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Transcriptional activation of a *ras*-like gene (*kir*) by oncogenic tyrosine kinases

(differential gene expression)

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ABSTRACT **We report the characterization of a member of the *ras* gene family that is overexpressed in cells transformed by *abl* tyrosine kinase oncogenes. The gene, named *kir* (for kinase-inducible *ras*-like), is induced at the transcriptional level. *kir* mRNA has a rapid turnover and encodes a protein of 33 kDa with guanine nucleotide-binding activity but undetectable intrinsic GTPase activity. *kir* was cloned by differential screening of genes present in fully malignant versus growth factor-independent cell lines expressing wild-type or mutant forms of BCR/ABL. BCR/ABL and v-Abl induce transcription of the *kir* gene via specific signaling pathway(s), but *kir* overexpression alone is not sufficient to mediate transformation.**

BCR/ABL is a fusion gene resulting from the t(9;22) chromosomal translocation, a cytogenetic marker of chronic myelogenous leukemia (CML) (1). Fusion of N-terminal BCR sequences upstream of the Src homology 3 (SH3) domain of c-Abl is sufficient to activate the tyrosine kinase and F-actin-binding activities of c-Abl and render the chimeric protein oncogenic (2–4). BCR/ABL proteins are related to the v-*abl* oncogene product of Abelson murine leukemia virus, a retrovirus that causes B-cell lymphoma in mice (5). *BCR/ABL* can induce leukemias in mice and transform hematopoietic cells and fibroblasts *in vitro* (6–12).

To isolate genes which may function as downstream effectors of *BCR/ABL*, we used a differential screening approach aimed at isolating tumor-promoting genes rather than growth-related genes, based on the following strategy. Pre-B lymphoid cells which express either wild-type *BCR/ABL* (P210-WT) or a mutant carrying a single amino acid change at the major autophosphorylation site of the protein (P210-Y1294F) can grow *in vitro* in the absence of growth factors (13). Cells expressing the mutant protein cause infrequent, long-latency, low-morbidity leukemias *in vivo*, whereas cells transformed by P210-WT develop into short-latency, highly metastatic, lethal leukemias (13). Our rationale was that cells transformed by P210-WT or P210-Y1294F would express similar sets of genes accounting for their growth factor-independent phenotype but should differ in expression of genes related to the fully malignant phenotype.

The present work reports the cloning of a gene, *kir* (for kinase-inducible *ras*-like),** whose expression is tightly regulated by BCR/ABL and v-Abl oncogenic protein-tyrosine kinases. While this paper was under review, Maguire *et al.* (14) reported the cloning of *gem*, an immediate-early gene expressed in mitogen-stimulated T cells. *kir* appears to be highly related to *gem* and is a close relative of the type II diabetes-associated *rad* gene (14, 15). *kir* and *gem* are almost identical in their coding sequences but diverge in their 5′ untranslated sequences. The cloning of two genes that encode identical or highly related proteins and are inducible by oncogenic tyrosine kinases or phorbol esters suggests an important role for this subfamily of small guanine nucleotide-binding proteins in the control of cell responses to growth stimuli.

MATERIALS AND METHODS

Retroviruses and Growth Factor-Independent Cell Lines. The construction of retroviral vectors encoding BCR/ABL wild-type and mutant proteins has been reported (13, 16). The interleukin 7 (IL-7)-independent clone H cell line and the granulocyte/macrophage-colony-stimulating factor (GM-CSF)-independent DAGM cell line expressing mutants of BCR/ABL were derived as reported (13). The parental, growth factor-dependent clone H and DAGM cells were cultured in media supplemented with recombinant IL-7 or GM-CSF, respectively.

Differential Display of mRNAs by Polymerase Chain Reaction (PCR). The technique was performed as described by Liang and Pardee (17). Reverse transcription reactions were performed with 2 μg of RNase-treated RNA from clone H/P210-WT and clone H/P210-Y1294F and were amplified as described (17). P10 (5′-GATGTTCCACT-3′) and $T_{12}GC$ were the primer pair used to detect *kir*. The *kir* PCR product was eluted from the sequencing gel, reamplified, and subcloned with the TA cloning system (Invitrogen) as reported (17).

cDNA Cloning and Sequencing. The *kir* fragment was removed from the TA vector and used as a probe to screen the 70Z/3 pre-B lymphoid mouse cDNA library kindly provided by Michael Gilly and Randolph Wall (University of California, Los Angeles). The filters (≈10^6 plaques) were hybridized overnight at 42°C with labeled *kir* DNA (specific activity, 10^9 cpm/μg) at 3–5 × 10^6 cpm/ml in 50% formamide/5× SSPE/0.2% SDS/6% dextran sulfate containing herring sperm DNA at 100 μg/ml (1× SSPE is 150 mM NaCl/10 mM sodium phosphate, pH 7/1 mM EDTA). The filters were washed under high-stringency conditions (15 mM NaCl/1.5 mM sodium citrate, pH 7/0.1% SDS at 55°C for the final washes). Six of 140 positive plaques were selected for secondary and tertiary screening. Plasmids (pBS KS; Strat-

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte/macrophage-colony-stimulating factor; GST, glutathione *S*-transferase; SH2 and SH3, Src homologies 2 and 3.

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**The nucleotide sequence reported in this paper has been deposited in the GenBank data base (accession no. U13053).

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agene) containing cDNA inserts of 2 kb were recovered from λZAP phagemids. Subclones of the 2-kb insert were sequenced by the dideoxy technique (Sequenase; United States Biochemical) using insert-flanking primers (T7 and T3) and internal primers.

RNA Analysis by Northern Blotting and S1 Nuclease Protection. The 103/BCL-2/4 cell line used in the time-course experiments has been described (18). Northern filters were hybridized under the conditions described above for the library screening, with full-length *kir* or c-*abl* cDNAs as probes. S1 nuclease protection assays were performed according to Weaver and Weissman (19), with 25 μg of total RNA from the indicated clone H or DAGM cell lines. The *kir* probe corresponded to a 778-bp *Pvu* I–*Hin*dIII fragment from pBS/kir, 5′-end-labeled at the *Hin*dIII site with [γ-^{32}P]ATP (6000 Ci/mmol; Amersham; 1 Ci = 37 GBq) and T4 polynucleotide kinase (20 units; Pharmacia). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe corresponded to a *Pvu* II–*Nco* I fragment (464 bp) 5′-end-labeled at the *Nco* I site in the coding sequence of rat GAPDH (20). The RNA samples were incubated overnight at 52°C with 50,000 cpm of radiolabeled *kir* and GAPDH probes. The S1 digestion was performed as described (19), and products of the reaction were separated by electrophoresis in a 5% polyacrylamide sequencing gel. The gel was dried and exposed on Kodak film for 24–48 hr at 70°C.

RESULTS

***kir*, a Member of the *ras* Superfamily, Is Overexpressed in Cells Transformed by a Tyrosine Kinase Oncogene.** Total RNA from clone H/P210-WT and clone H/P210-Y1294F cell lines was screened by the technique of differential display of mRNAs by PCR (17). From a series of 100 experiments (≈5000 mRNAs displayed), 46 partial cDNA clones were overrepresented in the clone H/P210-WT RNA population. Five of these displayed differential expression by Northern blot analysis and were further studied. This relatively low number was consistent with the rationale that both clone H/P210-WT and clone H/P210-Y1294F were closely matched cell populations expressing very similar sets of genes.

One particular clone, called *kir*, showed the highest quantitative variation of expression and was selected for detailed analysis (Fig. 1). A full-length cDNA encoding this mRNA was isolated and sequenced. Comparison with the GenBank data base (December 1993) revealed 40–75% homology at the amino acid level with members of the *ras* gene family. The size of full-length *kir* cDNA is 2010 bp. It is composed of an open reading frame of 888 bp surrounded by 120 bp of 5′ and 1002 bp of 3′ untranslated sequences. Sequences adjacent to the first of three in-frame ATG codons match best with the consensus Kozak sequence (21). The long 3′ untranslated sequence contains a polyadenylylation signal preceding the poly(A) tail, and four perfect repetitions of the ATTTA motif conferring mRNA instability (22).

Murine *kir* and *gem* (14) nucleotide sequences are 98.4% identical in their coding sequence and most likely encode the same protein or very highly related proteins, referred to from now as Kir/Gem. The 5′ untranslated sequence of murine *kir* diverges significantly from the 5′ untranslated sequence of murine *gem* (Fig. 2*A*). The *kir* and *gem* transcripts may be two spliced variants encoded by the same locus or may be encoded by two different loci with high exonic sequence conservation.

The Kir/Gem protein has an estimated molecular weight of 33,838 and is composed of 295 amino acid residues. The Kir/Gem sequence shows a high degree of homology with Ras-like GTPases, including mammalian Rap2A, RalA, and R-Ras proteins (Fig. 2*B*). The homology is particularly high

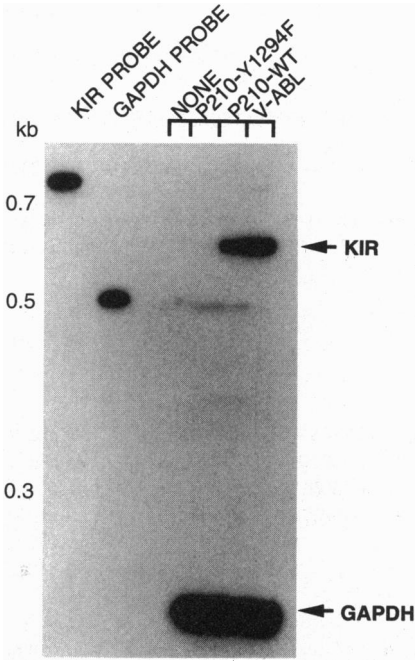

FIG. 1. Differential expression of the *kir* clone. Total RNA (25 μg) from the parental lymphoid cell line clone H (none), or clone H cell lines expressing the autophosphorylation mutant P210-Y1294F, P210-WT, or the v-*abl* oncogene product were analyzed by S1 nuclease protection assay to measure the abundance of *kir* mRNA. The protected fragments representative of *kir* and GAPDH mRNAs are indicated. The amounts of RNA analyzed were equivalent as judged by the levels of GAPDH mRNA in each sample.

between Kir/Gem and Rad (14, 15). Kir/Gem and Rad show 61% amino acid identity and 74% conservation. Kir/Gem and Rad diverge in the conserved G1 and G3 domains involved in GTP hydrolysis. The substitutions commonly found in transforming Ras proteins affect residues including Gly-12, Ala-59, and Gln-61 (23, 26). These mutations reduce the intrinsic GTPase activity of Ras proteins, reduce their sensitivity to GTPase-activating proteins (GAPs), and lock the proteins in a GTP-bound active state. None of these three residues are conserved in Kir/Gem and Rad, reminiscent of eukaryotic translation elongation factors (27). These proteins may cleave GTP by a different mechanism or simply lack GTPase activity. The G2 domain involved in effector function and interaction of Ras with GAPs (28–31) is not conserved in Kir/Gem and Rad. Kir/Gem has the conserved ED(S/T)Y sequence corresponding to the carboxyl half of G2 but diverges in the first half of the domain, which contains residues critical for GAP interaction (32). This implies that Kir/Gem and Rad may not be regulated by known GAP-like proteins. Kir/Gem and Rad do not end with one of the three lipid-modification motifs—CAAX (where A represents an aliphatic residue), CXC, or CC—typical of most Ras-like GTPases but have a cysteine residue at position 289. Kir/Gem and Rad may be modified by a different enzyme or may not be modified.

A GST–Kir fusion protein was tested for nucleotide binding and GTPase activity (Fig. 2*C* and data not shown). The GST–Kir protein exhibited GDP binding activity with an estimated K_d of 7 μM, a value significantly higher than that of Ras proteins (10 nM). The binding was specific for guanine nucleotides, since nonradioactive GDP or GTP, but not ATP, UTP, or CTP, competed with [^{3}H]GDP (data not shown). Hydrolysis of GTP by GST–Kir was undetectable under the conditions used.

***kir* Overexpression Is Associated with Specific Signaling Events Triggered by BCR/ABL.** If *kir* overexpression were inherent to secondary events associated with the transformed

A

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Mu-kir    CCTGCCAGCGGACATCACACAGCCTCGGACTGCGGAGCCCGCATCGCAGACGTCAGGCGTTG
          ::: :  :     :::          :::: :  :  :::  :   : :        :
Mu-gem    ATACCCATCAAATGAAGCACCAAAGACAACTGTGTTGAGCGCCCCTGGGCCTGGCTCATTCT

Mu-kir    CAGCAGGCGGATCGACGGCAGACGCCCACCCAGCGCCCAGTGCAGCCAAGATCCCCGCAATG
           :           :   :   :     : :: : :     : : ::::::::::::::
Mu-gem    TA-----------ATCATATTGCCATCTTTACTGCTCTGATT-CCTAGGATCCCCGCAATG
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B

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Kir       MTLNNVTMRQGTVGMQPQQRWSIPADARHLMVQKDPHPCNLRNRHSTAPEEHCRRSWSSDSt
Rad                           MPVDERDLQAALTPGALTAAAAGTGTQGPRLDWPEDSEDs
RalA                                                              Ma
RRas                                                   MSSGAASGTGRGRPRGg
Rap2A
N-Ras

Kir       DSVISSESGNtYYrVVli GEQGVGKS tLaNIFAGVHDSM dSdCEVLGEDtY ErTlVV
Rad       LSSGGSDSDEsV-k-lll -AP----- a-aRI-GGVEDGP eAeA  AGHt- DrSi--
RalA      ANKPKGQNSLaLHk-imv -SG----- a-tLQ-MYDEFVE dYePT KA-s- RkKv-l
RRas      GPGPGDPPPSETHkl-vv -GG----- a-tIQ-IQSYFVS dY-PT I--s- TkICS-
Rap2A             MRE-k--vl -SG----- a-tVQ-VTGTFIE KY-PT I--F- RkEiE-
N-Ras             MtE-kl-vv -AG----- a-tIQLIQNHFVD eY-PT I--s- RkQv-i

Kir       DGEsaTIIllDmW eNKG  En EWlhDHCMQVdayLiVySITdRASFeKAsElRIQlRRA
Rad       ---EaSLMvY-iW eQD-    GRWlPGHC-AMdayvi-y-vTdKG--eKAsEl-V-lR-A
RalA      ---Ev   Qi-iL dTA- Q-dYAAir-NYFRSegf-C-f--TeME--AATaDF-E-iL-V
RRas      --Ipa   R--iL dTA- Q-eFGAmreQY-RAHgf-l-fa-NdRQ--nEVgKlFT-iL-V
Rap2A     -sSps   v-eiL dTA- T-qFASmr-LYiKNqgfil-y-lVnQQ--qDYKPm-D-iI-V
N-Ras     ---tC   l--iL dTA- Q-eYSAmr-QY-RTegf-C-fa-NnSK--ADINLY-E-iK-V

Kir       rqTeDiPii LVGNKSDL VRCReVSVSEgrACAVVfDCK FIETSA AVQHNVKelFEGiV
Rad       rqTd-v-ii -------- VRS-e--VD-gr-C-VVfDCK ------ ALHH--QAl-EGv-
RalA      keDenv-Fl -------- EDK-q--VE-akNR-EQwNVN yv---- KTRA--DKv-FDlm
RRas      kdRd-F-vv -----a-- ESQ-q-pRS-aS-FgASHHVA yF-a-- KLRL--DeA-EQl-
Rap2A     kRYekv-vi -----V-- ESE-e--SS-gr-L-EEwGCP -m---- KSKTM-Del-AEi-
N-Ras     kdSd-v-mv -----C-- PT RT-DTKqahEL-KSyGIP ------ KTRQG-EdA-YTl-

Kir       RqvRLRr  DSKeKnERRLAYQKRRESIPRKARRFWGKIVAKNNKNMAFKLKSKS CHDLSVL
Rad       -qi-LRr  -SKeAnARRQAGTRRRESLGKKAKRFLGRIVARNSRKMAFRAKSKS CHDLSVL
RalA      -eI-ARkME-SKeKnGKKKRKSLAKRIRER CCIL
RRas      -Av-KYQEQeLPPSPPSAPRKKGGGCP CVLL
Rap2A     -qmNYAAQP-KDdPC CSACNIQ
N-Ras     -ei-QYrMKKLNSSdDGTQGCMGLP CVVM
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C

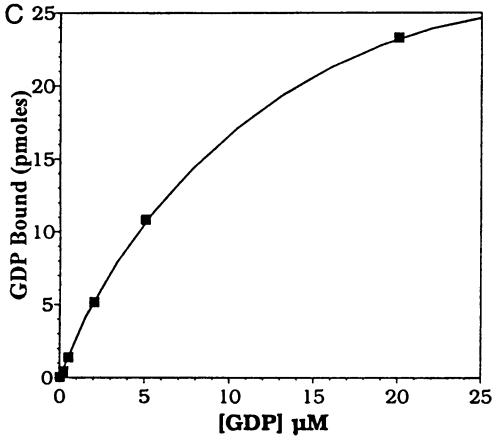

FIG. 2. Characterization of the *kir* gene. (*A*) *kir* and *gem* have different 5′ untranslated sequences. The 5′ untranslated sequence of murine *gem* was retrieved from GenBank (accession no. U10551). The sequences depicted include the first ATG start codon. Colons indicate nucleotide identity. Gaps were introduced to maximize the alignment. (*B*) Comparison of amino acid sequences of murine Kir with human Rad and with RalA, R-Ras, Rap2A, and N-Ras. The murine Gem sequence was identical to murine Kir except for four amino acid residues: Kir residues Ile-23, Ser-56, Arg-236, and Arg-241 are Met-23, Thr-56, Glu-236, and Pro-241 in Gem (14). Dashes indicate the regions of identity and lowercase letters the regions of homology. These regions are defined by amino acids conserved in Kir and at least three of the six Ras-like proteins represented. The five domains (G1–G5) conserved in all GTP-binding proteins are in boxes. Bold letters represent highly conserved residues found mutated in transforming Ras (23). The lipid-modification sites are in italics. Gaps (blank spaces) were introduced to maximize the alignment. The search for homology was performed through the National Center for Biotechnology Information (Bethesda, MD) with the BLAST network service. The complete sequences were retrieved from the GenBank data base. (*C*) A glutathione *S*-transferase (GST)–Kir fusion protein was constructed by subcloning a DNA fragment encoding the carboxyl-terminal 277 amino acids of Kir into pGEX-3X (Pharmacia); fusion proteins expressed in BL21 cells were purified as described (24). The filter binding assays were done as described (25). GST–Kir (27 pmol) was incubated with the indicated concentrations of [^{3}H]GDP (8 Ci/mmol; NEN) at 22°C until binding reached equilibrium (typically 30 min). The reactions were stopped and the mixtures were filtered through nitrocellulose (25), and the radioactivity retained on the filters was measured by scintillation counting.

phenotype, we would expect cells expressing any nontransforming mutant of BCR/ABL to lack *kir* mRNA. If *kir* expression were induced by a specific signaling pathway, BCR/ABL mutants affected in distinct pathways should differentially induce *kir*. Several signaling-deficient mutants of BCR/ABL-P185, including an autophosphorylation mutant (P185-Y813F) (13, 16), a SH2 mutant (P185-R552L) (16), and a Grb2-binding-site mutant (P185-Y177F) (16, 33), have been described. In complementation assays, only the transforming activity of the SH2 mutant can be rescued by hyperexpression of c-*myc*, indicating that the SH2 domain signals through a distinct pathway (16). High levels of *kir* expression were detected in cells transformed by wild-type P185 and in cells expressing the SH2 mutant when compared with the parental DAGM cell line (Fig. 3, lanes 1, 2, and 4). In contrast, cells expressing P185-Y813F or P185-Y177F showed reduced or undetectable levels of *kir* mRNA (lanes 3 and 5). These results suggest that the autophosphorylation

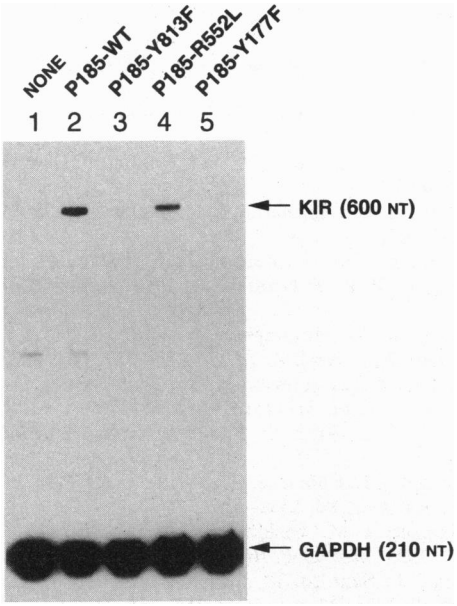

FIG. 3. Relative abundance of *kir* mRNA in growth factor-independent myeloid cell lines expressing various signaling-deficient mutants of BCR/ABL-P185. The S1 nuclease protection assay was performed (as in Fig. 1) on total RNA from DAGM expressing the indicated BCR/ABL proteins (lanes 2–5). Lane 1 (none) represents total RNA from the parental GM-CSF-dependent DAGM cells. The protected fragments representative of *kir* and GAPDH mRNAs are indicated. The background band above the GAPDH signal is a product of partial digestion of the GAPDH probe.

site and the Grb2-binding site of BCR/ABL trigger a common or converging pathway(s) which leads to *kir* expression, whereas the SH2 domain activates a distinct pathway involving c-*myc* but is not essential for *kir* expression.

Kinetics of *kir* Induction by a Temperature-Sensitive v-Abl Tyrosine Kinase. To further document the requirement for Abl tyrosine kinase activity for kir expression, we used a cell line expressing a temperature-sensitive mutant of v-*abl* (18). In cells shifted from nonpermissive (39.5°C) to permissive (34°C) temperature, recovery of Abl tyrosine kinase activity occurred within 1 hr after the shift (data not shown). *kir* was induced by the temperature downshift, and the kinetics of *kir* expression followed the kinetics of reactivation of the kinase (Fig. 4*A*). Quantitation of the data showed that *kir* mRNA increased ≈200-fold after 25 hr at 34°C relative to levels at 39.5°C. Levels of GAPDH mRNA also increased (≈30-fold) as a result of cells reentering the cell cycle (data not shown and ref. 18), whereas *abl* mRNA did not increase as much (≈3-fold) during the course of the experiment (Fig. 4*A*). In the reverse experiment, *kir* mRNA decreased rapidly in cells shifted from 34°C to 39.5°C (data not shown).

To determine whether *kir* induction was transcriptional and/or was due to an increase in mRNA stability, total RNA from 103/BCL-2/4 cells treated with actinomycin D and grown at 39.5°C or 34°C (18) was analyzed for *kir* expression. Although kir mRNA levels were higher at 34°C than at 39.5°C, *kir* mRNA appeared to decay rapidly in cells kept at both temperatures after addition of actinomycin D (Fig. 4*B*). The half-life of the *kir* message was estimated to be ≈60 min in cells grown at 34°C and ≈30 min in cells grown at 39.5°C. The data were consistent with *kir* mRNA having a rapid turnover as inferred from the presence of the ATTTA motifs in its 3′ untranslated sequence. That *kir* mRNA stability is not dramatically increased at the permissive temperature suggests that *kir* induction is mostly transcriptional.

Assessing *kir* Function and Its Role in Transformation. To test whether Kir possessed transforming activity on its own,

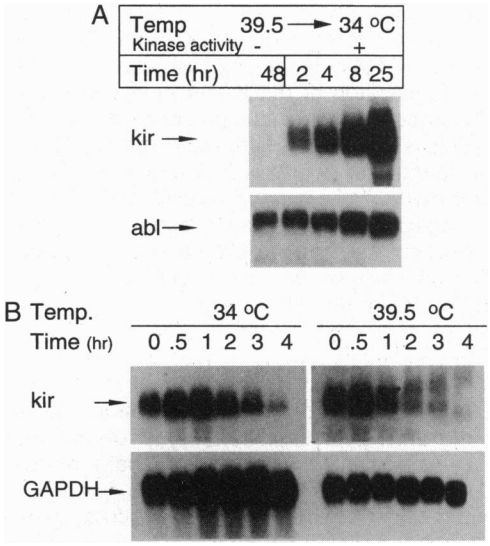

FIG. 4. (*A*) Kinetics of induction of *kir* expression by a temperature-sensitive v-Abl tyrosine kinase. The lymphoid cell line 103/BCL-2/4 expressing the temperature-sensitive v-Abl was shifted from nonpermissive (39.5°C) to permissive (34°C) temperature, and RNA samples were harvested at various times after the shift (18). Northern blots of the RNA samples were probed consecutively with radiolabeled *kir* and *abl* cDNAs. Levels of the respective mRNAs were quantified on a PhosphorImager (Molecular Dynamics). The fold induction was calculated as the ratio of RNA levels after the shift vs. RNA levels at 39.5°C (time 0). (*B*) 103/BCL-2/4 cells were grown at 34°C continuously or at 39.5°C for 20 hr prior to addition of actinomycin D (18). After addition of the inhibitor, cells were kept incubating at 34°C or 39.5°C, and RNAs were harvested at different times. Northern blots of the RNA samples were probed with *kir* and GAPDH cDNAs. The autoradiographs of the *kir* probe were exposed for 12 hr for the low-temperature samples and 72 hr for the high-temperature samples. The exposure time for the GAPDH probe was 5 hr for all samples. To measure *kir* half-life, levels of *kir* mRNA were quantified on a PhosphorImager and normalized to the levels of GAPDH mRNA.

retroviruses encoding murine *kir* and the neomycin-resistance gene were generated and tested in fibroblast transformation assays (11). From four independent experiments, we found that NIH 3T3 or Rat-1 fibroblasts expressing *kir* did not form large colonies in soft agar (data not shown). The lack

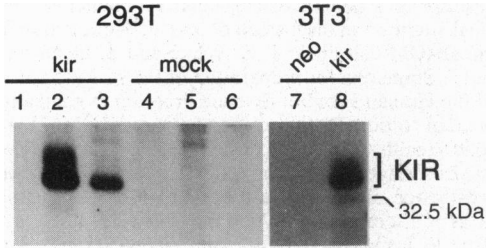

FIG. 5. Kir expression is maintained in NIH 3T3/Kir stable cell lines. Full-length *kir* cDNA was introduced into the pSRαMSVtkNeo retroviral vector (2) to produce *kir* retroviruses. 293T cells transfected with pSRαKIRtkNeo (lanes 1–3) or mock transfected (lanes 4–6) were labeled with [^{35}S]methionine/cysteine (Trans^{35}S-label; Amersham) for 90 min before lysis and immunoprecipitation (34) with polyclonal antisera (αk1 and αk2) raised against GST–Kir fusion protein. Lanes 1 and 4, preimmune serum; lanes 2 and 5, αk1; lanes 3 and 6, αk2. NIH 3T3/Kir cell lines were obtained after infection with *kir* retroviruses and G418 selection of the infected cells and were used in agar assays (11). Expression of the Kir protein in the control cell line NIH 3T3/Neo (lane 7) or in NIH 3T3/Kir (lane 8) cells was monitored by immunoprecipitation as described above, with the αk1 antiserum.

of clear transforming activity was not due to a toxic effect of *kir*, since cell lines stably expressing *kir* (NIH 3T3/Kir) were obtained after G418 selection of cells infected with *kir* retroviruses. Expression of the Kir protein was maintained in NIH 3T3/Kir cells grown in the presence of G418 for 12 days (Fig. 5). This is in contrast with the results of Maguire *et al.* (14), who found that overexpression of *gem* had a negative effect on growth of normal and transformed NIH 3T3 fibroblasts. Although this discrepancy could be explained by a difference in strength of the promoters driving *kir* or *gem* expression, further studies are required to determine the effects of Kir/Gem on cell growth.

DISCUSSION

The correlation between *kir* overexpression and the highly tumorigenic and metastatic phenotype of the *BCR/ABL*-expressing pre-B cells suggests that *kir* may be involved in processes of invasion or metastasis.

We found that the Kir/Gem protein on its own was not toxic *in vitro*. We tested whether Kir/Gem could synergize with BCR/ABL in fibroblast or hematopoietic cell transformation assays (data not shown). Increasing *kir* dosage did not have a dramatic effect on the transforming activity of *BCR/ABL*, nor did it complement the transforming activity of *BCR/ABL* mutants. *kir* may require two signals to function downstream of *BCR/ABL* in transformation, one leading to its transcriptional induction, and the other causing a structural alteration in the protein leading to its activation. *kir* inhibition by a dominant negative approach similar to that used to analyze Ras function (35) will be more definitive in determining a role for Kir in *BCR/ABL* transformation.

We noted that *kir* shares some characteristics with immediate-early genes such as c-*fos* and c-*myc*, including its inducibility and its short half-life, as well as its sustained expression in transformed cells (36). Although *kir* and *gem* probably encode the same protein, the two genes (isolated from a pre-B-cell and a T-cell library, respectively) possess two distinct 5′ untranslated sequences. *kir* and *gem* may represent cell type-specific transcripts encoding highly related proteins, similar to the T- and B-cell specific Itk and Btk tyrosine kinases (37). Alternatively, they may represent two alternative spliced forms that are differentially produced in cells by phorbol ester- or tyrosine kinase-mediated pathways.

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