpublished). These results suggest that TEL and BCR sequences have different properties in directing the distribution of neoplastic myeloid cells in mice. The significance of these differences between BCR–ABL and TEL–ABL in corresponding human diseases is not known.

Naturally occurring isoforms of BCR–ABL all induce MPD in mice

As introduced earlier, various BCR–ABL isoforms including p185, p210, and p230 show a preferential association with different types of leukemia. However all three forms of BCR–ABL were found capable of inducing CML-like MPD in mice when targeted into same pool of bone marrow cells from 5-FU-treated donor mice (Li *et al*., 1999 and Zhang and Ren unpublished). Compared to BCR–ABL/p210-induced disease in mice, the disease latency is consistently shorter for BCR–ABL/p185 mice and longer for BCR–ABL/p230 mice under the same experimental conditions. All three isoforms could also induce B-ALL in mice with much greater differences in disease latency when bone marrow cells from non-5-FU-treated donors were used in the model system (Li *et al*., 1999). These experiments indicate that all BCR–ABL isoforms can induce the same disease under the condition in current model system and that additional BCR sequences in BCR–ABL/p210 and BCR–ABL/p230 modulate the leukemogenic potential of BCR–ABL. The mechanism of preferential association of different isoforms of BCR–ABL with different types of leukemia in patients remains unknown.

The SH2 domain affects the phenotype and latency of BCR–ABL-induced disease

Besides the ABL kinase domain and BCR sequences, other domains/motifs of ABL also play roles in transformation by the *ABL* oncogenes. The SH2 domain is a modular unit present in many signaling proteins (Pawson, 1995). Its main function is to mediate specific protein-protein interactions in signaling pathways by binding phosphotyrosine-containing peptides. It has been shown that the ABL SH2 domain is required for transformation of fibroblast cells but not for transformation of hematopoietic cells by BCR–ABL (Afar *et al*., 1994; Cortez *et al*., 1995; Goga *et al*., 1995; Ilaria and Van Etten, 1995; Oda *et al*., 1995). We and others have investigated the function of the ABL SH2 domain in the induction of MPD in mice by BCR–ABL (Zhang *et al*., 2001b; Roumiantsev *et al*., 2001). We found that both the SH2 point mutant (changing the arginine residue in the phosphotyrosine-binding pocket of the ABL SH2 domain to lysine) and the SH2 deletion mutant of BCR–ABL retained the ability to induce a fatal MPD, but with an extended latency compared to wt BCR–ABL (Zhang *et al*., 2001b). Interestingly, in contrast to wt BCR–ABL-induced disease, which is rapid and monophasic, the disease caused by the BCR–ABL SH2 mutants is biphasic, consisting of an initial B-lymphoproliferative disorder followed by a fatal MPD. Dr Van Etten's group also found that the SH2 domain mutations attenuate the BCR–ABL's oncogenic potential, but they showed that the SH2 mutants of BCR–ABL induced mainly B-ALL with a longer disease latency (Roumiantsev *et al*., 2001). As discussed earlier, the different phenotypes observed might be due to different viral titers and transduction protocols, which appears to affect targeting of various hematopoietic cells (reviewed in Ren, 2002). Standardizing the experimental conditions and sorting out the target cells from which BCR–ABL induces distinct diseases may improve the model system.

It is interesting that the SH2 mutant of BCR–ABL induced expansion of both immature and mature B-lymphocytes, unlike wt BCR–ABL-induced B-ALL under the condition of using bone marrow cells from non-5-FU treated donor or under the condition of low retroviral titers using bone marrow cells from 5-FU-treated donor (Hao and Ren, 2000; Zhang *et al*., 2001b). This result suggests that the ABL SH2 domain may play a role in blocking differentiation of B cells.

Since the point mutation in the SH2 domain of BCR–ABL does not affect the ABL kinase activity, attenuation of the signaling pathways linked through the ABL SH2 domain is likely responsible for the phenotypes of the SH2 mutants of BCR–ABL. In addition, the disappearance of the B-lymphoproliferative disorder upon development of MPD in the BCR–ABL SH2 mutant mice suggests that expansion of myeloid cells may suppress lymphopoiesis. Indeed we found that the B-lymphocyte expansion was diminished in experiments that mixed *BCR–ABL*/Δ*SH2*- and wt *BCR–ABL*-infected bone marrow cells (Zhang *et al*., 2001b). This mechanism might explain the lack of expansion of Ph-positive B lymphoid cells in CML patients.

In summary, the structure/function analysis of BCR–ABL using the mouse bone marrow transduction and transplantation model for CML demonstrated the importance of delineating the molecular mechanism of CML *in vivo*. The *in vivo* model is particularly important in revealing the different oncogenic potentials of the *ABL* oncogenes toward different blood lineages. These studies demonstrated that activation of the ABL tyrosine-kinase activity is essential but not sufficient to induce CML-like disease, and that domains/motifs of BCR–ABL outside of the ABL kinase catalytic site play important roles in determining both the efficiency of BCR–ABL in inducing diseases (e.g. latency and severity of disease) and the specificities of the disease induced (e.g. myeloid versus lymphoid neoplasia). A comparison of the oncogenic potential of various forms and mutants of the *ABL* oncogenes assayed by different *in vitro* and *in vivo* model systems is presented in Figure 2. Further structure/function analysis of the *ABL* oncogenes using the *in vivo* leukemia model will continue to provide insights into the molecular mechanisms involved in the pathogenesis of CML and will contribute to our understanding of the role of signal transduction pathways in regulating the development of blood lineages.

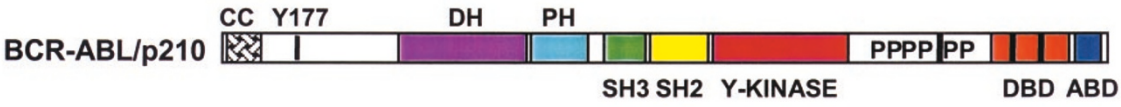
Roles of BCR–ABL-activated signaling pathways in the pathogenesis of the CML-like MPD

The role of BCR–ABL-activated signaling proteins in the pathogenesis of MPD in mice has also been examined using the bone marrow transduction and transplantation model for CML. It has been shown that STAT5 is constitutively activated in BCR–ABL-expressing cells and a dominant negative STAT5 inhibited the growth of K562 and BCR–ABL-expressing 32D cells (Carlesso *et al*., 1996; Frank and Varticovski, 1996; Ilaria and Van Etten, 1996; de Groot, 1999; Nieborowska-Skorska *et al*., 1999; Shuai *et al*., 1996; Sillaber *et al*., 2000). Gene targeting experiments have shown that the two related Stat5 proteins, Stat5a and Stat5b, are not essential for the overall organismal development (Teglund *et al*., 1998). However, Stat5a/b play a role in myelopoiesis and erythropoiesis in non-steady state conditions, such as during the rapid expansion of cells under stress/pathological conditions or during embryonic development (Kieslinger *et al*., 2000; Socolovsky *et al*., 1999). They also contribute to IL-7-induced expansion of B-cell precursors (Sexl *et al*., 2000).

The role of STAT5 in BCR–ABL leukemogenesis was further examined using Stat5a/b-deficient mice by a mouse bone marrow transduction and transplantation approach (Sexl *et al*., 2000). It was shown that expression of BCR–ABL in bone marrow cells from non-5-FU-treated Stat5a/b-deficient mice was still capable of inducing a fatal MPD. However, BCR–ABL induced more B-ALL in Stat5a/b-deficient mice than that in wt mice, suggesting that the ability of BCR–ABL to induce MPD was attenuated in the Stat5a/b-deficient mice. These experiments indicate that Stat5a/b are either not essential or redundant in induction of MPD by BCR–ABL, but they seem to contribute to the efficient induction of MPD by BCR–ABL. This experiment further demonstrates that BCR–ABL induces MPD via multiple signaling pathways and there is a great deal of redundancy among these pathways. Further assessment of the roles of other downstream signaling proteins is needed to understand the molecular mechanism underlying the pathogenesis of CML.

Autocrine and paracrine effects in the pathogenesis of CML

Coexpression of the green fluorescent protein (GFP) with BCR–ABL in the current bone marrow transduction and transplantation model for CML has greatly facilitated the analysis of BCR–ABL leukemogenesis (Zhang and Ren, 1998; Pear *et al*., 1998). We found that in mice with the BCR–ABL-induced MPD, both *BCR–ABL*-infected and non-infected myeloid cells were massively expanded (Zhang and Ren, 1998). This bystander effect suggests that excess growth factor(s) may be produced in BCR–ABL mice and that such growth factor(s) may play an important role in autocrine and/or paracrine stimulation of growth of myeloid cells in CML. Indeed we have found that in mice with BCR–ABL-induced CML-like disease, *BCR–ABL*-infected cells expressed excess IL-3 and GM-CSF. In CML patients, increased serum levels of GM-CSF and increased gene expression of IL-3 in CML primitive hematopoietic progenitor cells have also been reported (el-Ahmady *et al*., 1997; Jiang *et al*., 1999; Jonuleit *et al*., 1998). It was shown that the factor-independent growth of human primitive CML progenitor cells requires the autologous production of IL-3 (Jiang *et al*., 1999). However, it was found that BCR–ABL induced a similar CML-like MPD in IL-3- and GM-CSF-deficient mice as that in wt mice (Li *et al*., 2001 and Zhang and Ren, unpublished). These results demonstrated that IL-3 and GM-CSF were not required for BCR–ABL to induce MPD in mice. Interestingly, we found that massive expansion of the bystander myeloid cells still occurred in IL-3- and GM-



Oncogenic potentials of the *ABL* oncogenes and *BCR-ABL* mutants

	Fibroblast cell lines	Hematopoietic cell lines	In mice
wt	+	+	MPD
kinase$^-$	-	-	-
ΔSH3	+	+	MPD
ΔSH3-c-Abl	+	+	B/T leukemia/lymphoma (L.L.)
v-Abl	+	+	B-ALL
ΔCC	-	+	T leukemia/lymphoma (L.L.)
ΔCC/ΔSH3	-	+	MPD (L.L.)
Y177F	-	+	MPD (L.L., non-fatal) → T leukemia/lymphoma (L.L.)
CC-ABL	-	+	MPD (L.L., non-fatal) → T leukemia/lymphoma (L.L.)
BCR$^{1-222}$-ABL	+	+	MPD + B-ALL
SH2 mut	-	+	LPD → MPD (L.L.)

Figure 2 Summary of structure-function analysis of the *ABL* oncogenes and *BCR–ABL* mutants in transformation both *in vitro* (transformation of fibroblast cell lines and inducing factor-independent growth of hematopoietic cell lines) and in mice. Domains/motifs of BCR–ABL/p210 are shown (abbreviations are the same as that shown in Figure 1). The transformation potentials of the *ABL* oncogenes and *BCR–ABL* mutants in cell lines are indicated qualitatively only. The *in vivo* experiments were done under the conditions (using bone marrow cells from 5-FU-treated donor mice) where wild-type BCR–ABL/p210 (wt) induces a fatal MPD. Kinase: kinase-deficient BCR–ABL/p210; ΔSH3: SH3-deleted BCR–ABL/p210; ΔCC: Coiled-coil domain-deleted BCR–ABL/p210; ΔCC/ΔSH3: Coiled-coil domain-deleted and SH3-deleted BCR–ABL/p210; CC-ABL: Coiled-coil domain of BCR fused directly to the ABL part of BCR–ABL; Y177F: Y177F mutant of BCR–ABL/p210; SH2 mut: SH2 point or deletion mutant of BCR–ABL/p210; MPD: myeloproliferative disorder; L.L.: long latency; B-ALL, acute B-lymphoblastic leukemia; LPD: lymphoproliferative disorder. Arrows represent transitions from an intial phase disease to a late phase disease as indicated before and after the arrows. Data on BCR–ABL/p185 and BCR–ABL/p230, which also induce a fatal MPD in mice, are not shown

CSF-deficient mice with BCR–ABL-induced MPD (Zhang and Ren, unpublished). These results suggest that BCR–ABL may induce overproduction of other cytokines and/or dysregulate the microenvironment in hematopoietic organs. Further identification of the host factor(s) causing the expansion of bystander myeloid cells in BCR–ABL mice is important to understand the pathogenesis of CML and may help to develop novel CML therapies.

Molecular mechanism of IFN-α treatment of CML

IFN-α induces hematological response in a majority of CML patients but induces complete cytogenetic remission only in a minority of patients (reviewed in Bonifazi *et al*., 2001). Elucidating the molecular mechanisms of IFN-α treatment and identifying the specific mediators of the IFN-α response in treating CML is important for developing improved therapies for CML. IFNs are a family of multi-functional cytokines that play important roles in induction of antiviral activities, inhibition of cell growth, induction of cell differentiation, and immunomodulation (reviewed in Pfeffer *et al*., 1998). It is likely that IFN-α suppresses CML through multiple mechanisms. It has been shown that IFN-α may inhibit CML progenitors by restoring their normal adhesion to bone marrow stroma and by down-regulating BCR–ABL transcription (Bhatia and Verfaillie, 1998; Pane *et al*., 1999). More recent data indicate that IFN-α also intervenes in the development of CML through activating/inducing transcription factors that regulate myeloid cell growth and anti-tumor immunity.

IFNs induce a group of transcriptional factors called interferon regulatory factors (IRFs) (reviewed in Harada *et al*., 1998). The IRF protein family includes IRF-1, IRF-2, IRF-3, IRF-4/ICSAT/Pip, IRF-5, IRF-6 and IRF-7, ISGF-3γ (Interferon Stimulated Gene Factor-3γ), and ICSBP (Interferon Consensus Sequence

Binding Protein) (reviewed in Nguyen *et al*., 1998). Genetic analyses in mice showed that IRF-1, IRF-2 and IRF4 play important roles in lymphopoiesis (Matsuyama *et al*., 1993; Mittrucker *et al*., 1997). ICSBP, a ~50 kDa protein expressed predominantly in hematopoietic cells, on the other hand, plays an important role in regulating immune responses and myelopoiesis – ICSBP-deficient mice exhibit enhanced susceptibility to viral and intracellular parasite infections, possibly due to impaired IL-12 production, and manifest a CML-like syndrome (Holtschke *et al*., 1996). It has been shown that IFN-α can induce *ICSBP* gene expression *in vivo* (Schmidt *et al*., 1998). To assess the direct anti-tumor potential of ICSBP, we examined the effect of ICSBP on BCR–ABL-induced CML-like disease in mice (Hao and Ren, 2000). We found that expression of the ICSBP protein was significantly decreased in mice with BCR–ABL-induced CML-like disease. Forced coexpression of ICSBP inhibited the BCR–ABL-induced colony-formation of bone marrow cells from 5-FU-treated mice *in vitro* and BCR–ABL-induced CML-like disease *in vivo*. These data provide direct evidence that ICSBP can act as a tumor suppressor to inhibit the growth of neoplastic cells. Interestingly, coexpression of ICSBP and BCR–ABL induced a transient B-lymphoproliferative disorder in the mouse model for BCR–ABL-induced CML-like disease. Consistently, overexpression of ICSBP slightly promotes, rather than inhibits, BCR–ABL-induced B-lymphoproliferation in a mouse model where bone marrow cells from non-5-FU-treated donors were used. These latter results indicate that ICSBP has a specific anti-tumor activity toward myeloid neoplasms.

The idea that ICSBP is an important mediator of IFN-α treatment is also supported by studies of CML patients. It was found that *ICSBP* transcripts were decreased in CML patients and that this reduction of *ICSBP* transcripts could be reversed by IFN-α treatment (Schmidt *et al*., 1998). In addition, ICSBP expression levels were found to correlate with the therapeutic response of CML to IFN-α (Schmidt *et al*., 2001). The precise mechanism by which ICSBP specifically regulates myeloid cell growth is not known. But it has been shown that ICSBP modulates survival of myeloid cells through regulating apoptosis-related genes (Gabriele *et al*., 1999). It was also found that ICSBP promotes the terminal differentiation of macrophages but represses granulocyte differentiation in bipotential myeloid progenitor cells (Tamura *et al*., 2000).

In addition to a direct role in regulating growth and/or survival of myeloid cells, ICSBP may also mediate IFN-α treatment by regulating anti-tumor immunity. It was shown that BCR–ABL-transformed BaF3 (an IL-3 dependent myeloid progenitor cell line) cells with enforced expression of ICSBP stimulated a protective CD8-positive cytotoxic T-cell response specifically against the parental transformed cells in mice (Deng and Daley, 2001). However, this effect of ICSBP was limited to BCR–ABL-transformed BaF3 cells in syngeneic Balb/c mice, since ICSBP expression was unable to stimulate the same protective immunity against BCR–ABL-transformed 32D (another IL-3 dependent myeloid progenitor cell line) in syngeneic C3H/HeJ mice. Nevertheless, this study raised the possibility that IFN-α may stimulate an anti-BCR–ABL immunity through ICSBP.

STAT1 and STAT2 play important roles in transducing IFNs' signals (Bromberg and Darnell, 2000). STAT1-deficient mice show no overt developmental abnormalities, but display a complete lack of responsiveness to IFN-α and IFN-γ (Meraz *et al*., 1996). It has been shown that CML cells resistant to IFN-α lack STAT1 expression, suggesting that STAT1 may be a determinant for clinical response to IFN-α in certain CML patients (Landolfo *et al*., 2000). Further evaluating the role of IFN-α signaling proteins and responsive genes in mediating IFN-α treatment of CML using mouse models for CML and may help to elucidate the mechanism of IFN-α treatment of CML and to identify targets for developing new CML therapies.

Oncogene cooperation in blast transformation of CML

The molecular mechanism of the blast transformation of CML is poorly understood. It is clear that BCR–ABL alone does not block differentiation of myeloid cells. Blast transformation of CML must involve acquisition of additional genetic abnormalities (reviewed in Faderl *et al*., 1999). Some of the genetic changes found in CML patients in blast phase involve mutations in p53, RB, and $p16^{INK4A}$, overexpression of EVI1 and c-MYC, and/or generation of fusion genes such as *AML1/MDS1/EVI1* (resulting from the t(3;21)(q26;q22) translocation), *AML1/ETO* (resulting from the t(8;21)(q22;q22) translocation), *NUP98–HOXA9* (resulting from the t(7;11)(p15;p15) translocation) and *CBFβ/SMMHC* [resulting from inv(16)(p13;q22)] (reviewed in Ahuja *et al*., 2001; Colovic *et al*., 1998; Deininger *et al*., 2000; Faderl *et al*., 1999). Investigating the role of these mutant genes in leukemogenesis and the potential cooperation between BCR–ABL and these secondary mutations is critical for understanding the molecular mechanism of CML blast transformation.

In an earlier mouse bone marrow transduction and transplantation model, B-lymphoblastic leukemia developed in secondary transfer mice (Daley *et al*., 1991). However, the efficiency of this earlier model system to develop MPD was very low, which hindered its use in the study of blast transformation of CML. In the current mouse bone marrow transduction and transplantation model, BCR–ABL induces MPD in 100% of recipient mice. However, BCR–ABL mice die quickly with MPD and no blast transformation was observed in the primary recipient mice. Serial transfer experiments have been performed to examine the transplantability of BCR–ABL-induced MPD and to examine whether the tumor cells could undergo blast

transformation. It was shown that a clonal T-cell leukemia/lymphoma can be developed from the BCR–ABL induced MPD (Pear *et al*., 1998). However, T-cell leukemia/lymphoma is rarely observed in human CML. Therefore, this system does not satisfactorily model the blast transformation of CML under current experimental conditions. The same problem exists in a recently described transgenic mouse model for the blast crisis of human CML (Honda *et al*., 2000). It was shown in this transgenic model that acquired loss of p53 in BCR–ABL-expressing hematopoietic cells induced a T-cell leukemia/lymphoma. The mechanism of the high susceptibility of mouse T cells to a leukemogenesis is not known.

Another way to study the blast transformation of CML is to examine whether BCR–ABL can cooperate in leukemogenesis with genes that are altered in CML patients during the blast phase. To this end, we tested whether BCR–ABL and the AML1/MDS1/EVI1 fusion protein cooperate to induce acute myelogenous leukemia using the mouse bone marrow transduction and transplantation model. We first examined the leukemogenic potential of AML1/MDS1/EVI1 and found that AML1/MDS1/EVI1 can induce an AML in mice but with a greatly extended latency (Cuenco *et al*., 2000). The disease latency suggests that additional mutations are required for induction of AML by AML1/MDS1/EVI1.

It has been shown that the CBF subunits RUNX1/AML1 and CBFβ are essential for definitive hematopoiesis and AML1/ETO and CBFβ/SMMHC fusion proteins can function as dominant negative alleles of normal CBF subunits (Castilla *et al*., 1996; Niki *et al*., 1997; Okuda *et al*., 1996, 1998; Wang *et al*., 1996; Yergeau *et al*., 1997). In cell culture models, AML1-containing fusion genes have been shown to block myeloid cell differentiation and to immortalize myeloid progenitors (Sood *et al*., 1999; Tanaka *et al*., 1995; Westendorf *et al*., 1998). These results have led to a generally held belief that AML-associated transcription factor fusion proteins block hematopoietic differentiation. However, disrupting function of CBF seems to block hematopoiesis at the level of HSC precursors. The effect of CBF mutations in HSC and in hematopoiesis thereafter was not clear.

In an analysis of the direct effect of AML1/MDS1/EVI1 on hematopoiesis during the 4-month preleukemia stage, we found that expression of AML1/MDS1/EVI1 alone in mouse bone marrow cells did not block myeloid differentiation *in vivo* (Cuenco and Ren, 2001). Likewise, expression of CBFβ/SMMHC in mouse bone marrow cells did not block myeloid differentiation (Cuenco and Ren, unpublished). These results suggest that some, if not all, AML-associated fusion proteins are not sufficient to block myeloid cell differentiation by themselves. Interestingly we found that co-expression of BCR–ABL and AML1/MDS1/EVI1 in mice rapidly induced an AML, suggesting that blocking myeloid differentiation and inducing AML involves cooperation between mutations that dysregulate protein tyrosine kinase signaling and mutations that disrupt hematopoietic gene transcription (Cuenco and Ren, 2001). We also found that coexpression of CBFβ/SMMHC with BCR–ABL was not sufficient to induce AML in mice (Cuenco and Ren, unpublished). These results suggest that AML1/MDS1/EVI1 has a stronger oncogenic potential than CBFβ/SMMHC and that blocking myeloid differentiation and developing full-blown myelogenous leukemia, in many cases, involves cooperation of multiple genetic abnormalities.

A differentiation block leading to the accumulation of immature hematopoietic cells characterizes both AML and the blast phase of CML. Development of both of these malignancies may require accumulation of genetic abnormalities that disrupt regulation of cell proliferation, apoptosis and differentiation. The different initiating mutational event – one with activating signaling proteins and the other with disruption of transcription factors – may underlie the different clinical phenotypes observed. CML manifests a myeloproliferative disorder in chronic phase as a result of expressing an activated protein tyrosine kinase, while the initial AML-associated genetic abnormalities may be more latent or sometimes induce a myelodysplasia prior to the development of a full-blown AML. Despite this, the mechanism of multi-step development of both diseases may involve cooperation between transcription and signaling types of oncogenes as well as tumor suppressor genes. Indeed, in addition to mutated transcription factors, disruption of signal transduction pathways has also been found in AML. For example, activating point mutations of *RAS* have been found in approximately 25% of cases of AML (Ahuja *et al*., 1990; Neubauer *et al*., 1994). In addition, about 30% of AML cases have an activating internal tandem duplication mutation or point mutation of the FLT3 protein tyrosine kinase receptor (Griffin, 2001). Further analysis of the mechanism of the cooperation between BCR–ABL and AML1/MDS1/EVI1 should provide insights into the molecular mechanism of blast transformation of CML as well as the general mechanism in the pathogenesis of AML.

Conclusions

The mouse bone marrow transduction and transplantation model for CML has enabled us to study the molecular pathogenesis of CML in a pathophysiologically relevant *in vivo* system. Continuing to use this model system to identify BCR–ABL signaling pathways and target genes that are important for BCR–ABL leukemogenesis, and to study the cooperation in malignant transformation between BCR–ABL and mutated genes found in CML patients during blast phase will provide new insights into the molecular mechanisms of CML as well as AML, and will help to further design rational therapeutic interventions for myelogenous leukemias. Since development of CML involves disruption of regulation in stem cell biology,

blood lineage determination and/or selection, as well as hematopoietic cell proliferation, survival, differentiation, adhesion and migration, the *in vivo* analysis of the molecular mechanism of CML will also help to uncover fundamental rules governing normal hematopoiesis.

Acknowledgments
I thank Alec W Gross and Ramesh Subrahmanyam for their critical reading of this review. This work was supported by National Cancer Institute grant CA68008 and by ACS Grant RSG-02-059-01-LIB. R Ren is a recipient of The Leukemia and Lymphoma Society Scholar Award.

References

Afar DEH, Goga A, McLaughlin J, Witte ON and Sawyers CL. (1994). *Science,* **264,** 424–426.
Ahuja HG, Foti A, Bar-Eli M and Cline MJ. (1990). *Blood,* **75,** 1684–1690.
Ahuja HG, Popplewell L, Tcheurekdjian L and Slovak ML. (2001). *Genes Chromosomes Cancer,* **30,** 410–415.
Bhatia R and Verfaillie CM. (1998). *Leuk. Lymphoma,* **28,** 241–254.
Bonifazi F, de Vivo A, Rosti G, Guilhot F, Guilhot J, Trabacchi E, Hehlmann R, Hochhaus A, Shepherd PC, Steegmann JL, Kluin-Nelemans HC, Thaler J, Simonsson B, Louwagie A, Reiffers J, Mahon FX, Montefusco E, Alimena G, Hasford J, Richards S, Saglio G, Testoni N, Martinelli G, Tura S and Baccarani M. (2001). *Blood,* **98,** 3074–3081.
Bromberg J and Darnell Jr JE. (2000). *Oncogene,* **19,** 2468–2473.
Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Druker BJ and Lydon NB. (1996). *Cancer Res.,* **56,** 100–104.
Carlesso N, Frank DA and Griffin JD. (1996). *J. Exp. Med.,* **183,** 811–820.
Castellanos A, Pintado B, Weruaga E, Arevalo R, Lopez A, Orfao A and Sanchez-Garcia I. (1997). *Blood,* **90,** 2168–2174.
Castilla LH, Wijmenga C, Wang Q, Stacy T, Speck NA, Eckhaus M, Marin-Padilla M, Collins FS, Wynshaw-Boris A and Liu PP. (1996). *Cell,* **87,** 687–696.
Colovic M, Jankovic G, Bila J, Djordjevic V and Wiernik PH. (1998). *Med. Oncol.,* **15,** 199–201.
Cortez D, Kadlec L and Pendergast AM. (1995). *Mol. Cell. Biol.,* **15,** 5531–5541.
Cuenco GM, Nucifora G and Ren R. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 1760–1765.
Cuenco GM and Ren R. (2001). *Oncogene,* **20,** 8236–8248.
Daley GQ, Van Etten RA and Baltimore D. (1990). *Science,* **247,** 824–830.
Daley GQ, Van Etten RA and Baltimore D. (1991). *Proc. Natl. Acad. Sci. USA,* **88,** 11335–11338.
de Groot RP, Raaijmakers JA, Lammers JW, Jove R and Koenderman L. (1999). *Blood,* **94,** 1108–1112.
Deininger MW, Goldman JM and Melo JV. (2000). *Blood,* **96,** 3343–3356.
Deng M and Daley GQ. (2001). *Blood,* **97,** 3491–3497.
Druker BJ, Sawyers CL, Capdeville R, Ford JM, Baccarani M and Goldman JM. (2001). *Hematology (Am. Soc. Hematol. Educ. Program)* 87–112.
Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J and Lydon NB. (1996). *Nat. Med.,* **2,** 561–566.
el-Ahmady O, Mansour M, Kamel H and Baker A. (1997). *Anticancer Res.,* **17,** 3179–3182.
Elefanty AG and Cory S. (1992). *Mol. Cell. Biol.,* **12,** 1755–1763.
Elefanty AG, Hariharan IK and Cory S. (1990). *EMBO J.,* **9,** 1069–1078.
Era T and Witte ON. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 1737–1742.
Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R and Kantarjian HM. (1999). *N. Engl. J. Med.,* **341,** 164–172.
Frank DA and Varticovski L. (1996). *Leukemia,* **10,** 1724–1730.
Franz WM, Berger P and Wang JYJ. (1989). *EMBO J.,* **8,** 137–147.
Gabriele L, Phung J, Fukumoto J, Segal D, Wang IM, Giannakakou P, Giese NA, Ozato K and Morse III HC. (1999). *J. Exp. Med.,* **190,** 411–421.
Ghaffari S, Daley GQ and Lodish HF. (1999). *Leukemia,* **13,** 1200–1206.
Gishizky ML, Johnson-White J and Witte ON. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 3755–3759.
Goga A, McLaughlin J, Afar DE, Saffran DC and Witte ON. (1995). *Cell,* **82,** 981–988.
Goldman JM and Druker BJ. (2001). *Blood,* **98,** 2039–2042.
Golub TR, Goga A, Barker GF, Afar DE, McLaughlin J, Bohlander SK, Rowley JD, Witte ON and Gilliland DG. (1996). *Mol. Cell. Biol.,* **16,** 4107–4116.
Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN and Sawyers CL. (2001). *Science,* **293,** 876–880.
Griffin JD. (2001). *Blood,* **97,** A2193.
Gross AW and Ren R. (2000). *Oncogene,* **19,** 6286–6296.
Gross AW, Zhang X and Ren R. (1999). *Mol. Cell. Biol.,* **19,** 6918–6928.
Hao SX and Ren R. (2000). *Mol. Cell. Biol.,* **20,** 1149–1161.
Harada H, Taniguchi T and Tanaka N. (1998). *Biochimie,* **80,** 641–650.
Hawley RG. (1994). *Ann. NY Acad. Sci.,* **716,** 327–330.
He Y, Wertheim JA, Xu L, Miller JP, Karnell FG, Choi JK, Ren R and Pear WS. (2002). *Blood,* **99,** 2957–2968.
Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA and Zigler AJ. (2000). *Blood,* **96,** 925–932.
Heisterkamp N, Jenster G, Kioussis D, Pattengale PK and Groffen J. (1991). *Transgenic Research,* **1,** 45–53.
Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK and Groffen J. (1990). *Nature,* **344,** 251–253.
Holtschke T, Löhler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, Lou J, Knobeloch K-P, Gabriele L, Waring JF, Bachmann MF, Zinkernagel RM, Morse 3rd HC, Ozato K and Horak I. (1996). *Cell,* **87,** 307–317.
Honda H, Fujii T, Takatoku M, Mano H, Witte ON, Yazaki Y and Hirai H. (1995). *Blood,* **85,** 2853–2861.
Honda H, Oda H, Suzuki T, Takahashi T, Witte ON, Ozawa K, Ishikawa T, Yazaki Y and Hirai H. (1998). *Blood,* **91,** 2067–2075.
Honda H, Ushijima T, Wakazono K, Oda H, Tanaka Y, Aizawa S, Ishikawa T, Yazaki Y and Hirai H. (2000). *Blood,* **95,** 1144–1150.
Huettner CS, Zhang P, Van Etten RA and Tenen DG. (2000). *Nature Genetics,* **24,** 57–60.
Ilaria Jr RL and Van Etten RA. (1995). *Blood,* **86,** 3897–3904.

Ilaria Jr RL and Van Etten RA. (1996). *J. Biol. Chem.,* **271,** 31704 – 31710.
Jackson P and Baltimore D. (1989). *EMBO J.,* **8,** 449 – 456.
Janssen JW, Ridge SA, Papadopoulos P, Cotter F, Ludwig WD, Fonatsch C, Rieder H, Ostertag W, Bartram CR and Wiedemann LM. (1995). *Br. J. Haematol.,* **90,** 222 – 224.
Jiang X, Lopez A, Holyoake T, Eaves A and Eaves C. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 12804 – 12809.
Jonuleit T, Peschel C, Schwab R, van der Kuip H, Buchdunger E, Fischer T, Huber C and Aulitzky WE. (1998). *Br. J. Haematol.,* **100,** 295 – 303.
Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M and Druker B. (2002). *N. Engl. J. Med.,* **346,** 645 – 652.
Kelliher MA, McLaughlin J, Witte ON and Rosenberg N. (1990). *Proc. Natl. Acad. Sci. USA,* **87,** 6649 – 6653.
Kelliher MA, Weckstein DJ, Knott AG, Wortis HH and Rosenberg N. (1993). *Oncogene,* **8,** 1249 – 1256.
Kieslinger M, Woldman I, Moriggl R, Hofmann J, Marine JC, Ihle JN, Beug H and Decker T. (2000). *Genes Dev.,* **14,** 232 – 244.
Landolfo S, Guarini A, Riera L, Gariglio M, Gribaudo C, Cignetti A, Cordone I, Montefusco E, Mandelli F and Foa R. (2000). *Hematol. J.,* **1,** 7 – 14.
Li S, Gillessen S, Tomasson MH, Dranoff G, Gilliland DG and Van Etten RA. (2001). *Blood,* **97,** 1442 – 1450.
Li S, Ilaria RL, Million RP, Daley GQ and Van Etten RA. (1999). *J. Exp. Med.,* **189,** 1399 – 1412.
Matsuyama T, Kimura T, Kitagawa M, Pfeffer K, Kawakami T, Watanabe N, Kundig TM, Amakawa R, Kishihara K and Wakeham A. (1993). *Cell,* **75,** 83 – 97.
McLaughlin J, Chianese E and Witte ON. (1987). *Proc. Natl. Acad. Sci. USA,* **84,** 6558 – 6562.
McWhirter JR, Gaalasso DL and Wang JYJ. (1993). *Mol. Cell. Biol.,* **13,** 7587 – 7595.
Melo JV. (1997). *Baillieres Clin. Haematol.,* **10,** 203 – 222.
Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Aguet M and Schreiber RD. (1996). *Cell,* **84,** 431 – 442.
Million RP and Van Etten RA. (2000). *Blood,* **96,** 664 – 670.
Mittrucker HW, Matsuyama T, Grossman A, Kundig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS and Mak TW. (1997). *Science,* **275,** 540 – 543.
Neubauer A, Dodge RK, George SL, Davey FR, Silver RT, Schiffer CA, Mayer RJ, Ball ED, Wurster-Hill D, Bloomfield CD and Liu ET. (1994). *Blood,* **83,** 1603 – 1611.
Nguyen H, Hiscott J and Pitha PM. (1998). *Cytokine Growth Factor Rev.,* **8,** 293 – 312.
Nieborowska-Skorska M, Wasik MA, Slupianek A, Salomoni P, Kitamura T, Calabretta B and Skorski T. (1999). *J. Exp. Med.,* **189,** 1229 – 1242.
Niki M, Okada H, Takano H, Kuno J, Tani K, Hibino H, Asano S, Ito Y, Satake M and Noda T. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 5697 – 5702.
O'Dwyer ME and Druker BJ. (2001). *J. Intern. Med.,* **250,** 3 – 9.
Oda T, Tamura S, Matsuguchi T, Griffin JD and Druker BJ. (1995). *Leukemia,* **9,** 295 – 301.
Okuda T, Cai Z, Yang S, Lenny N, Lyu CJ, van Deursen JM, Harada H and Downing JR. (1998). *Blood,* **91,** 3134 – 3143.
Okuda T, van Deursen J, Hiebert SW, Grosveld G and Downing JR. (1996). *Cell,* **84,** 321 – 330.
Pane F, Mostarda I, Selleri C, Salzano R, Raiola AM, Luciano L, Saglio G, Rotoli B and Salvatore F. (1999). *Blood,* **94,** 2200 – 2207.
Pawson T. (1995). *Nature,* **373,** 573 – 580.
Pear WS, Nolan GP, Scott ML and Baltimore D. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 8392 – 8396.
Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML and Baltimore D. (1998). *Blood,* **92,** 3780 – 3792.
Pendergast AM, Quilliam LA, Cripe LD, Bassing CH, Dai Z, Li N, Batzer A, Rabun KM, Der CJ, Schlessinger J and Gishizky ML. (1993). *Cell,* **75,** 175 – 185.
Pfeffer LM, Dinarello CA, Herberman RB, William BRG, Borden EC, Bordens R, Walter MR, Nagabhushan TL, Trotta PP and Pestka S. (1998). *Cancer Res.,* **58,** 2489 – 2499.
Pluk H, Dorey K and Superti-Furga G. (2002). *Cell,* **108,** 247 – 259.
Ren R. (2002). *Leukemia and Lymphoma,* **8,** 1549 – 1561.
Rosenberg N and Witte ON. (1988). *Advances in virus research* Vol. 35. New York: Academic Press, Inc, pp 39 – 81.
Roumiantsev S, de Aos IE, Varticovski L, Ilaria RL and Van Etten RA. (2001). *Blood,* **97,** 4 – 13.
Sawyers CL. (1997). *Baillieres Clin. Haematol.,* **10,** 223 – 231.
Sawyers CL. (2001). *Semin. Hematol.,* **38,** 15 – 21.
Schmidt M, Hochhaus A, Nitsche A, Hehlmann R and Neubauer A. (2001). *Blood,* **97,** 3648 – 3650.
Schmidt M, Nagel S, Proba J, Thiede C, Ritter M, Waring JF, Rosenbauer F, Huhn D, Wittig B, Horak I and Neubauer A. (1998). *Blood,* **91,** 22 – 29.
Sexl V, Piekorz R, Moriggl R, Rohrer J, Brown MP, Bunting KD, Rothammer K, Roussel MF and Ihle JN. (2000). *Blood,* **96,** 2277 – 2283.
Shuai K, Halpern J, ten Hoeve J, Rao X and Sawyers CL. (1996). *Oncogene,* **13,** 247 – 254.
Sillaber C, Gesbert F, Frank DA, Sattler M and Griffin JD. (2000). *Blood,* **95,** 2118 – 2125.
Socolovsky M, Fallon AE, Wang S, Brugnara C and Lodish HF. (1999). *Cell,* **98,** 181 – 191.
Sood R, Talwar-Trikha A, Chakrabarti SR and Nucifora G. (1999). *Leukemia,* **13,** 348 – 357.
Talpaz M. (2001). *Semin. Hematol.,* **38,** 22 – 27.
Tamura T, Nagamura-Inoue T, Shmeltzer Z, Kuwata T and Ozato K. (2000). *Immunity,* **13,** 155 – 165.
Tanaka T, Mitani K, Kurokawa M, Ogawa S, Tanaka K, Nishida J, Yazaki Y, Shibata Y and Hirai H. (1995). *Mol. Cell. Biol.,* **15,** 2383 – 2392.
Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G and Ihle JN. (1998). *Cell,* **93,** 841 – 850.
Van Etten RA. (2001). *Blood Cells Mol. Dis.,* **27,** 201 – 205.
Verfaillie CM, Hurley R, Lundell BI, Zhao C and Bhatia R. (1997). *Acta Haematol.,* **97,** 40 – 52.
Voncken JW, Griffiths S, Greaves MF, Pattengale PK, Heisterkamp N and Groffen J. (1992). *Cancer Res.,* **52,** 4534 – 4539.
Voncken JW, Kaartinen V, Pattengale PK, Germeraad WT, Groffen J and Heisterkamp N. (1995). *Blood,* **86,** 4603 – 4611.
Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH and Speck NA. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 3444 – 3449.
Westendorf JJ, Yamamoto CM, Lenny N, Downing JR, Selsted ME and Hiebert SW. (1998). *Mol. Cell. Biol.,* **18,** 322 – 333.
Wolff NC and Ilaria Jr RL. (2001). *Blood,* **98,** 2808 – 2816.
Wong S and Witte ON. (2001). *Oncogene,* **20,** 5644 – 5659.
Yaish P, Gzit A, Gilon C and Levitzki A. (1988). *Science,* **242,** 933 – 935.

Yergeau DA, Hetherington CJ, Wang Q, Zhang P, Sharpe AH, Binder M, Marin-Padilla M, Tenen DG, Speck NA and Zhang DE. (1997). *Nat. Genet.,* **15,** 303–306.

Zhang X and Ren R. (1998). *Blood,* **92,** 3829–3840.

Zhang X, Subrahmanyam R, Wong R, Gross AW and Ren R. (2001a). *Mol. Cell. Biol.,* **21,** 840–853.

Zhang X, Wong R, Hao SX, Pear WS and Ren R. (2001b). *Blood,* **97,** 277–287.

Zhao X, Ghaffari S, Lodish H, Malashkevich VN and Kim PS. (2002). *Nat. Struct. Biol.,* **9,** 117–120.

Zou X and Calame K. (1999). *J. Biol. Chem.,* **274,** 18141–18144.