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Genetics

# *TAL2*, a helix–loop–helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia

(chromosome translocation/helix–loop–helix protein)

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**ABSTRACT** **Tumor-specific alteration of the *TAL1* gene occurs in almost 25% of patients with T-cell acute lymphoblastic leukemia (T-ALL). We now report the identification of *TAL2*, a distinct gene that was isolated on the basis of its sequence homology with *TAL1*. The *TAL2* gene is located 33 kilobase pairs from the chromosome 9 breakpoint of t(7;9)(q34;q32), a recurring translocation specifically associated with T-ALL. As a consequence of t(7;9)(q34;q32), *TAL2* is juxtaposed with sequences from the T-cell receptor β-chain gene on chromosome 7. *TAL2* sequences are actively transcribed in SUP-T3, a T-ALL cell line that harbors the t(7;9)(q34;q32). The *TAL2* gene product includes a helix–loop–helix protein dimerization and DNA binding domain that is especially homologous to those encoded by the *TAL1* and *LYL1* protooncogenes. Hence, *TAL2*, *TAL1*, and *LYL1* constitute a discrete subgroup of helix–loop–helix proteins, each of which can potentially contribute to the development of T-ALL.**

Alteration of the *TAL1* gene is the most common genetic lesion known to be associated with T-cell acute lymphoblastic leukemia (T-ALL). Tumor-specific alterations of *TAL1* arise by either of two distinct mechanisms. First, almost 25% of T-ALL patients exhibit a nearly precise 90-kilobase-pair (kbp) deletion of upstream sequences from one allele of the *TAL1* locus (1, 2). The site specificity of this deletion is apparently mediated by aberrant activity of the immunoglobulin recombinase (1, 3). Second, an additional 3% of T-ALL patients harbor t(1;14)(p34;q11), a chromosome translocation that transposes *TAL1* from its normal location on chromosome 1 into the T-cell receptor α/δ-chain complex on chromosome 14 (4–8). Since structural lesions of *TAL1* are commonly and exclusively associated with T-ALL, *TAL1* gene alteration is likely to be a critical factor in T-cell leukemogenesis.

The *TAL1* gene product is homologous to a number of proteins that are involved in the control of cell growth and differentiation (7, 8). The region of homology is restricted to a 56-amino acid domain that has the potential to form two amphipathic helices separated by an intervening loop (9). Several helix–loop–helix (HLH) proteins are proposed to function as transcriptional regulatory factors based on their ability to bind *in vitro* to the E-box motif (CANNTG) of eukaryotic transcriptional enhancers (reviewed in refs. 9–12). The enhancer-binding HLH proteins include E47 and E12, two distinct but related polypeptides encoded by the *E2A* gene (9). The E47 protein binds the E box as a homodimer that is apparently stabilized by protein–protein interactions involving the amphipathic helices of its HLH domain. Interestingly, the *E2A* gene products (E47 and E12) can form heterologous complexes, presumably heterodimers, with other HLH proteins, and these heterodimers also bind the E-box sequence with high affinity (10). We recently observed that *TAL1* polypeptides form heterologous complexes *in vitro* with either E47 or E12; since the resultant *TAL1*/E47 and *TAL1*/E12 heterodimers specifically recognize the E-box motif, the *TAL1* gene product may also function *in vivo* as a transcriptional regulatory factor (13).

The HLH proteins all share a moderate level of sequence homology that is likely to be required for adoption of the domain's proposed tertiary structure (9). However, those proteins that were identified on the basis of similar functional properties exhibit far more dramatic similarities in their HLH domains. It is noteworthy, therefore, that the HLH domain of *TAL1* is especially related to that encoded by *LYL1*, a gene on chromosome 19 that was also identified on the basis of tumor-specific rearrangement in human T-cell leukemia (14). The HLH domains of *TAL1* and *LYL1* share an 87% level of amino acid sequence identity. Hence, these genes represent a subgroup of HLH proteins that can potentially promote the formation of T-ALL. It is conceivable that this subgroup includes members that have not as yet been recognized, and that these may also play a role in leukemogenesis—especially among the ≈70% of T-ALL patients who do not have detectable alterations of *TAL1* or *LYL1*. In this report we describe *TAL2*, a HLH gene on chromosome 9 that is transcriptionally activated by t(7;9)(q34;q32), a recurring translocation associated with human T-ALL (15–18).§

## MATERIALS AND METHODS

**PCR.** DNA amplification was performed by the PCR (19) using oligonucleotide primers HLH3 (MGRMGRRTXTTY-ACXAAYWSYMGRGARMG) and HLH4 (YARRAAX-YYRATRTAYTTCATXGC) (where M = A or C; R = A or G; X = A, C, G, or T; Y = C or T; W = A or T; and S = G or C). The reaction conditions have been described (20). The PCR products were fractionated by polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. After elution from the gel, the amplification products either were reamplified by PCR or were cloned into the *Sma* I site of M13mp18 for nucleotide sequence analysis.

**DNA Analysis and Cloning.** DNA analysis and cloning were conducted by standard procedures (21), essentially as described (1). RNase protection assays were performed with the RPA kit (Ambion, Austin, TX).

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Abbreviations: HLH, helix–loop–helix; T-ALL, T-cell acute lymphoblastic leukemia.

§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M81077 (mouse) and M81078 (human)].

**Fluorescence *in Situ* Chromosomal Hybridization.** Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. The *TAL2* probes (T33SS and T31SS) were 15 and 7 kbp, respectively, genomic fragments cloned in the vector pUC12 (see Fig. 1). The procedure used for fluorescence *in situ* hybridization is a modification (22) of the method described by Lichter *et al.* (23). Biotin-labeled probes were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics). Hybridization was detected with fluorescein-conjugated avidin, and chromosomes were identified by staining with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI).

## RESULTS

**Identification of the *TAL2* Gene.** To identify genes related to *TAL1* and *LYL1*, we used the PCR to amplify HLH-coding sequences from human genomic DNA. The PCRs were primed with a pair of degenerate oligonucleotides complementary to sequences encoding amino acids on the amino-terminal flank of helix I (oligonucleotide HLH3: 29 residues, 4096-fold degeneracy) and amino acids of helix II (oligonucleotide HLH4: 24 residues, 4096-fold degeneracy). The degeneracies were designed to allow equal hybridization with *TAL1* and *LYL1* and to accommodate silent changes in the second and third positions of their codons. After two successive rounds of PCR under typical thermal conditions, a DNA band of the expected size (≈150 bp) was visible by ethidium bromide staining. The DNA was isolated and cloned into the M13mp18 phage vector for nucleotide sequence analysis. Although half the clones contained *TAL1* gene sequences, the remaining clones contained a unique amplified sequence (not including primer sequences) that was distinct but related to both *TAL1* (82 of 103 identical nucleotides) and *LYL1* (79 of 103 identical nucleotides). Since most of the differences represent silent codon changes, this sequence (designated *TAL2*) potentially encodes a HLH domain with >78% amino acid identity to those of *TAL1* and *LYL1* (see below).

To evaluate the genomic structure of *TAL2*, the 156-bp PCR amplification product was used as a probe to screen a phage λ library of human DNA from the RPMI 8402 cell line (24). The four recombinant clones isolated in this manner were shown by restriction analysis to contain distinct but overlapping genomic DNA inserts; the inserts of two of these clones (λT3.2 and λT3.3) are illustrated in Fig. 1. A 0.75-kbp genomic DNA fragment (T3.1B2S) that encompasses the human HLH-coding sequence was then isolated (Fig. 1). Upon Southern analysis of mouse genomic DNA the T3.1B2S probe hybridized to a 13.6-kbp *Bam*HI fragment that presumably represents the murine homologue of *TAL2* (data not shown). A phage λ library of *Bam*HI-digested mouse genomic DNA was constructed, and recombinant clones that contain the 13.6-kbp *Bam*HI fragment were isolated by screening with T3.1B2S. The HLH-coding sequences of both human and mouse *TAL2* genomic DNA were then determined by nucleotide sequence analysis. As illustrated in Fig. 2*A*, the HLH motif of *TAL2* is found within a conserved open reading frame of 111 residues that is preceded by a splice acceptor signal and followed by a termination codon. Although this reading frame contains a potential initiator methionine codon (Fig. 2*A*), cDNA analysis will be required to ascertain the full coding capacity of spliced *TAL2* mRNA. Nevertheless, the phylogenetic conservation of *TAL2* coding potential is striking; the amino acid identities of human and mouse *TAL2* are nearly 85% for the entire open reading frame (94 of 111 residues) and 96% for the HLH motif (54 of 56 residues). The sequence alignment in Fig. 2*B* illustrates the high levels of amino acid identity between the human *TAL2* HLH domain and those encoded by *TAL1* (86%) and *LYL1* (79%).

**Expression of the *TAL2* Gene.** We initially developed a RNase protection assay to screen human cell lines for *TAL2* gene expression. Hence, genomic sequences that span the putative splice acceptor site upstream of the *TAL2* reading frame (Fig. 2) were inserted into the pBluescript vector. The resultant plasmid (H*tal*2HE-0.5/pBS) was used as a template for transcription with T7 RNA polymerase to generate a radiolabeled RNA fragment of 550 nucleotides. The radiolabeled probe was then annealed with RNAs extracted from various cell lines, digested with RNase, and fractionated by denaturing polyacrylamide gel electrophoresis (Fig. 3*A*). The probe includes 470 nucleotides of antisense polarity ranging from the splice acceptor site (Fig. 2) to an *Eco*RI site located downstream of the human *TAL2* coding sequence. As shown in Fig. 3*A*, a radiolabeled fragment of the appropriate size was protected from RNase digestion by RNA from SUP-T3, one of the four human T-ALL cell lines examined. Moreover, Northern hybridization of SUP-T3 RNA with a human genomic DNA probe (T3.1B2S; Fig. 1) revealed two polyadenylylated *TAL2* transcripts of approximately 5.0 and 3.5 kbp (Fig. 3*B*). In total, 29 human cell lines representing a spectrum of different tumor types were examined for *TAL2* gene expression by either Northern analysis or the RNase protection assay; of these, only SUP-T3 harbored detectable levels of *TAL2* RNA transcripts.

**Chromosomal Localization of the Human *TAL2* Gene.** To determine the chromosomal localization of human *TAL2*, a

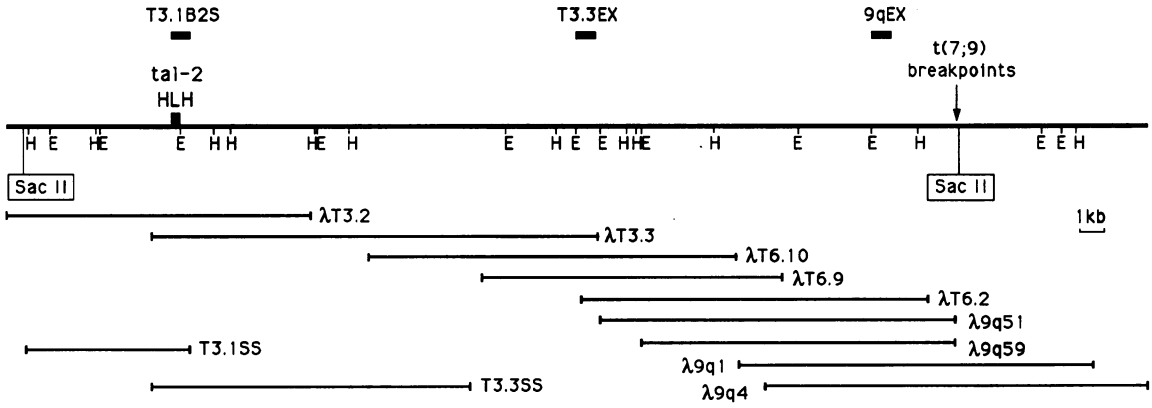


FIG. 1. A map of *TAL2* genomic DNA indicating linkage with the t(7;9)(q34;q32) breakpoint region. DNA probes derived from the *TAL2* locus are illustrated above the restriction map. The open reading frame encompassing the *TAL2* HLH domain is represented by a solid box (cf. Fig. 2*A*). The chromosome 9 breakpoints of t(7;9)(q34;q32) are indicated by a vertical arrow. The overlapping genomic λ phage clones were generated by chromosome walking on a library of RPMI 8402 DNA as described in the text. The plasmid clones T3.1SS and T3.3SS were used as probes for fluorescent *in situ* chromosome hybridization. E, *Eco*RI; H, *Hin*dIII.

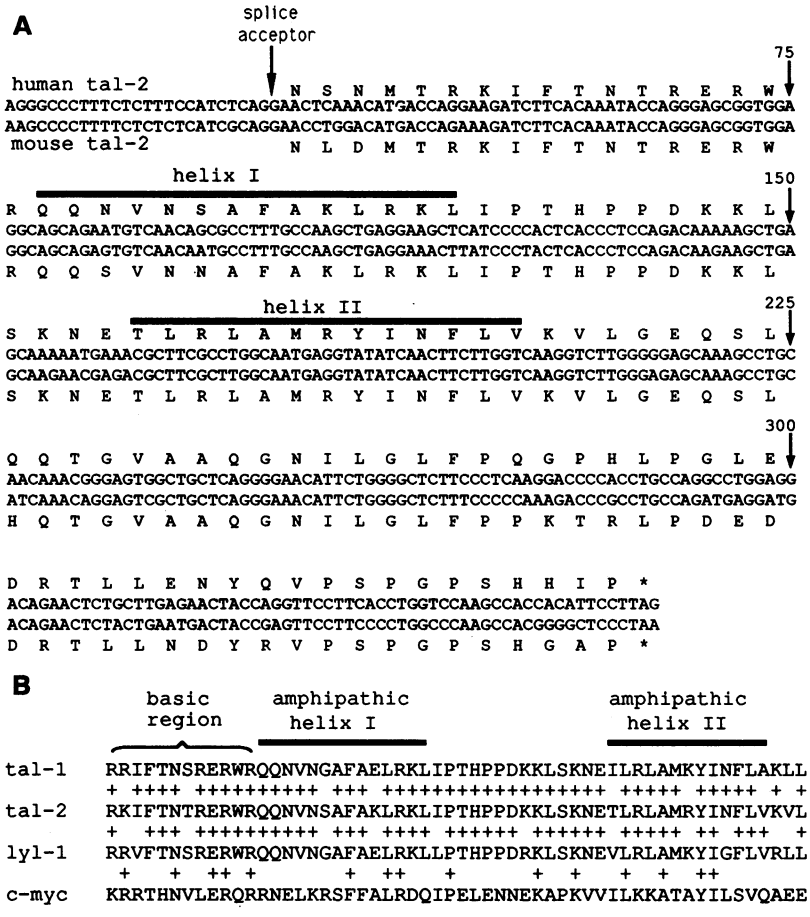


FIG. 2. (*A*) Genomic *TAL2* sequences of human and mouse are compared. The open reading frame of 111 amino acids is flanked by a splice acceptor site (vertical arrow) and a termination codon (asterisk). The 103-bp DNA fragment generated by PCR amplification (see text) corresponds to nucleotide residues 71–173 of the human *TAL2* sequence. (*B*) Alignment of the HLH domains of *TAL1*, *TAL2*, and *LYL1*. The HLH domain of the MYC protein is included for comparison. Residues that comprise the basic region and the amphipathic helices of the HLH motif are indicated.

genomic DNA fragment (T3.1B2S; Fig. 1) was used as a probe in Southern hybridization with DNAs from a panel of 17 human–hamster somatic cell hybrids containing randomly segregated human chromosomes (25). The hybridization of T3.1B2S showed 100% concordance with the presence or absence of chromosome 9 in the hybrids, whereas other chromosomes were present randomly with respect to a positive signal with the probe (18–59% discordance). The regional localization of *TAL2* was determined by fluorescent *in situ* hybridization of biotin-labeled *TAL2* probes to normal

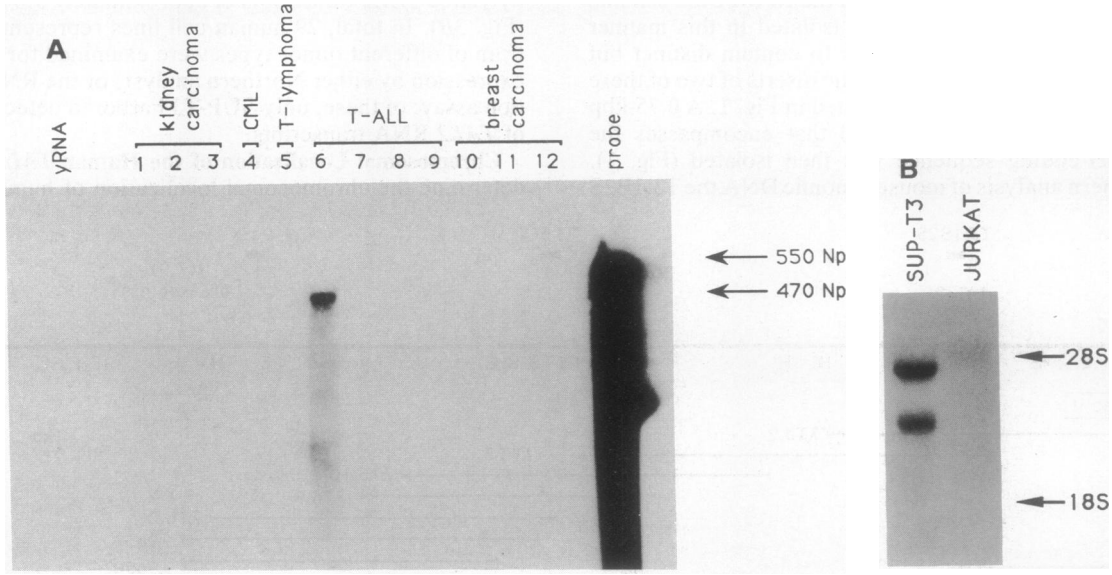


FIG. 3. Transcription of the human *TAL2* gene. (*A*) The 550-nucleotide (Np) $^{32}$P-labeled RNA probe was generated by *in vitro* transcription of genomic human *TAL2* DNA. The radiolabeled probe was annealed with 10 μg of either yeast tRNA (yRNA) or total RNA from various human cell lines. After RNase treatment and denaturing polyacrylamide gel electrophoresis, radiolabeled RNA fragments were detected by autoradiography. An aliquot of untreated RNA probe was fractionated in the lane marked probe. Cell lines: lane 1, 293; lane 2, SK-NEP-1; lane 3, G401; lane 4, K562; lane 5, SUP-T1; lane 6, SUP-T3; lane 7, Jurkat; lane 8, Molt-3; lane 9, Molt-13; lane 10, SK-BR-3; lane 11, MCF7; lane 12, DU4675. (*B*) Northern hybridization of the T3.1B2S probe (see Fig. 1) with 20 μg of total RNA from the SUP-T3 or Jurkat cell lines. Mobilities of the 28S and 18S rRNAs are indicated. Identical hybridization profiles were obtained with poly(A)-selected RNA (data not shown).

human metaphase chromosomes. Fluorescent signals from biotin-labeled probes are visualized as discrete green-yellow dots on unstained chromosomes; a specific signal is frequently observed on all four chromatids. Hybridization of the T3.3SS probe resulted in specific labeling only of chromosome 9 (Fig. 4). Specific labeling of 9q31 was observed on one (2 cells), two (6 cells), three (8 cells), or all four (9 cells) chromatids of the chromosome 9 homologues in 25 cells examined. Similar results were obtained in a second hybridization experiment with this probe and in hybridizations with the T3.1SS probe. Thus, the *TAL2* gene is localized to chromosome 9 band q31.

**Linkage between *TAL2* and the t(7;9)(q34;q32) Breakpoint.** Previous studies have shown that t(7;9)(q34;q32) is a recurring chromosome translocation associated with human T-ALL (15–18). Tycko *et al.* characterized t(7;9) at the molecular level in three patients and found that the chromosome 9 breakpoints were tightly clustered within a 31-bp sequence (18). Consequently, in each patient a specific region of chromosome 9 was juxtaposed with sequences of the T-cell receptor β-chain gene from chromosome 7. Although an appropriate candidate has not been identified, it was proposed that chromosome 9 harbors a protooncogene whose malignant potential is unleashed in T-ALL by t(7;9)(q34;q32) (16–18). Three factors now indicate that *TAL2* may be the putative oncogene. First, *TAL2* is highly related to *TAL1* and *LYL1*, both of which were identified on the basis of chromosomal rearrangement in T-ALL. Second, *TAL2* transcripts are specifically expressed in SUP-T3, a T-ALL cell line that carries t(7;9)(q34;q32). Third, the *TAL2* gene resides at band 9q31, which, at the cytogenetic level, approximates the chromosome 9 breakpoints of t(7;9)(q34;q32).

If *TAL2* is indeed the putative oncogene activated by t(7;9)(q34;q32), then it should be possible to establish linkage at the molecular level between *TAL2* and the translocation breakpoint region of chromosome 9. A 60-bp sequence spanning the breakpoints had previously been determined by Tycko *et al.* (18). Therefore, radiolabeled synthetic oligonucleotides with this sequence were used to screen the phage λ library of RPMI 8402 human DNA. In this manner, we isolated several clones encompassing the breakpoint region (e.g., λ9q1 and λ9q4; Fig. 1), from which a suitable probe was obtained for Southern hybridization (9qEX; Fig. 1). Normal human DNA was then digested with rare-cutting restriction endonucleases, fractionated by transverse alternating field electrophoresis, and analyzed by Southern hybridization with 9qEX and the *TAL2* probe T31B2S (Fig. 1). This analysis revealed cohybridization of these probes with genomic DNA fragments generated by three different restriction endonucleases (*Bss*HII, 150 kbp; *Sac* II, 45 kbp; *Sfi* I, 65 kbp; data not shown). The cohybridization with a 45-kbp *Sac* II fragment was particularly informative because restriction analysis of the recombinant λ clones had identified *Sac* II restriction sites ≈6 kbp upstream of T31B2S and 3 kbp downstream of 9qEX (Fig. 1). Thus, the chromosome 9 breakpoints of t(7;9)(q34;q32) occur ≈35 kbp downstream from the HLH-coding sequences of *TAL2*.

Direct linkage between the *TAL2* gene and the t(7;9)(q34;q32) breakpoint region was confirmed by chromosome walking. The chromosome walk was initiated by screening the phage λ library of RPMI 8402 human DNA with T3.3EX, a probe derived from the λT3.3 phage clone (Fig. 1). The three recombinant clones detected with this probe (λT6.10, λT6.9, and λT6.2) were shown by restriction analysis to contain distinct but overlapping genomic DNA inserts (Fig. 1). The same phage λ library was also screened with the translocation breakpoint probe 9qEX. This probe hybridized with the λT6.2 clone as well as with two additional clones (λ9q51 and λ9q59). Restriction mapping and Southern analysis revealed substantial overlap between the clones identified with the T3.3EX probe (λT6.10, λT6.9, and λT6.2) and those identified with 9qEX (λT6.2, 9q51, 9q59) (see Fig. 1). Therefore, this series of overlapping genomic DNA clones extends from the *TAL2* gene to the t(7;9)(q34;q32) breakpoints located 33 kbp downstream. Although these clones represent human DNA from a leukemic cell line (RPMI 8402), extensive Southern analysis of nonleukemic DNAs from a variety of human sources confirms that the restriction map in Fig. 1 corresponds to the normal configuration of the *TAL2* locus (data not shown).

## DISCUSSION

**Activation of *TAL2* Gene Expression by t(7;9)(q34;q32).** The results indicate that *TAL2* may correspond to the proposed

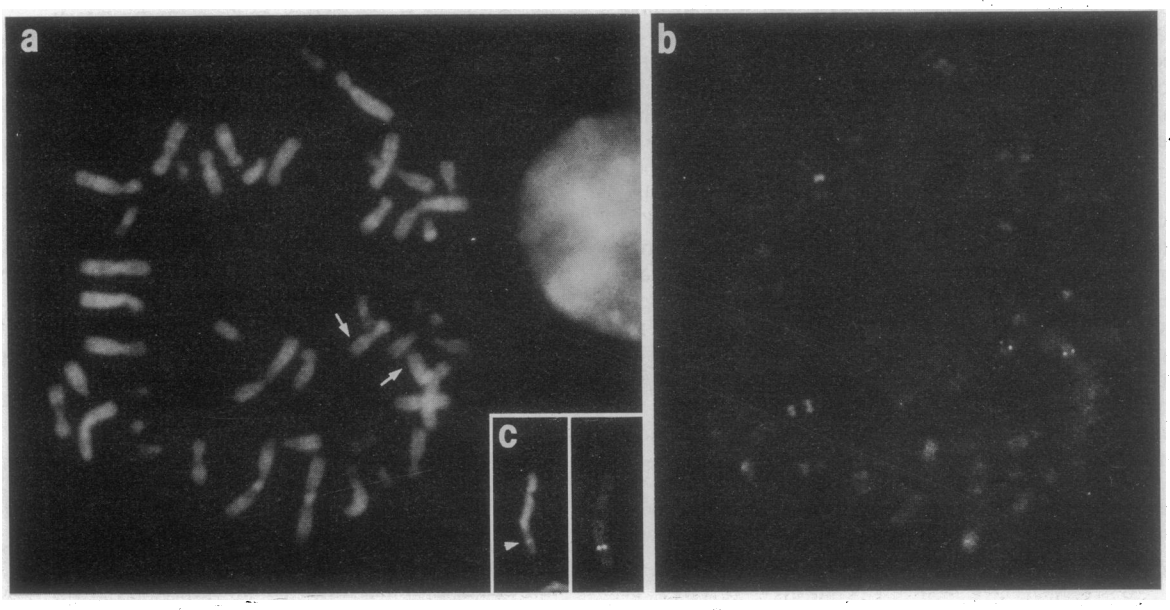


FIG. 4. *In situ* hybridization of a biotin-labeled *TAL2* probe to human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes. (*a*) Counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride. (*b*) Detection of the probe with fluorescein isothiocyanate-conjugated avidin. (*c*) Partial karyotype of a chromosome 9 homologue illustrating specific labeling at 9q31. The chromosome 9 homologues are identified with arrows.

oncogene activated by t(7;9)(q34;q32) in T-ALL patients (15–18). The chromosome 9 breakpoints of this translocation occur 33 kbp downstream of sequences that encode the *TAL2* HLH domain. Hence, translocated alleles of *TAL2* are juxtaposed with transcriptional regulatory elements within the T-cell receptor β-chain locus. It is noteworthy, therefore, that our initial survey of human neoplastic cell lines only uncovered *TAL2* transcripts in SUP-T3, a T-ALL line that harbors the t(7;9)(q34;q32). A preliminary analysis of normal tissues dissected from adult mice revealed restricted *TAL2* gene transcription in testes and a notable absence of expression in hematopoietic organs such as spleen and thymus (unpublished data). These data are consistent with the hypothesis that *TAL2* transcription is activated ectopically in lymphoid cells as a result of t(7;9)(q34;q32) and that the inappropriate expression of *TAL2* in these cells promotes development of T-ALL.

**A Subgroup of HLH Proteins Implicated in Human T-ALL.** *TAL1*, *LYL1*, and *TAL2* constitute a discrete subgroup of HLH proteins, each of which has been implicated in human T-ALL. Although their HLH domains are highly related (Fig. 2*B*), these proteins share little detectable homology outside the HLH region. Therefore, if the TAL-related proteins foster T-ALL by a common mechanistic route, then the malignant potential of these proteins is likely to reside largely within their HLH domains. Most HLH proteins contain clusters of basic residues at the amino-terminal flank of the HLH domain that potentially mediate sequence-specific DNA recognition (9–12). Recent studies have shown that the basic regions of myogenic HLH proteins (e.g., MyoD and myogenin) also contain residues that are essential for muscle-specific transcription of subordinate genes during myogenesis (11, 26, 27). Likewise, the basic regions of TAL-related proteins may dictate cell type-specific transcription of a distinct subset of subordinate genes. If so, then the inappropriate expression of these subordinate genes in T-lineage cells may constitute a leukemogenic program that is activated in T-ALL by malignant alteration of one of the *TAL*-related loci.

**The Genetic Basis of T-ALL.** Certain lymphoid tumors (e.g., Burkitt lymphoma and follicular B-cell lymphoma) are characterized by a unique chromosome abnormality that is present in a significant proportion of the patient population. In contrast, cytogenetic studies have not uncovered a major karyotypic defect associated with T-ALL but instead have defined a number of chromosome translocations, each of which recurs in a relatively small proportion of the T-ALL population (reviewed in refs. 28 and 29). Recent molecular studies have identified several putative protooncogenes that are altered as a result of these translocations. In addition to the *TAL*-related loci, these include the *HOX11* homeobox gene (30, 31); *RHOM1/TTG1* and *RHOM2*, related genes that encode cysteine-rich LIM motifs (24, 32–34); and *MYC*, which encodes a HLH domain distantly related to those present in the TAL-related proteins (35). It may be significant that each of the genes implicated in T-ALL encodes a protein homologous to known transcriptional regulatory factors. Transcriptional control of a given locus is usually achieved by the interplay of multiple distinct regulatory factors. Thus, it is possible that the different oncoproteins implicated in T-ALL may regulate expression of common subordinate genes whose activity in turn elicits leukemogenesis.

**Note Added in Proof.** The designations *TAL1* and *TAL2* have been adopted as gene symbols by the Human Genome Mapping Workshop.

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