Tyrosyl Phosphorylation and DNA Binding Activity of Signal Transducers and Activators of Transcription (STAT) Proteins in Hematopoietic Cell Lines Transformed by Bcr/Abl

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Summary

Bcr/Abl is a chimeric oncogene that can cause both acute and chronic human leukemias. Bcr/Abl–encoded proteins exhibit elevated kinase activity compared to c-Abl, but the mechanisms of transformation are largely unknown. Some of the biological effects of Bcr/Abl overlap with those of hematopoietic cytokines, particularly interleukin 3 (IL-3). Such effects include mitogenesis, enhanced survival, and enhanced basophilic differentiation. Therefore, it has been suggested that p210Bcr/Abl and the IL-3 receptor may activate some common signal transduction pathways. An important pathway for IL-3 signaling involves activation of the Janus family kinases (JAKs) and subsequent tyrosyl phosphorylation of STAT proteins (signal transducers and activators of transcription). This pathway directly links growth factor receptors to gene transcription. We analyzed JAK activation, STAT protein phosphorylation, and the formation of specific DNA-binding complexes containing STAT proteins, in a series of leukemia cell lines transformed by *Bcr/Abl* or other oncogenes. We also examined these events in cell lines transformed by a temperature sensitive (ts) mutant of Bcr/Abl, where the kinase activity of Abl could be regulated. STAT1 and STAT5 were found to be constitutively phosphorylated in 32D, Ba/F3, and TF-1 cells transformed by Bcr/Abl, but not in the untransformed parental cell lines in the absence of IL-3. Phosphorylation of STAT1 and STAT5 was also observed in the human leukemia cell lines K562 and BV173, which express the *Bcr/Abl* oncogene, but not in several Bcr/Abl–negative leukemia cell lines. Phosphorylation of STAT1 and STAT5 was directly due to the tyrosine kinase activity of Bcr/Abl since it could be activated or deactivated by temperature shifting of cells expressing the Bcr/Abl *ts* mutant. DNA–STAT complexes were detected in all Bcr/Abl–transformed cell lines and they were supershifted by antibodies against STAT1 and STAT5. DNA-STAT complexes in 32Dp210Bcr/Abl cells were similar, but not identical, to those formed after IL-3 stimulation. It is interesting to note that JAK kinases (JAK1, JAK2, JAK3, and Tyk2) were not consistently activated in Bcr/Abl–positive cells. These data suggest that STATs can be activated directly by Bcr/Abl, possibly bypassing JAK family kinase activation. Overall, our results suggest a novel mechanism that could contribute to some of the major biological effects of Bcr/Abl transformation.

Bcr/Abl is a chimeric oncoprotein generated by a reciprocal translocation between chromosomes 9 and 22 and implicated in the pathogenesis of Philadelphia chromosome positive (Ph^+)[1] human leukemias (1, 2). Fusion proteins of either 210 (p210) or 185 kD (p185) are generated and are associated with chronic myelogenous leukemia (CML) or acute lymphocytic leukemia, respectively.

Both p210 and p185 Bcr/Abl proteins exhibit increased tyrosine kinase activity and transforming properties compared with the normal c-Abl protein (3, 4). p210Bcr/Abl can cause a CML-like syndrome in mice in vivo (5), converts IL-3– and GM-CSF–dependent hematopoietic cell lines to factor independence, and transforms immature hematopoietic cells in vitro (6–9). The elevated tyrosine kinase activity is essential for its transforming activity, but the mechanisms involved in p210Bcr/Abl–mediated transformation are unknown. Some biological effects of p210Bcr/Abl are known to overlap with those of IL-3. For example, chronic administration of IL-3 or unregulated IL-3 gene

[1] *Abbreviations used in this paper:* CML, chronic myelogenous leukemia; FBS, fetal bovine serum; IRF-1, interferon-responsive factor 1; JAK, Janus kinase; Ph^+, Philadelphia chromosome; STAT, signal transducer and activator of transcription; *ts*, temperature sensitive.

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expression in mice results in an expansion of myeloid cells similar to that seen in CML (10–12). Further, p210Bcr/Abl expression and IL-3 stimulation induce tyrosine phosphorylation of an overlapping set of membrane and cytoplasmic proteins, suggesting that similar biological effects of p210Bcr/Abl and IL-3 may in part result from activation of common signal transduction pathways (13). For instance, Shc, Vav, Fes, Paxillin, and SHPTP2 are known to be tyrosyl phosphorylated by both IL-3 and p210Bcr/Abl in myeloid cells (14–18). However, the role of these phosphoproteins in both IL-3–mediated mitogenesis and Bcr/Abl–mediated transformation is still undetermined.

Recently, a signal transduction pathway that involves Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) has been shown to be regulated by several hematopoietic cytokine receptors, including IL-3R and GM-CSFR (for reviews see references 19 and 20). The JAK family members (JAK1, JAK2, JAK3, and Tyk2) are ~130-kD cytosolic tyrosine kinases that are phosphorylated and activated in response to cytokine stimulation. JAK activation leads to phosphorylation of STAT proteins (21–23) which then dimerize, translocate to the nucleus, and bind specific promoter elements to regulate gene expression. STAT proteins, therefore, function as a direct link between growth factor receptors and the nucleus (24). The STAT family includes at least six members that can be activated by one or more JAK family kinase in response to specific cytokines. Each cytokine receptor tends to signal through a subset of STATs, typically leading to the phosphorylation of one to three different STATs. For example, IL-3 and GM-CSF induce tyrosine phosphorylation of STAT5 (25), STAT6 (26), and STAT1 (27), predominantly through activation of JAK2 (28).

Since IL-3 stimulation and Bcr/Abl expression have similar effects in hematopoietic cells and result in tyrosyl phosphorylation of common substrates, we investigated the potential involvement of the JAK-STAT pathway in Bcr/Abl–mediated transformation. It is interesting that we found that Bcr/Abl induces both the tyrosine phosphorylation of selected STAT proteins and the formation of STAT-containing DNA–binding complexes. Despite leading to STAT phosphorylation, Bcr/Abl did not significantly or consistently increase phosphorylation of JAKs.

Material and Methods

Cells and Cell Culture. The 32Dc13 cell line (29) was obtained from Joel Greenberger (University of Pittsburgh, Pittsburgh, PA). The Ba/F-3 cell line (30) was obtained from Alan D'Andrea (Dana-Farber Cancer Institute). The BV173 cell line (31) was obtained from Bruno Calabretta (Jefferson Medical School, Philadelphia, PA). The RL and NALM6 cell lines (32, 33) were obtained from John Gribben (Dana-Farber Cancer Institute). TF-1 (34), K562 (35), HL60 (36), and U937 (37) cells were obtained from the American Type Culture Collection (Rookville, MD). K562, HL-60, and U937 were grown in RPMI 1640 medium (Mediatech, Washington, D.C.) containing 10% fetal bovine serum (FBS). IL-3–dependent 32Dc13 and Ba/F-3 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 10% WEHI-3B conditioned medium as source of murine IL-3. GM-CSF–dependent TF-1 cells were cultured in RPMI 1640 supplemented with 10% FBS and human 1 ng/ml GM-CSF (Genetics Institute, Cambridge, MA).

pGDp210 (5), a p210Bcr/Abl expression vector, was obtained from George Daley and David Baltimore (Massachusetts Institute of Technology, Cambridge, MA). The p210Bcr/Abl temperature sensitive (*ts*) mutant (p210Bcr/AblTS-1) was generated by introducing two point mutations in the tyrosine kinase domain of Abl as described (38); it was subsequently cloned into pGD. Sublines expressing pGDp210Bcr/Abl or pGDp210 Bcr/AblTS-1 were generated by transfection of the corresponding vector into 32Dc13, Ba/F-3, or TF-1 cells by electroporation, as previously described (5) using a Gene Pulser (Bio-Rad, Richmond, CA) and selecting for G418-resistant cell lines. All cell lines transfected with pGDp210 Bcr/Abl became factor independent and were thereafter cultured in RPMI 1640 containing 10% FBS. 32D cells expressing p210Bcr/AblTS-1 were IL-3 dependent when maintained at the nonpermissive temperature (39°C), and therefore were cultured in RPMI supplemented with 10% FBS and 10% WEHI-3B conditioned medium as a source of IL-3.

Cytokine Starvation and Stimulation. Before lysis, the factor-dependent cell lines 32D, Ba/F-3, and TF-1 cells were cytokine deprived for 18 h and then stimulated for 15 min with control buffer, 10 ng/ml murine IL-3 (Upstate Biotechnologies, Inc., Lake Placid, NY), 10 ng/ml human GM-CSF (Genetics Institute), or 100 U/ml murine IFN-α (Sigma Chemical Co., St. Louis, MO). The 32Dp210Bcr/AblTS-1 cell line was cytokine deprived for 18 h at the nonpermissive temperature (39°C) and then shifted for various lengths of time to the permissive temperature (33°C). The K562, BV173, HL-60, U937, RL, NALM6 and the p210Bcr/Abl transfected cell lines were maintained in RPMI 1640 medium supplemented with 10% FBS.

Antibodies. An antiphospho-STAT1 antibody was raised to a mouse STAT1 peptide, containing amino acids 694–705 in which the tyrosine residue in position 701 was phosphorylated (39). The antiphosphotyrosine antibody (4G10) was a gift from B. Druker (Oregon Health Science University, Portland, OR). The anti-STAT1 p91 COOH terminus and anti-STAT5 antibodies used for supershift experiments were a gift from C. Schindler (Columbia University, New York) and from J. Rosen (Baylor College of Medicine, Houston, TX), respectively. The anti-JAK3 antibody was a gift from J. Ihle (St. Jude's Childrens Cancer Research Institute, Memphis, TN). Antibodies against STAT1α + β, STAT3, STAT4, STAT6, and Tyk2 were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-STAT5 was from Transduction Lab (Lexington, KY); and antibodies to JAK1 and JAK2 were from Upstate Biotechnologies, Inc.

Immunoblotting and Immunoprecipitation. Cells were lysed in 1% NP-40, 50 mM Hepes, 0.5 mM EGTA, and 10% glycerol lysis solution containing 1 mM PMSF, 20 μg/ml aprotinin, and 1 mM sodium Na_3VO_4 at 10^8 cells/ml. Lysates were adjusted to contain equal amounts of total protein, using the Bradford assay (Bio-Rad), and were either boiled for 5 min in an equal volume of SDS sample buffer before loading on a SDS-polyacrylamide gel or were used for immunoprecipitation. 50–100 μl of each lysate was incubated with 50 μl of the antiphosphotyrosine antibody 4G10 coupled to protein A–Sepharose beads for 3 h at 4°C and then washed three times with lysis solution. For JAK immunoprecipitation, cells were lysed in 1% Brij 96, 50 mM Tris, 150 mM NaCl, and 5 mM EDTA lysis solution containing 5 mM leupeptin, 10 μg/ml aprotinin, 1 mM Na_3VO_4, 1 mM PMSF, 10

mM NaF, and 100 mg/ml trypsin inhibitor at 10^8 cells/ml. After incubation with the JAK antibody and protein A–Sepharose beads for 3 h at 4°C, immunoprecipitates were washed three times with 1:10 diluted lysis buffer. Proteins were separated by 7.5% SDS-PAGE and electrophoretically transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH). Western blots were performed as described (16). Immunoreactive bands were visualized with the enhanced chemiluminescence (ECL) Western blotting system (Amersham Corp., Arlington Heights, IL) or with the alkaline phosphatase method as described (40).

Nuclear Extracts. Cells were plated on ice, washed once with cold PBS, resuspended, and incubated on ice for 5 min in 5 ml hypotonic buffer (10 mM Tris, pH 7.4, 10 mM NaCl, and 6 mM $MgCl_2$). Cells were then pelleted, resuspended in 0.8 ml hypotonic buffer containing 1 mM 2-ME, 10 mg/ml PMSF, and 1 mM Na_3VO_4 and disrupted by shearing in a Dounce homogenizer (type B pestle, 40 strokes; Kontes Glass Co., Vineland, NJ). Nuclei were collected by a 10-s centrifugation at 12,000 *g*, washed once with hypotonic buffer, resuspended in 3 vol of high salt buffer (20 mM Hepes, pH 7.9, 420 mM NaCl, 25% glycerol, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM βME, 1 mM PMSF, and 1 mM Na_3VO_4) and mixed at 4°C for 30 min. After centrifugation at 12,000 *g* for 3 min at 4°C, supernatants containing the nuclear proteins were recovered.

Electrophoretic Mobility Shift Assay. A DNA binding assay was performed as described (39), using a ^{32}P-labeled oligonucleotide 5′-AGCCTGATTTCCCCGAAATGACGGC-3′, derived from the interferon-responsive 1 (IRF-1) promoter sequence. To detect the presence of specific proteins in the DNA protein complexes, 0.5 μl of control antisera, anti-STAT1, or anti-STAT5 was added to the reaction for 30 min at 4°C before loading. DNA–protein complexes were separated by Tris-borate-EDTA/5% PAGE and visualized by autoradiography.

Results

Tyrosyl Phosphorylation of STAT Proteins in Bcr/Abl–positive Cell Lines. To analyze STAT protein phosphorylation, we used an antiserum generated to a phosphopeptide containing the tyrosine phosphorylation site of STAT1, Tyr 701 (39). We have previously shown that this antibody specifically recognizes the tyrosine-phosphorylated forms of STAT1 and STAT5 (Frank, D.A., unpublished observations). STAT protein phosphorylation was examined in three different IL-3– or GM-CSF–dependent hematopoietic cell lines, and in p210Bcr/Abl–transformed subclones of each cell line. As shown in Fig. 1 *A*, the antiphospho-STAT antibody detected 91–95-kD tyrosyl-phosphorylated STAT proteins in nontransformed Ba/F3, 32D, and TF-1 cells after stimulation with IL-3 or GM-CSF, (lanes *4–6*), but not in the absence of factor stimulation (lanes *1–3*). In contrast, in the Bcr/Abl–transformed cell lines, tyrosyl-phosphorylated STAT proteins were detected in the absence of factor stimulation (lanes *7–9*). It is interesting to note that the addition of IL-3 or GM-CSF to any of the three Bcr/Abl–transformed cell lines, did not further increase reactivity with the antiphospho-STAT1 antibody (data not shown). As shown in Fig. 1 *B*, factor-independent STAT tyrosyl phosphorylation correlated with the expression of p210Bcr/Abl. STAT protein phosphorylation was

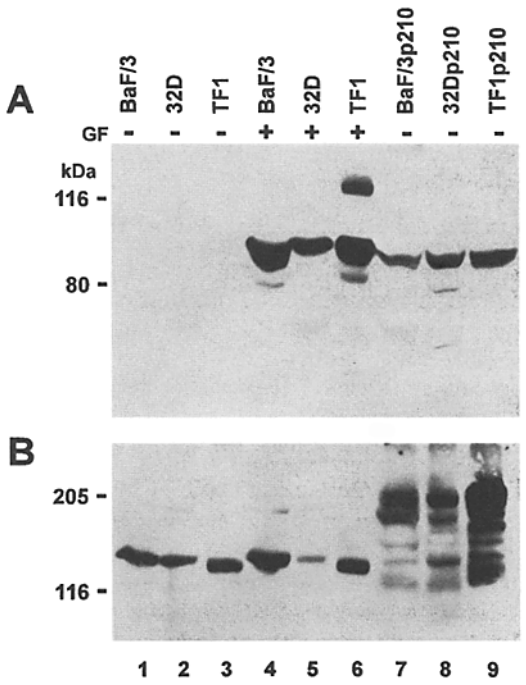

Figure 1. (*A*) Tyrosyl phosphorylation of STAT proteins in p210Bcr/Abl–transfected cells. Untransfected cells from 32D, Ba/F3, and TF-1 cell lines, were growth factor deprived for 18 h and then stimulated with control buffer (lanes *1–3*) or growth factor: 10 ng/ml murine recombinant IL-3 (*BaF/3* and *32D* cells, lanes *4–5*) or 10 ng/ml human recombinant GM-CSF (*TF1* cells, lane *6*) for 15 min, before lysis. All Bcr/Abl–transfected sublines (lanes *7–9*) did not require growth factor for survival or proliferation and were grown in RPMI with 10% FBS before lysis. Cellular proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antiphospho-STAT1 antiserum. (*B*) c-Abl and p210Bcr/Abl expression. The nitrocellulose membrane in *A* was stripped and reprobed with an antibody against c-Abl. Control levels of c-Abl in the untransfected cells (lanes *1–6*) and of c-Abl and p210Bcr/Abl in the transfected sublines (lanes *7–9*) are shown.

also analyzed in two human Ph^+ leukemia-derived cell lines, K562 and BV173, and compared with two Ph^- myeloid leukemia cell lines, HL-60 and U937, and two Ph^- lymphoid leukemia cell lines, RL and Nalm6. As shown in Fig. 2, the antiphospho-STAT1 antiserum recognized tyrosyl-phosphorylated STAT proteins only in the Ph^+ cell lines, suggesting that constitutive tyrosyl phosphorylation of STAT proteins is an event specifically associated with Bcr/Abl transformation.

Tyrosyl Phosphorylation of STAT Proteins Is Dependent on Bcr/Abl Tyrosine Kinase Activity. To further analyze the relation between Bcr/Abl expression and STAT protein tyrosyl phosphorylation, we examined STAT protein phosphorylation in 32D cells transfected with a *ts* mutant of the p210Bcr/Abl kinase (p210TS-1). The Bcr/Abl *ts* kinase is minimally active at 39°C (nonpermissive temperature) and has increased activity at 33°C (permissive temperature) (38). In parallel with the Bcr/Abl kinase activation, 32Dp210TS-1 cells are completely IL-3 dependent at the nonpermissive temperature and IL-3–independent for survival (but not for proliferation) at the permissive temperature (38). In ab-

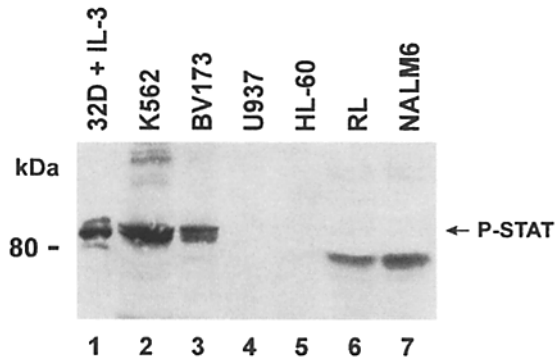

Figure 2. Tyrosyl phosphorylation of STAT proteins in Ph+ cell lines. Cells were grown in RPMI with 10% FCS before lysis. Cellular proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antiphospho-STAT1 antiserum as in Fig. 1 *A*. (Lane *1*) IL-3–stimulated 32D cells as positive control. The ~70-kD bands observed in lanes *6* and *7* were not recognized by any specific antibody against known STATs.

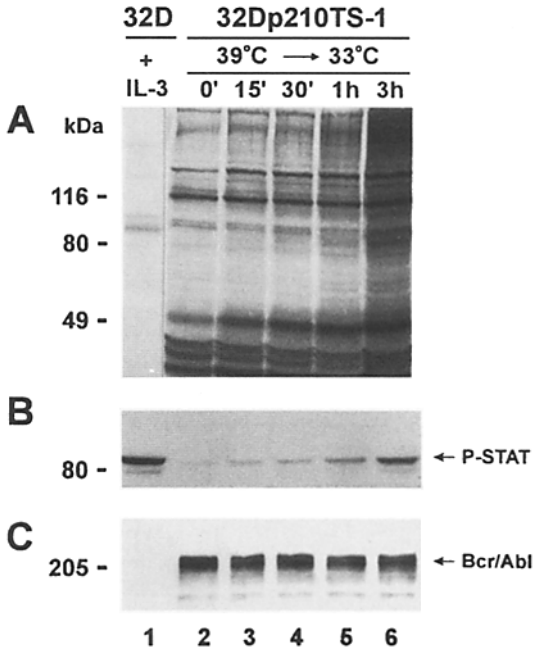

Figure 3. (*A*) Levels of phosphotyrosine in cellular proteins in cells expressing p210Bcr/AblTS. 32Dp210Bcr/Ab1TS-1 cells growing at 39°C were growth factor deprived for 18 h (lane *2*) and shifted to 33°C for 15 and 30 min, 1 and 3 h before lysis (lanes *3–6*). Untransfected 32D cells, used as control, were factor deprived for 18 h and stimulated with IL-3 (10 ng/ml for 15 min), (lane *1*) before lysis. Lysates were treated as in Fig. 1 *A* and immunoblotted with an antiphosphotyrosine antibody. (*B*) Levels of phosphotyrosine in STAT proteins from cells expressing p210Bcr/AblTS-1. Lysates from the same experiment as in *A* were probed with the antiphospho-STAT1 antiserum. (*C*) p210Bcr/AblTS-1 expression in the transfected cells. The nitrocellulose membrane from *B* was stripped and reprobed with an anti c-Abl antibody.

sence of growth factor, tyrosyl phosphorylation of cellular proteins increases rapidly after shifting 32Dp210TS-1 cells to the permissive temperature (Fig. 3 *A*), consistent with previous studies (38, 41). No detectable effects on cellular protein tyrosine phosphorylation were observed after a similar temperature shift in control 32D cells transfected with the vector pGD alone (38). In 32Dp210TS-1 transfectants, tyrosyl phosphorylation of STAT proteins correlated with p210Bcr/Abl kinase activation (Fig. 3 *B*). STAT proteins were tyrosyl phosphorylated in the absence of growth factor as early as 15 min after the temperature shift (Fig. 3 *B*, lane *3*) and the level of phosphorylation continued to increase over time (Fig. 3 *B*, lanes *4–6*; Fig. 4, lanes *7* and *8*) reaching a plateau at ~6–12 h. (Fig. 3 *C* shows comparable levels of p210Bcr/AblTS-1 protein in the samples). Phosphorylation of STAT proteins was not detected at the nonpermissive temperature in the absence of factor stimulation (Fig. 3 *B*, lane *2*; Fig. 4, lane *6*), although it could be efficiently induced by IL-3 addition (Fig. 4, lane *4*). Phosphorylation of STAT proteins was rapidly reduced (within 15 min) as the cells were shifted from the permissive to the nonpermissive temperature (Fig. 4, lanes *9* and *10*). These results indicate that tyrosyl phosphorylation of STAT proteins is dependent on the tyrosine kinase activity of Bcr/Abl.

However, one possible explanation for the observed tyrosyl phosphorylation of STAT proteins in p210Bcr/Abl–expressing cells is autocrine secretion of IL-3 or other growth factors, a phenomenon that has been observed in some cell lines transformed by Bcr/Abl (42), but not in others (43). Two observations suggest that the phosphorylation of STAT-related proteins in these Bcr/Abl–expressing cells is not likely due to autocrine secretion of growth factors after Bcr/Abl activation. First, increased phosphorylation of STAT proteins in 32Dp210TS-1 was observed very rapidly after temperature shift (<15 min; Fig. 3 *B*, lane *3*). Second, after a temperature shift from the permissive to the nonpermissive temperature, the phosphorylation of STAT proteins declined within 15 min (Fig. 4, lane *9*). Such very rapid changes in STAT protein phosphorylation with the temperature shift are not consistent with an autocrine response.

To address more directly the possibility of an autocrine mechanism, supernatants from 32Dp210 or 32Dp210TS-1 cells grown at the permissive temperature were tested for the presence of factors that could induce either STAT phosphorylation or S-phase entry in growth factor–deprived 32D cells. Cells were washed, incubated either at 37°C (32Dp210) or 33°C (32Dp210TS-1), and supernatants were collected after 6, 12, and 24 h of incubation. The supernatants, undiluted or diluted (1:2, 1:4) failed to sustain survival or induce proliferation of factor-deprived 32D cells (data not shown). These supernatants also failed to induce tyrosyl phosphorylation of STAT proteins in either cytokine-deprived 32D or 32Dp210TS-1 cells (Fig. 4, lanes *3* and *5*).

These results demonstrate that in these cell lines, p210Bcr/Abl does not induce secretion of a cytokine in sufficient amounts to induce STAT phosphorylation in an autocrine manner.

Identification of the STAT Proteins Phosphorylated in Bcr/Abl–positive Cell Lines. To further characterize STAT proteins phosphorylated in Bcr/Abl–positive cells, cell lysates

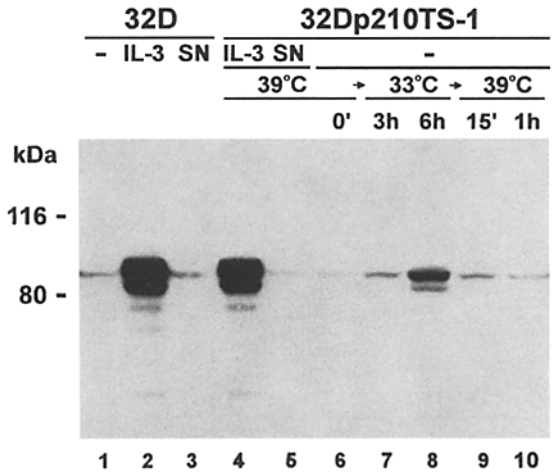

Figure 4. Levels of phosphotyrosine in STAT proteins in cells expressing p210Bcr/AblTS-1. 32Dp210Bcr/AblTS-1 cells growing at 39°C were growth factor deprived for 18 h (lane *6*) and shifted to 33°C for 3 and 6 h (lanes *7* and *8*) before lysis. 32Dp210Bcr/AblTS-1 cells growing at 33°C in the absence of growth factor for 6 h were shifted back to 39°C for 15 and 30 min (lanes *9* and *10*) before lysis. 32p210Bcr/AblTS-1 cells (at 39°C) and 32D parental cells were factor deprived for 18 h and stimulated with 10 ng/ml IL-3 for 15 min (lanes *2* and *4*) or with the supernatants of 32Dp210 cells and of 32Dp210TS-1 cells at 33°C, respectively (lanes *3* and *5*), before lysis. Lysates were treated as in Fig. 1 *A* and probed with the antiphospho-STAT1 antiserum.

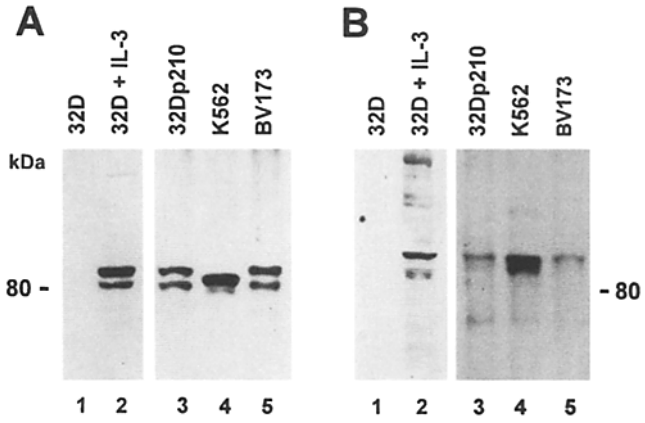

Figure 5. STAT1 and STAT5 tyrosyl phosphorylation in p210Bcr/Abl cells. 32D cells were growth factor deprived for 18 h and then stimulated with control buffer (lane *1*) or 10 ng/ml murine recombinant IL-3 (lane *2*) before lysis. 32Dp210, K562, and BV173 cells were grown in RPMI with 10% FCS before lysis. Lysates were immunoprecipitated with the antiphosphotyrosine antibody 4G10 and then subjected to Western blot analysis with anti-STAT1 (*A*) and with anti-STAT5 (*B*) antibodies.

were immunoprecipitated with antiphosphotyrosine antibody 4G10, followed by Western blotting with antibodies against STAT1, STAT3, STAT4, STAT5, and STAT6. As shown in Fig. 5 *A*, anti-STAT1 antibody detected two proteins, p91 and p84, in antiphosphotyrosine immunoprecipitates from 32D cells stimulated with IL-3, 32Dp210, and BV173 cells (lanes *2*, *3*, and *5*). In K562 cells, a single band of ~90 kD was detected (Fig. 5 *A*, lane *4*). Anti-STAT5 antibody detected a 93-kD protein in IL-3–stimulated 32D cells, 32Dp210, K562, and BV173 cells (Fig. 5 *B*, lanes *2–5*). In K562 cells, the STAT5 antibody also detected a lower molecular weight band of ~91 kD. STAT3, STAT4, and STAT6 antibodies did not detect any protein in any of the antiphosphotyrosine immunoprecipitates (data not shown).

Overall, these data suggest that the STATs phosphorylated in response to p210Bcr/Abl are similar to the STAT proteins phosphorylated by IL-3. However, it is also possible that other STAT-related proteins, not yet characterized, are involved in IL-3 or p210Bcr/Abl signaling.

JAK Phosphorylation Is Not Consistently Increased in Bcr/Abl–transformed Cell Lines. To determine if phosphorylation of STAT proteins in p210Bcr/Abl–transformed cells was secondary to activation of JAK family kinases, we analyzed tyrosyl phosphorylation of the JAK proteins in p210Bcr/Abl–expressing cells. Cell lysates from 32D, 32Dp210, K562, or BV173 cells were analyzed simultaneously for tyrosyl phosphorylation of JAK1, JAK2, Tyk2, and JAK3 and for tyrosyl phosphorylation of STAT proteins. As shown in Fig. 6 *A*, IL-3 induced strong tyrosyl phosphorylation of JAK1 and JAK2 in 32D cells (lanes *2* and *7*). However, tyrosyl phosphorylation of JAK1 and JAK2 was not detected in 32Dp210 and K562 cell lines (Fig. 6 *A*, lanes *3*, *4*, *8*, and *9*). In BV173 cells, a small amount of tyrosyl phosphorylation of JAK1 was detected (Fig. 6 *A*, lane *5*) whereas JAK2 was not phosphorylated (Fig. 6 *A*, lane *10*). In K562 cells a small amount of tyrosyl phosphorylation of Tyk2 was detected (Fig. 6 *A*, lane *14*). Phosphorylation of JAK3 was not observed in any of the cell lines analyzed (data not shown). Approximately equal amounts of JAK1 and JAK2 protein were detected in all cell lines (Fig. 6 *B*, lanes *1–10*), whereas Tyk2 was found to be expressed only in K562 cells (Fig. 6 *B*, lane *14*). Despite differences in levels of JAK phosphorylation, comparable amounts of STAT protein tyrosyl phosphorylation were observed in factor-stimulated 32D cells and in 32Dp210, K562, and BV173 cells, as shown in Fig. 6 *C*.

It is interesting to note that tyrosyl phosphorylation of JAK1 and JAK2 in these p210Bcr/Abl–transformed cell lines could not be induced or augmented by addition of IL-3 or GM-CSF (data not shown).

Overall, these results show that, in contrast with STAT protein phosphorylation, there is no consistent increase in the steady state tyrosyl phosphorylation of the JAKs in cells transformed by p210Bcr/Abl.

Bcr/Abl Activates DNA-binding Activity Involving STATs. After cytokine-induced tyrosyl phosphorylation, STAT proteins translocate to the nucleus and modulate gene expression by binding to specific promoter sequences (19–23). To examine Bcr/Abl–transformed cells for the presence of STAT-containing DNA-binding complexes, nuclear extracts were prepared from IL-3–deprived and IL-3–stimulated 32D cells, and from 32Dp210, BV173, K562, and U937 cell lines. Proteins in the extracts were examined for their ability to bind a sequence from the IRF-1 promoter. This sequence has been shown previously to bind phos-

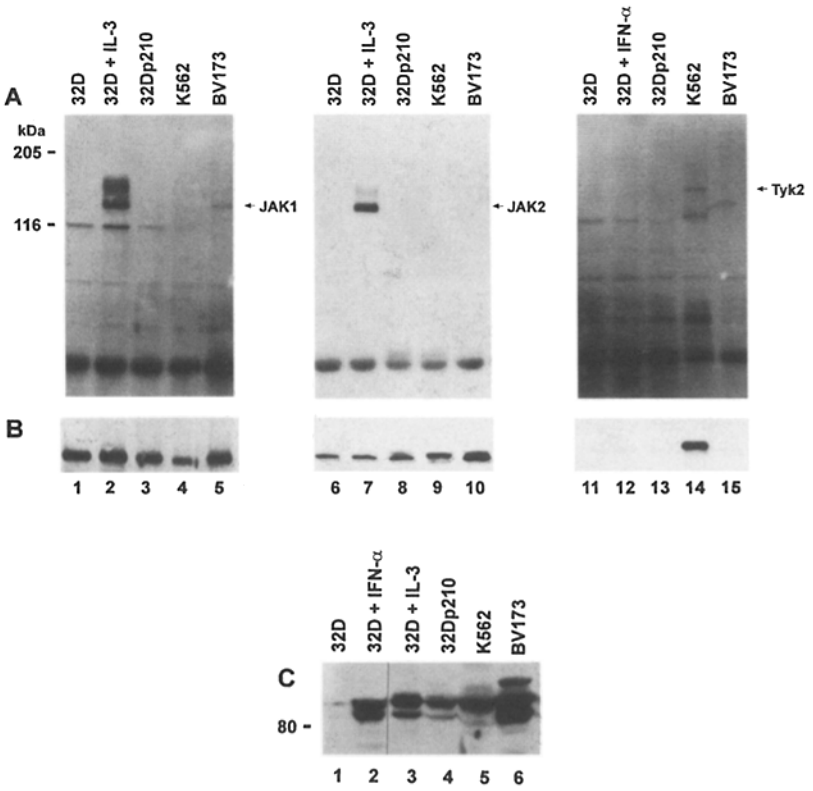

Figure 6. JAK kinase phosphorylation in p210Bcr/Abl cells. 32D cells were starved and stimulated with control buffer (lanes *1*, *6*, and *11*), with 10 ng/ml IL-3, as a control for JAK1 and JAK2 activation, (lanes *2* and *7*) or with 100 U/ml IFN-α, as a control for Tyk2 activation, (lane *12*) before lysis. 32Dp210, K562, and BV173 were maintained in RPMI with 10% medium before lysis. (*A*) Lysates were immunoprecipitated with anti-JAK1 (lanes *1–5*), anti-JAK2 (lanes *6–10*), and anti-Tyk2 antisera (lanes *11–15*) and subjected to Western blot analysis with an antiphosphotyrosine antibody. (*B*) JAK protein levels were determined using the respective antisera after stripping of the nitrocellulose membrane in *A*. (*C*) Lysates were analyzed for STAT protein phosphorylation using the antiphospho-STAT1 antibody.

phorylated STAT1, STAT3, and other related STAT proteins, and to mediate a STAT-dependent transcriptional response (39, 44). As shown in Fig. 7, *A* and *B* (lanes *1* and *6*), no DNA-binding activity could be detected in nuclear extracts from IL-3–deprived 32D cells, or in the U937 cell line (which does not express Bcr/Abl). In IL-3–stimulated 32D cells and in the p210Bcr/Abl–transformed cells, we observed formation of DNA-binding complexes. These complexes were specific for the IRF-1 promoter sequence, since they could be competed by the addition of excess unlabeled probe (data not shown). In 32D cells, IL-3 stimulated the formation of a single complex (complex I; Fig. 7, *A* and *B*, lane *2*), whereas p210Bcr/Abl induced the formation of three distinct complexes (IIIa, IIIb, and IV; Fig. 7, *A* and *B*, lane *3*). In K562 cells, a single complex was observed (II; Fig. 7, *A* and *B*, lane *4*), whereas the complexes in BV173 cells were similar to those in 32Dp210 cells (Fig. 7, *A* and *B*, lane *5*). To determine if the DNA-binding complexes observed contained STAT proteins, supershift experiments were carried out by adding anti-STAT antibodies to the binding reaction. Antibody against STAT1 (Fig. 7 *A*) led to the formation of supershifted complexes (*A*) in all samples. Antibodies against STAT5 (Fig. 7 *B*) completely abolished complex I in IL-3–stimulated 32D cells (lane *8*), complex II in K562 cells (lane *10*), and complex IIIa in 32Dp210 and BV173 cells (lanes *9* and *12*), leading to formation of supershifted complexes (*A*). In 32Dp210 and in BV173 cells, complexes IIIb and IV (Fig. 7, *A* and *B*, lanes *9* and *12*) were not supershifted by antibody against either STAT1 or STAT5, or by a combination of the two antibodies. The observation that the anti-STAT5 antibody completely supershifts complexes I and II, which also contain STAT1, suggests that STAT1-STAT5 heterodimers may be present and more efficiently bound by anti-STAT5 antibodies.

In conclusion, these data suggest that specific DNA-binding activity of STAT1 and STAT5 is induced in response to Bcr/Abl.

Discussion

Activation of the JAK family kinases, followed by STAT protein phosphorylation, represents a newly defined signal transduction pathway that directly links cytokine receptors to gene expression (21–23). The JAK/STAT pathway appears to be distinct and independent from the p21ras pathway and is activated by many different cytokines with different biological actions (for reviews, see references 19 and 20). The function of STAT proteins and the mechanism through which specificity is reached in each particular cytokine-signaling system have not yet been clarified. However, there is growing evidence that STAT proteins are likely to play an important role in mitogenesis, survival, or differentiation. Therefore, it would not be surprising if JAK or STAT proteins are involved in malignant transformation. Recently, this hypothesis has been supported by two studies that report constitutive activation of STAT proteins in transformed cells (45, 46), suggesting that aberrant STAT phosphorylation in response to oncogenic tyrosine kinases could contribute to malignant cell growth.

Although *Bcr/Abl* was one of the first human oncogenes described to encode a tyrosine kinase (3), its mechanism of

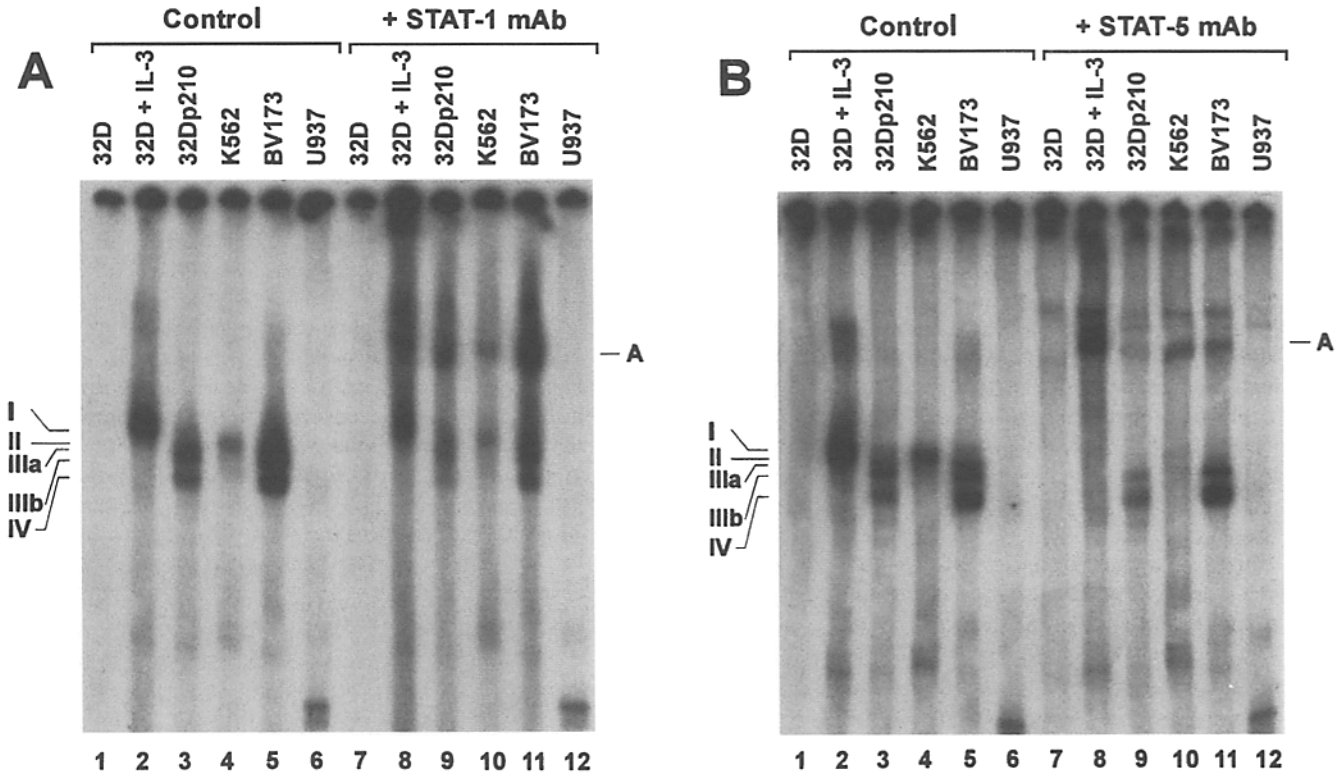

Figure 7. EMSA. Nuclear extracts from 32D cells, unstimulated (*A* and *B*, lanes *1*) and IL-3 stimulated (*A* and *B*, lanes *2*), 32Dp210 (*A* and *B*, lanes *3*), K562 (*A* and *B*, lanes *4*), BV173 (*A* and *B*, lanes *5*), and U937 (*A* and *B*, lanes *6*) cells were incubated with ^{32}P-labeled probe and analyzed by electrophoresis. (*A*) Antibodies against STAT1 were included in the binding incubation in lanes *7–12*. (*B*) Antibodies against STAT5 were included in the binding incubation in lanes *7–12*.

transformation is still poorly understood. The *p210BCR/ABL* oncogene is expressed in hematopoietic stem cells, and causes massive expansion of progenitor cell pools, particularly in the blood and spleen (47, 48). Differentiation along the granulocytic pathway may be enhanced, whereas there is little or no augmentation of erythropoiesis or lymphopoiesis in most patients. In some patients, there is increased production or accumulation of basophils, eosinophils, or platelets. These apparent lineage-restricted effects of a stem cell oncogene are so far unexplained. When primary cells are analyzed in vitro, their phenotype is also subtle, mainly characterized by a decreased susceptibility to apoptosis and adhesion defects (38, 41, 49). In experimental models, in contrast to the human disease, p210Bcr/Abl is associated with a block in hematopoietic differentiation and/or acquisition of factor independence (6–9).

In this study, we show that STAT1 and STAT5, or closely related proteins, are persistently phosphorylated in cell lines transformed by Bcr/Abl, whereas STAT3, STAT4, and STAT6 are not. These results are of interest because STAT5, and to a lesser extent STAT1, are transiently phosphorylated on tyrosine residues in factor-dependent cells in response to cytokines such as IL-3 and GM-CSF (25, 27). Thus, STAT phosphorylation may represent an important point of convergence between the Bcr/Abl and IL-3/GM-CSF signaling pathways that could contribute to the known overlap in the biological effects of IL-3 and Bcr/Abl.

To understand the potential significance of Bcr/Abl–induced tyrosine phosphorylation of STAT proteins, we first asked if this phenomenon was directly due to Bcr/Abl tyrosine kinase activity. Cell lines transformed by Bcr/Abl tend to accumulate cellular mutations (50), and some events observed in cultured cell lines are likely to be due to secondarily acquired genetic alterations. In an effort to define the primary consequences of Bcr/Abl kinase activity and to distinguish these from secondary events associated with transformation, we developed a system in which the tyrosine kinase activity of p210Bcr/Abl could be induced by temperature shift and the direct effects of Bcr/Abl expression observed in the IL-3–dependent hematopoietic murine cell line 32D (38). In the present study, we found that the tyrosine phosphorylation of STAT proteins was directly dependent on the level of tyrosine kinase activity of Bcr/Abl. This result suggests that the increased tyrosine phosphorylation of STAT proteins in Bcr/Abl–transformed cell lines is due directly to Bcr/Abl and not to a secondary mutation. Furthermore, these results indicate that STAT phosphorylation is not sufficient for cell proliferation, but could be involved in other events such as prolonged survival.

A critical question in these studies is whether Bcr/Abl–mediated tyrosyl phosphorylation of STAT proteins occurs on specific tyrosine residues that are important for the formation of STAT-containing DNA-binding complexes.

This issue was examined in two ways. First, STAT tyrosyl phosphorylation was surveyed in CML cell lines using a previously described antibody that recognizes STAT1 phosphorylated at tyrosine residue 701 (39). Phosphorylation of Y701 has been shown to be critical for translocation of STAT1 to the nucleus and for subsequent transcriptional activation (23). The antibody also reacts with phospho-STAT5, but not with any STAT protein in quiescent hematopoietic cells (Frank, D.A., unpublished observations). The reactivity of the phospho-STAT antibody with the STAT proteins in cells transformed by Bcr/Abl suggests that these STAT proteins are likely to be phosphorylated in response to Bcr/Abl at a biologically important tyrosine residue. It is interesting that the SDS-PAGE mobility of STAT proteins detected by this antibody in Bcr/Abl–transformed cell lines was similar but not identical to the mobility of STAT proteins detected in nontransformed cells after IL-3 stimulation.

A second observation, which supports the notion that Bcr/Abl–induced STAT protein phosphorylation takes place on biologically important tyrosine residues, is the detection of STAT-containing DNA-binding complexes in Bcr/Abl–transformed cell lines. Using gel shift assays with an IRF-1 promoter sequence that can bind STAT1-containing protein complexes, we readily detected DNA–protein complexes that could be supershifted with antibodies directed against STAT1 and STAT5. It is interesting that these complexes were distinct from those observed after IL-3 stimulation in the same cell line. Overall, these results suggest that the observed tyrosyl phosphorylation of STAT proteins in Bcr/Abl–transformed cells is associated with the formation of DNA-binding complexes that have the potential to affect gene transcription. We also detected complexes in 32Dp210Bcr/Abl and in BV173 cells that could not be supershifted with anti-STAT1 or anti-STAT5 antibodies, suggesting that either as yet unidentified STAT related proteins or non-STAT proteins participate in the formation of the DNA-binding complex. It is interesting that we observed heterogeneity in DNA–complex formation among Bcr/Abl–transformed cell lines. This could be due to species or cell lineage differences in the availability of STATs and other proteins.

The mechanism of increased tyrosine phosphorylation of STAT proteins in response to Bcr/Abl is not yet known. It is possible that Bcr/Abl activates a JAK family kinase or a cytokine receptor. We did not observe any consistent increases in tyrosyl phosphorylation of JAK1, JAK2, JAK3, and Tyk2. However, tyrosine phosphorylation of Tyk2 in K562 cells and JAK1 in BV173 cells was increased over background levels, raising the possibility that these JAKs may contribute to STAT phosphorylation in these cell lines. It is also possible that Bcr/Abl activates one or more cytokine receptors, and studies to directly assess this possibility are warranted. An equally attractive hypothesis is that STAT proteins are phosphorylated directly by Bcr/Abl, as may be the case for v-src (46). In fact, STAT proteins contain SH2 domains (51), and since Bcr/Abl is heavily phosphorylated on tyrosine residues, it is possible that one or more STAT proteins bind to Bcr/Abl directly through their SH2 domains. Although we were not able to coprecipitate Bcr/Abl and any STAT protein (our unpublished observations), STAT proteins may be substrates of Bcr/Abl without physically binding or only transiently binding to the kinase. A third possibility is that increased STAT phosphorylation is due primarily to inhibition of one or more tyrosine phosphatases. There is good evidence that Bcr/Abl interacts with at least two phosphatases, SHPTP2 (Syp, PTP1C) and SHPTP1 (HCP) (18). At the present time, it is not known if the association of Bcr/Abl with either one of these enzymes affects its phosphatase activity.

Increased STAT phosphorylation is not a general property of leukemic transformation. Several other myeloid and lymphoid leukemia cell lines that do not express Bcr/Abl (U937, HL-60, RL, NALM6) have been analyzed, and no increased tyrosine phosphorylation of STAT proteins was observed. Overall, our results suggest a novel mechanism contributing to transformation by a human oncogene. We suggest that some of the unique effects of Bcr/Abl on myeloid lineage cells, such as enhanced basophil differentiation, are particularly likely to be due to STAT activation.

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References

1. Nowell, P.C., and D.A. Hungerford. 1960. A minute chromosome in human chronic granulocytic leukemia. *Science (Wash. DC).* 132:1497.
2. Rowley, J.D. 1973. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa banding. *Nature (Lond.).* 243: 290–291.
3. Konopka, J.B., S.M. Watanabe, and O.N. Witte. 1984. An

alteration of the human c-abl protein in K562 unmasks associated tyrosine kinase activity. *Cell.* 37:1035–1042.
4. Konopka, J.B., and O.N. Witte. 1985. Detection of c-abl tyrosine kinase activity in vitro permits direct comparison of normal and altered abl products. *Mol. Cell. Biol.* 5:3116–3123.
5. Daley, G.Q., R.A. Van Etten, and D. Baltimore. 1990. Induction of chronic myelogenous leukemia in mice by the p210Bcr/Abl gene of the Philadelphia chromosome. *Science (Wash. DC).* 247:824–830.
6. Daley, G.Q., and D. Baltimore. 1988. Transformation of an interleukin 3–dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl. *Proc. Natl. Acad. Sci. USA.* 85:9312–9316.
7. Gishizky, M.L., and O.N. Witte. 1992. Initiation of deregulated growth of multipotent progenitor cells by bcr-abl in vitro. *Science (Wash. DC).* 256:836–839.
8. McLaughlin, J., E. Chianese, and O.N. Witte. 1987. In vitro transformation of immature hematopoietic cells by the p210Bcr/Abl oncogene product of the Philadelphia chromosome. *Proc. Natl. Acad. Sci. USA.* 84:6558–6562.
9. Young, J.C., and O.N. Witte. 1988. Selective transformation of primitive lymphoid cells by the Bcr/Abl oncogene expressed in long-term lymphoid or myeloid cultures. *Mol. Cell. Biol.* 8:4079–4087.
10. Metcalf, D., C.G. Begley, G.R. Johnson, N.A. Nicola, A.F. Lopez, and D.J. Williamson. 1986. Effects of purified bacterially synthesized murine multi-CSF (IL-3) on hematopoiesis in normal mice. *Blood.* 68:46–57.
11. Broxmeyer, H.E., D.E. Williams, S. Cooper, R.W. Shadduck, S. Gillis, A. Waheed, D.L. Urdal, and D.C. Bicknell. 1987. Comparative effects in vivo of recombinant murine interleukin 3, natural murine colony-stimulating factor-1, and recombinant murine granulocyte-macrophage colony-stimulating factor on myelopoiesis in mice. *J. Clin. Invest.* 79:721–730.
12. Wong, P.M., S.W. Chung, C.E. Dunbar, and D.M. Bodine. 1989. Retrovirus-mediated transfer and expression of the interleukin-3 gene in mouse hematopoietic cells result in a myeloproliferative disorder. *Mol. Cell. Biol.* 9:798–808.
13. Matulonis, U., R. Salgia, K. Okuda, B. Druker, and J.D. Griffin. 1993. IL-3 and p21bcr/abl activate both unique and overlapping pathways of signal transduction in a factor-dependent myeloid cell line. *Exp. Hematol.* 21:1460–1466.
14. Matsuguchi, T., R. Salgia, M. Hallek, M. Eder, B. Druker, T. Ernst, and J.D. Griffin. 1994. Shc phosphorylation in myeloid cells is regulated by granulocyte macrophage colony stimulating factor, interleukin-3, and steel factor and is constitutively increased by p210Bcr/Abl. *J. Biol. Chem.* 269: 5016–5021.
15. Matsuguchi, T., R. Inhorn, N. Carlesso, G. Xu, B. Druker, and J.D. Griffin. 1995. Tyrosine phosphorylation of p955VAV in myeloid cells is regulated by GM-CSF, IL-3, and Steel factor and is constitutively increased by p210Bcr/Abl. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:257–265.
16. Ernst, T.J., K.E. Slattery, and J.D. Griffin. 1994. p210Bcr/Abl and p160v-Abl induce and increase in the tyrosine phosphorylation of p93c-Fes. *J. Biol. Chem.* 269:5764–5769.
17. Salgia, R., J.L. Li, S.H. Lo, B. Brunkhorst, G.S. Kansas, E.S. Sobhany, Y. Sun, E. Pisick, M. Hallek, T. Ernst, et al. 1995. Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by p210Bcr/Abl. *J. Biol. Chem.* 270:5039–5047.
18. Tauchi, T., G.S. Feng, R. Shen, H.Y. Song, D. Donner, T. Pawson, and H.E. Broxmeyer. 1994. SH2-containing phosphotyrosine phosphatase Syp is a target of p210Bcr/Abl tyrosine kinase. *J. Biol. Chem.* 269:15381–15387.
19. Darnell, J.E., Jr., I.M. Kerr, and G.R. Stark. 1994. JAK-STAT pathways and transcriptional activation in response to IFNs and other extra-cellular signaling proteins. *Science (Wash. DC).* 264:1415–1421.
20. Ihle, J.N., B.A. Witthuhn, F.W. Quelle, K. Yamamoto, W.E. Thierfelder, B. Kreider, and O. Silvennoinen. 1994. Signaling by the cytokine receptor superfamily: JAKs and STATs. *Trends Biochem. Sci.* 19:222–227.
21. Schindler, C., K. Shuai, V.R. Prezioso, and J.E. Darnell, Jr. 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science (Wash. DC).* 257:809–813.
22. Shuai, K., C. Schindler, V.R. Prezioso, and J.E. Darnell, Jr. 1992. Activation of transcription by IFNγ: tyrosine phosphorylation of a 91 kD DNA binding protein. *Science (Wash. DC).* 259:1808–1812.
23. Shuai, K., G.R. Stark, I.M. Kerr, and J.E. Darnell, Jr. 1993. A single phosphotyrosine residue of STAT91 required for gene activation by interferon-γ. *Science (Wash. DC).* 261: 1744–1746.
24. Shuai, K., A. Ziemiecki, A.F. Wilks, A.G. Harpur, H.B. Sadowski, M.Z. Gilman, and J.E. Darnell, Jr. 1993. Polypeptide signaling to the nucleus through tyrosine phosphorylation of JAK and STAT proteins. *Nature (Lond.).* 366:580.
25. Mui, A.L.-F., H. Wakao, A.M. O'Farrell, N. Harada, and A. Miyajima. 1995. Interleukin-3, granlocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:1166–1175.
26. Quelle, F.W., K. Shimoda, W. Thierfelder, C. Fischer, A. Kim, S.M. Ruben, J. Cleveland, J.H. Pierce, A.D. Keegan, K. Nelms, et al. 1995. Cloning of murine STAT6 and human STAT6, STAT proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. *Mol. Cell. Biol.* 15:3336–3343.
27. Brizzi, M.F., M.G. Aronica, A. Rosso, P. Dentelli, Y. Yarden, and L. Pegoraro. Granulocyte-macrophage colony stimulating factor (GM-CSF) stimulates JAK2 signaling pathway and rapidly activates p93fes, STAT1 p91 and STAT3 p92 in polymorphonuclear leukocytes. *J. Biol. Chem.* In press.
28. Quelle, F.W., N. Sato, B.A. Witthuhn, R.C. Inhorn, M. Eder, A. Miyajima, J.D. Griffin, and J.N. Ihle. 1994. JAK2 associates with the beta c chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane proximal region. *Mol. Cell. Biol.* 14: 4335–4341.
29. Greenberger, J.S., M.A. Sakakeeny, R.K. Humphries, C.J. Eaves, and R.J. Eckner. 1983. Demonstration of permanent factor-dependent multipotential (erythroid/neutrophil/basophil) hematopoietic progenitor cell lines. *Proc. Natl. Acad. Sci. USA.* 80:2931–2935.
30. Palacios, R., and M. Steinmetz. 1985. IL-3–dependent mouse clones that express B-220 antigen, contain Ig genes in germ-line configuration, and generate B lymphocytes in vivo. *Cell.* 41:727–734.
31. Pegoraro, L., L. Matera, J. Ritz, A. Lewis, A. Palumbo, and G. Biagini. 1983. Establishment of a Ph1-positive human cell line (BV173). *J. Natl. Cancer Inst.* 70:447–453.

32. Beckwith, M., D.L. Longo, C.D. O'Connell, C.M. Moratz, and W.J. Urba. 1990. Phorbol ester-induced, cell cycle-specific, growth inhibition of human B-lymphoma cell lines. *J. Natl. Cancer Inst.* 82:501–509.
33. Minowada, J., G. Janossy, M.F. Greaves, T. Tsubota, B.I. Srivastava, S. Morikawa, and E. Tatsumi. 1978. Expression of an antigen associated with acute lymphoblastic leukemia in human leukemia-lymphoma cell lines. *J. Natl. Cancer Inst.* 60: 1269–1277.
34. Kitamura, T., T. Tange, T. Teresawa, S. Chiba, T. Kawai, K. Miyagama, Y.F. Piao, K. Miyazono, A. Urabe, and F. Takaku. 1989. Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3 or erythropoietin. *J. Cell. Physiol.* 140:323–334.
35. Lozzio, C.B., and B.B. Lozzio. 1975. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood.* 45:321–334.
36. Collins, S.J., R.C. Gallo, and R.E. Gallagher. 1977. Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature (Lond.).* 270:347–349.
37. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocitic lymphoma cell line. *Int. J. Cancer.* 17:565–577.
38. Carlesso, N., J.D. Griffin, and B. Druker. 1994. Use of a temperature-sensitive mutant to define the biological effects of the p210Bcr/Abl tyrosine kinase on proliferation of a factor-dependent murine myeloid cell line. *Oncogene.* 9:149–156.
39. Frank, D.A., M.J. Robertson, A. Bonni, J. Ritz, and M.E. Greenberg. 1995. Interleukin 2 signaling involves the phosphorylation of STAT proteins. *Proc. Natl. Acad. Sci. USA.* 92: 7779–7783.
40. Kanakura, Y., B. Druker, S.A. Cannistra, Y. Furukawa, Y. Torimoto, and J.D. Griffin. 1990. Signal transduction of the human granulocyte–macrophage colony-stimulating factor and interleukin-3 receptors involves tyrosine phosphorylation of a common set of cytoplasmic proteins. *Blood.* 76:706–715.
41. Kabarovsky, J.H.S., P.B. Allen, and L.M. Wiedemann. 1994. A temperature sensitive p210Bcr/Abl mutant defines the primary consequences of Bcr/Abl tyrosine kinase expression in growth factor dependent cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:5887–5895.
42. Sirard, C., P. Laneuville, and J.E. Dick. 1994. Expression of bcr-abl abrogates factor-dependent growth of human hematopoietic MO7E cells by an autocrine mechanism. *Blood.* 83:1575–1585.
43. Laneuville, P., N. Heisterkamp, and J. Goeffen. 1991. Expression of the chronic myelogenous leukemia–associated p210Bcr/Abl oncoprotein in a murine IL-3 dependent myeloid cell line. *Oncogene.* 6:275–282.
44. Bonni, A., D.A. Frank, C. Schindler, and M.E. Greenberg. 1993. Characterization of a pathway for ciliary neurotrophic factor signaling to the nucleus. *Science (Wash. DC).* 262: 1575–1579.
45. Migone, T.S., J.-X. Lin, A. Cereseto, J.C. Mulloy, J.J. O'Shea, G. Franchini, and W.J. Leonard. 1995. Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-1. *Science (Wash. DC).* 269:79–81.
46. Yu, C.-L., D.J. Meyer, G.S. Campbell, A.C. Larner, C. Carter-Su, J. Schwartz, and R. Jove. 1995. Enhanced DNA-binding activity of a STAT3-related protein in cells transformed by the Src oncoprotein. *Science (Wash. DC).* 269:81–83.
47. Fialkow, P.J., J.R. Jacobson, and T. Papayannopoulou. 1977. Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. *Am. J. Med.* 63:125–130.
48. Eaves, C.J., and A.C. Eaves. 1987. Bailliere's Clinical Haematology: Chronic Myeloid Leukemia. J.M. Goldman, editor. Bailliere Tindall Ltd., London, 931–961.
49. Verfaille, C.M., J.B. McCarthy, and P.B. McGlave. 1992. Mechanisms underlying abnormal trafficking of malignant progenitors in chronic myelogenous leukemia. *J. Clin. Invest.* 90:1232–1241.
50. Laneuville, P., G. Sun, M. Timm, and M. Vekemans. 1992. Clonal evolution in a myeloid cell line transformed to IL-3 independent growth by retroviral transduction and expression of p210bcr/abl. *Blood.* 80:1788–1797.
51. Fu, X.-Y. 1992. A transcription factor with SH2 and SH3 domains is directly activated by an interferon α-induced cytoplasmic protein tyrosine kinase(s). *Cell.* 70:323–335.