products were detected by fluorography. Quantitation was done with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

23. Glutathione-agarose beads (12.5 μl) were pre-incubated with 75 μg of each GST fusion protein (*22*) in 100 μl of phosphate-buffered saline. The beads were then washed twice in AP buffer [0.1 M MES (pH 7.0), 0.15 M NaCl, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.02% w/v NaN_3, and 0.6% w/v Triton X-100] and incubated with 30 μl of purified brain AP-2 (at 0.9 mg/ml) in AP buffer for 45 min at room temperature. After three more washes with AP buffer, bound proteins were resolved by SDS-PAGE on 12.5% acrylamide gels and processed for immunoblotting.
24. Synthetic oligonucleotides encoding $(SDYQRL)_3$, $(SDGQRL)_3$, SDYQRL, SDAQRL, ADYQRL, SAYQRL, SDYARL, SDYQAL, SDYQRA, $(AGYQTI)_3$, $(STYQPL)_3$, $(SSYTPL)_3$, $(LSYTRF)_3$, and $(DKQTLL)_3$ (*4*) were ligated in-frame to the 3′ end of GAL4bd coding sequences contained within the vector pGBT9. Constructs GAL4bd-TGN38 tail and GAL4bd-TGN38 tail (Y → A) were made by PCR with the use of the TGN38 cDNA as a template, and the products were cloned in-frame into pGBT9-GAL4bd. Gal4bd constructs having residues 324 to 347 of TGN38 followed by two alanine residues and the sequences YQRL, AQRL, or YTRF at the COOH-termini were constructed by PCR and ligation of synthetic oligonucleotides.
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26. SPR experiments were done with a BIAcore instrument (Pharmacia). GST-$(SDYQRL)_3$, GST-$(SDGQRL)_3$, or GST were adsorbed to the surface of a biosensor chip coated with monoclonal antibody 1F9 to GST. To remove potential aggregates, preparations of AP-2 (*12*) in AP buffer (*23*) were spun before each SPR experiment for 30 min at 300,000*g* in a TL100 centrifuge fitted with a TL100.4 rotor (Beckman Instruments). Under these conditions, only monomeric AP-2 complexes were detected by gel filtration. The supernatants were injected into the biosensor chamber at a concentration of 0.9 mg/ml in AP buffer.
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Jak-STAT Signaling Induced by the v-*abl* Oncogene

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The effect of the v-*abl* oncogene of the Abelson murine leukemia virus (A-MuLV) on the Jak-STAT pathway of cytokine signal transduction was investigated. In murine pre–B lymphocytes transformed with A-MuLV, the Janus kinases (Jaks) Jak1 and Jak3 exhibited constitutive tyrosine kinase activity, and the STAT proteins (signal transducers and activators of transcription) normally activated by interleukin-4 and interleukin-7 were tyrosine-phosphorylated in the absence of these cytokines. Coimmunoprecipitation experiments revealed that in these cells v-Abl was physically associated with Jak1 and Jak3. Inactivation of v-Abl tyrosine kinase in a pre–B cell line transformed with a temperature-sensitive mutant of v-*abl* resulted in abrogation of constitutive Jak-STAT signaling. A direct link may exist between transformation by v-*abl* and cytokine signal transduction.

The v-*abl* oncogene of A-MuLV encodes a fusion protein of viral Gag sequences and the COOH-terminus of the c-Abl protein [including the tyrosine kinase and the Src homology 2 (SH2) domains] (*1*). Loss of the c-Abl SH3 domain, addition of viral Gag sequences, and changes in the subcellular localization of the protein from the nucleus to the cytoplasm have all been implicated in contributing to the oncogenic activity of v-Abl (*2*). The tyrosine kinase and the SH2 domains of v-Abl are required for transformation (*3*, *4*), but the mechanism by which v-*abl* transforms cells and allows cytokine-independent growth is unclear (*5*).

The growth of pre–B lymphocytes, the most common in vivo target of transformation by v-*abl*, normally requires cytokines such as interleukin-7 (IL-7) produced by bone marrow stromal cells (*6*). To understand the possible mechanism underlying the cytokine-independent growth of A-MuLV–transformed pre–B cell lines (*7*), we studied signal transduction by cytokines important for growth and proliferation of B lymphoid cells. Signal transduction by IL-4 and IL-7 involves the activation of Jak1 and Jak3 nonreceptor tyrosine kinases, which in turn are thought to phosphorylate latent cytoplasmic transcription factors referred to as STATs (signal transducers and activators of transcription) (*8*). Phosphorylation allows STATs to dimerize and translocate to the nucleus,

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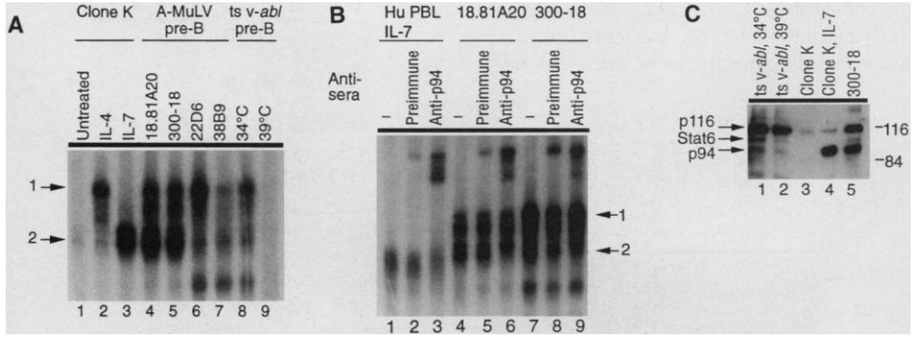

Fig. 1. Constitutive activation of STATs in pre–B cell lines transformed with v-*abl*. (**A**) Constitutive GAS binding activities in Abelson cell lines. EMSA was done with whole-cell extracts prepared from Clone K cells, either untreated or treated with IL-4 or IL-7 (lanes 1 to 3), A-MuLV–transformed pre–B cells derived from bone marrow cells (lanes 4 and 5) and fetal liver (lanes 6 and 7) (*25*), or cells expressing a temperature-sensitive (ts) mutant of v-*abl* grown at permissive temperature (34°C) (lane 8), or shifted to nonpermissive temperatures (39°C) for 15 hours (lane 9). Clone K cells were deprived of feeder cells for 4 hours before the addition of recombinant murine IL-4 (400 U/ml; DNAX) or IL-7 (10 ng/ml; Genzyme) for 15 min. The probe used was from the IRF-1 GAS element; 5′-gatcGATTTC-CCCGAAAT-3′ (*17*). For (A) and (B), arrow 1 indicates STF-IL4, and arrow 2 indicates STF-IL7. (**B**) Identification of DNA binding complexes in (A) with an antibody supershift assay. After incubation with the probe whole-cell extracts were incubated for 30 min with either preimmune serum or an antibody that recognizes the 94-kD isoform of Stat5 (anti-p94) (*14*) (1:20 dilution). Human peripheral blood lymphocytes (PBLs) were isolated by centrifugation on Ficoll-Hypaque (Sigma) and treated with IL-7 (lanes 1 to 3). Human STF-IL7 migrates faster than murine STF-IL7 (*14*). (**C**) Binding of proteins to GAS oligonucleotide. Cells extracts were incubated with multimerized GAS from the β casein gene linked to agarose beads (*17*). This GAS element binds STF-IL7 and STF-IL4 with high affinity (*11*). Bound material was eluted with 2M NaCl, fractionated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (7% gel), blotted with antibody to phosphotyrosine (4G10, Upstate Biotechnology), and detected with enhanced chemiluminescence (ECL, Amersham). Prestained molecular size markers (Sigma) are shown on the right in kilodaltons.

where they regulate transcription from promoters containing DNA sequences related to interferon γ activation sites (GAS) (9).

Electrophorectic mobility shift assay (EMSA) revealed that GAS binding activities similar to those induced by IL-4 and IL-7 [known as signal transducing factor (STF)–IL4 and STF-IL7] were present in several A-MuLV–transformed pre–B cell lines in the absence of these cytokines (Fig. 1A). Complexes similar to STF-IL4 and STF-IL7 were not constitutively active in a nontransformed, IL-7–dependent pre–B cell line [Clone K (*10*)] or in tumors of more mature B cells (*11*). We examined a pre–B cell line transformed with a temperature-sensitive (ts) v-*abl* mutant (*4*, *12*). We detected GAS binding activities similar to those of STF-IL4 and STF-IL7 in extracts derived from cells grown at permissive temperatures at which v-Abl is active (Fig. 1A). With a shift to nonpermissive temperatures and abrogation of v-Abl tyrosine kinase activity, the GAS binding complexes disappeared rapidly (Fig. 1A). The loss of GAS binding activity at nonpermissive temperatures was not a result of decreased cell viability (*12*, *13*). Therefore, we conclude that in A-MuLV–transformed pre–B cell lines, complexes similar to STF-IL4 and STF-IL7 are formed in a v-Abl–dependent manner.

To confirm that the GAS binding complexes were STF-IL4 and STF-IL7, we used antibody supershifts and oligonucleotide competition assays. An antibody that recognizes STF-IL7, but not STF-IL4 (*14*, *15*), specifically supershifted the higher mobility complex in A-MuLV–transformed pre–B cells (Fig. 1B). The complex with lower mobility was identified as STF-IL4 by competition with unlabeled oligonucleotides containing a specific STF-IL4 binding site from the I-ε germline promoter (*11*, *16*).

To examine the protein components of the constitutive GAS binding complexes, we used multimerized GAS elements linked to agarose beads to precipitate proteins with high affinity for the oligonucleotide (*17*). Tyrosine-phosphorylated proteins of 94, 100, and 116 kD were precipitated from whole-cell extracts prepared from A-MuLV–transformed pre–B cells (Fig. 1C). The tyrosine-phosphorylated protein of 94 kD (p94) was previously identified as a component of STF-IL2 and STF-IL7 by oligonucleotide-affinity precipitation and appears to be an isoform of Stat5 (*14*, *15*, *18*). The 100-kD protein may be Stat6, a component of STF-IL4 (*19*), because it was recognized by an antibody to Stat6 (*11*). In the pre–B cell line transformed with the temperature-sensitive mutant of v-*abl*, p94 and p100 (Stat5 and Stat6) were tyrosine-phosphorylated at permissive temperatures, and upon a shift to nonpermissive temperatures, the tyrosine phosphorylation of these proteins was reduced as detected on blots with antibody to phosphotyrosine (Fig. 1C). Tyrosine phosphorylation of p116 was observed at both temperatures. Stat1 (p91) did not appear to be activated in A-MuLV–transformed pre–B cells (*11*, *16*), suggesting that the transformation process by v-*abl* might specifically activate some but not all STAT signaling pathways. Abrogation of GAS binding activities at nonpermissive temperatures was apparently not the result of loss or inactivation of components of IL-4 or IL-7 signaling pathways. Even after 20 hours of growth at nonpermissive temperatures, IL-4 and IL-7 induced GAS binding activities in the temperature-sensitive cells (Fig. 2).

Because STATs are thought to be phosphorylated by Jak protein kinases (*20*), we investigated whether constitutive phosphorylation and activation of STATs in

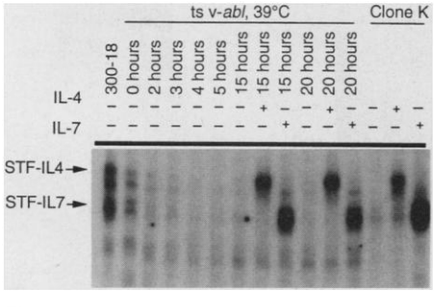

Fig. 2. Intact IL-4 and IL-7 signaling in cells expressing the temperature-sensitive mutant of v-Abl. Cells were grown at 39°C for the indicated times, IL-4 or IL-7 was added for 15 min, and whole-cell extracts were prepared and analyzed by EMSA (Fig. 1).

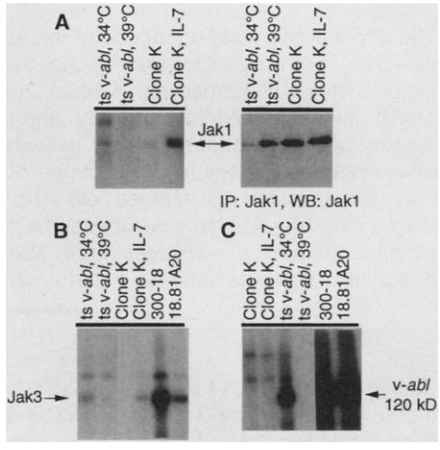

Fig. 3. Constitutive activation of Jak1 and Jak3 in A-MuLV–transformed pre–B cell lines. Jak1 (**A**), Jak3 (**B**), and Abl (**C**) were immunoprecipitated from Clone K cells that were either untreated or treated with IL-7, cells transformed with temperature-sensitive mutant of v-*abl* grown at permissive or nonpermissive temperatures, and A-MuLV–transformed pre–B cell lines 300-18 and 18.81A20. In vitro kinase assays were done as described (*26*), except that the wash and kinase buffers contained 20 mM Hepes, pH (7.4), 2 mM $MnCl_2$, 10 mM $MgCl_2$, 1 mM dithiothreitol, 150 mM NaCl, 0.1 mM sodium orthovanadate, and 0.4 mM phenylmethylsulfonyl fluoride. One-half of each sample was analyzed by SDS-PAGE (7% gel) and autoradiography, and the other half was blotted with antibodies to Jak1, Jak3, and Abl (*11*). Constitutive activation of Jak1 and Jak3 by tyrosine phosphorylation was also confirmed by protein immunoblotting of Jak1 and Jak3 immunoprecipitates with antibody to phosphotyrosine (*11*). IP, immunoprecipitating antibody; WB, immunoblotting antibody.

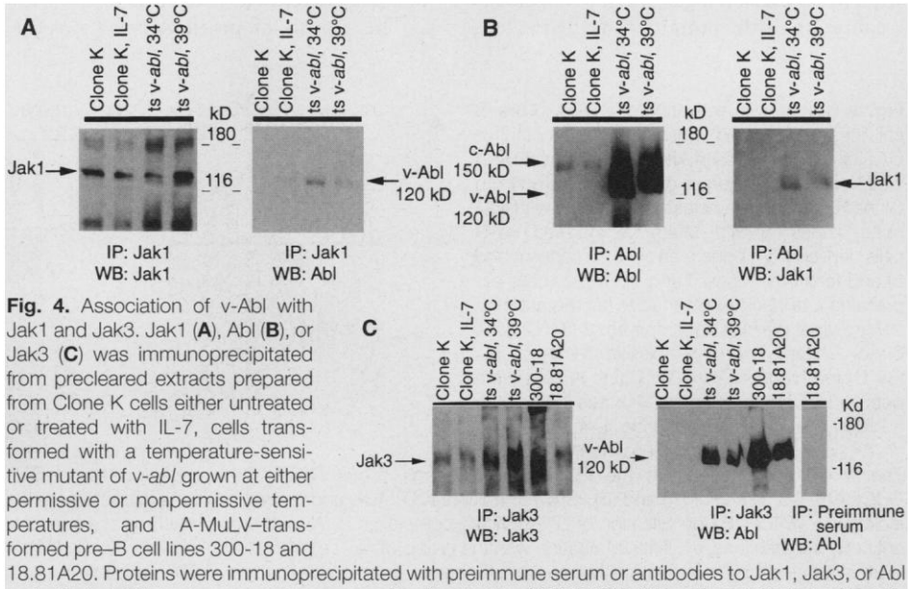

Fig. 4. Association of v-Abl with Jak1 and Jak3. Jak1 (**A**), Abl (**B**), or Jak3 (**C**) was immunoprecipitated from precleared extracts prepared from Clone K cells either untreated or treated with IL-7, cells transformed with a temperature-sensitive mutant of v-*abl* grown at either permissive or nonpermissive temperatures, and A-MuLV–transformed pre–B cell lines 300-18 and 18.81A20. Proteins were immunoprecipitated with preimmune serum or antibodies to Jak1, Jak3, or Abl as indicated (IP). Immune complexes were fractionated by SDS-PAGE (7% gel) and blotted with antibodies to Jak1, Jak3, or Abl. The blots were then stripped and probed with antibodies to Abl, Jak1, or Jak3 as indicated (WB). Immunoprecipitation with preimmune serum is shown as a control.

A-MuLV–transformed pre–B cells resulted from the activation of Jak1 and Jak3 in the absence of cytokines. Jak1 and Jak3 were activated in Clone K cells treated with IL-7, whereas in A-MuLV–transformed pre–B cell lines, these kinases were constitutively active (Fig. 3) (*11*). In pre–B cells transformed with a temperature-sensitive mutant of v-*abl*, Jak1 and Jak3 were active at permissive temperatures, and upon a shift to nonpermissive temperatures, they became inactive (Fig. 3). Thus, in A-MuLV–transformed pre–B cells, the activities of Jak1 and Jak3 correlate with that of the v-Abl protein tyrosine kinase.

We next investigated the possible interaction between v-Abl and Jak1 or Jak3. Jak1 or Jak3 was immunoprecipitated from extracts of pre–B cells expressing the temperature-sensitive mutant of v-Abl. The v-Abl protein was detected in Jak1 and Jak3 immune complexes (Fig. 4) (*21*). Jak1 was also detected in v-Abl immunoprecipitates (Fig. 4B). These observations were confirmed with several other antibodies to Jak1 and Jak3 (*11*). We were unable to coimmunoprecipitate either Jak1 or Jak3 with the 150-kD c-Abl protein in the non–A-MuLV–transformed pre–B cell line Clone K (Fig. 4).

Two models have been proposed for the mechanism of growth factor independence in cells transformed by oncogenic forms of Abl (*5, 7, 22, 23*). According to one model, v-*abl* may increase the synthesis of RNAs encoding cytokines. Secreted cytokines could then bind their receptors and transduce their signals. Alternatively, v-Abl may abrogate the need for cytokines to bind to their receptors by interacting with components of cytokine signal transduction pathways. In an attempt to distinguish between the two models, we assessed the amount of RNA encoding IL-4 and IL-7 in the pre–B cells transformed with the temperature-sensitive mutant of v-*abl*. We were unable to detect IL-4 or IL-7 mRNAs by reverse transcription–polymerase chain reaction (RT-PCR) in these cells (*11*). In addition, supernatants from cultures of A-MuLV–transformed pre–B cell lines did not induce GAS binding activities in Clone K cells (*11*). Although, we cannot exclude the possibility that these transformed cells produce other cytokines, the lack of mRNA for IL-4 and IL-7 and our observation that Jak1 and Jak3 coimmunoprecipitate with v-Abl are consistent with the latter model. Our results suggest that A-MuLV constitutively activates the IL-4 and IL-7 signaling pathways. Constitutive activation of Jak-STAT signaling has also been observed in T cells transformed with human T cell leukemia virus–I (*24*). Activation of cytokine signal transduction pathways might be a mechanism by which some transforming viruses induce proliferation of their target cells.

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21. Extracts from 18.81A20 cells were immunoprecipitated with antibodies against either Jak1 (directed to amino acids 797 to 817), Jak3 [directed to amino acids 1104 to 1124], or Abl (directed to the COOH-terminus of the Abl protein), and the immunoprecipitates were immunoblotted with a monoclonal antibody to Abl (Oncogene Science). Analysis by densitometry suggested that Jak3 and Jak1 immunoprecipitates contained ~10 to 20% of the v-Abl proteins found in the Abl immunoprecipitations. We find that the v-Abl antibody depletes ~90% of total cellular v-Abl from the 18.81A20 extracts. Coimmunoprecipitation of these proteins was not disturbed by increasing the NaCl concentration to 1M or by adding 0.1% SDS.
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Lateral Interactions in Primary Visual Cortex: A Model Bridging Physiology and Psychophysics

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Recent physiological studies show that the spatial context of visual stimuli enhances the response of cells in primary visual cortex to weak stimuli and suppresses the response to strong stimuli. A model of orientation-tuned neurons was constructed to explore the role of lateral cortical connections in this dual effect. The differential effect of excitatory and inhibitory current and noise conveyed by the lateral connections explains the physiological results as well as the psychophysics of pop-out and contour completion. Exploiting the model's property of stochastic resonance, the visual context changes the model's intrinsic input variability to enhance the detection of weak signals.

A characteristic feature of primary visual cortex (V1) in primates is its topographic organization into columns (*1*) of neurons responding to oriented stimuli within restricted regions of the visual field. A neuron's classical receptive field (CRF) is defined as that region of visual space in which an individual stimulus will elicit a response. Neuroanatomical studies (*2*), however, have revealed an extensive network of long-range horizontal processes that extend far beyond the representation of the classical receptive field in cortex. While these lateral connections primarily link regions of cortex whose neurons prefer stimuli with similar orientations, the contribution of these connections in the processing of visual information remains unclear. Gilbert

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