T-Cell Acute Lymphoblastic Leukemia—The Associated Gene SCL/tal Codes for a 42-Kd Nuclear Phosphoprotein

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SCL/tal is a putative oncogene originally identified through its involvement in the translocation t(1;14)(p32;q11) present in the leukemic cell line DU.528. Subsequent studies have shown an upstream deletion activating expression of SCL/tal to be one of the most common genetic lesions in T-cell acute lymphoblastic leukemia (T-ALL). The cDNA sequence of SCL/tal encodes a basic helix-loop-helix (bHLH) protein with regions of marked homology to lyl-1 and tal-2, two other bHLH proteins involved in T-ALL chromosomal translocations. The bHLH motif suggests that the SCL/tal product localizes to the nucleus, binds to specific DNA sequences, and regulates transcription of a specific array of target genes. Our studies directly identify the SCL/tal product as a 42-Kd phosphoprotein that efficiently localizes to the nucleus. Deletion mutagenesis has allowed identification of a region critical for nuclear localization, a region that corresponds to the DNA-binding basic domain within the bHLH motif. Because this domain is shared by lyl-1 and tal-2, these latter putative T-cell oncogenes probably use a nuclear localization mechanism identical to that of SCL/tal.

A novel transcriptional unit on chromosome number 1, band p32 has recently been shown to be involved in the translocation, t(1;14)(p32;q11) found in rare cases of T-cell acute lymphoblastic leukemia (T-ALL) and stem cell leukemia. Investigators at several laboratories, including the University of Minnesota, have independently cloned this translocational breakpoint and identified the associated transcriptional unit on chromosome 1p32, which we have designated SCL/tal based on previously published nomenclature. Several different kinds of chromosomal breakpoints affecting the SCL/tal gene have been observed in cases of acute leukemia. In the multipotent leukemic cell line, DU.528, the t(1;14)(p32;q11) is associated with disruption of the 3' untranslated sequences of exon VI. In one case report of T-ALL associated with t(1;7)(p32;q35) the breakpoint occurs 35 kb downstream of the entire SCL/tal locus. In approximately 2% to 3% of T-ALLs the t(1;14)(p32;q11) breakpoint disrupts or eliminates 5' non-coding exons. Finally, in 16% to 25% of cases of T-ALL, a submicroscopic interstitial deletion upstream of the SCL/tal locus juxtaposes noncoding exons from a novel gene expressed in thymocytes, SIL, with coding exons from SCL/tal, aberrantly placing the SCL/tal gene under the control of a promoter active in T cells.

Our own sequencing efforts support the previous reports, in which the derived amino acid sequence of SCL/tal includes a basic helix-loop-helix (bHLH) motif. This motif places SCL/tal in a family of proteins, identified in organisms ranging from yeast to humans, which bind specific DNA sequences and regulate the transcriptional activity of target genes. Other bHLH genes involved in leukemic translocations include C-myc and E2A, contributing to an emerging pattern of translocation-associated proto-oncogenes containing transcription factor motifs. Of great interest in this regard is the identification of a subset of bHLH proteins, lyl-1, SCL/tal, and tal-2, all of which share marked homology (> 85%) within the bHLH region and all of which have been associated with T-ALL-specific translocations.

In an effort to gain an understanding of the function of the SCL/tal gene in both normal and neoplastic development we have undertaken studies of the protein product encoded by this gene. As presented below, the nuclear localization of the SCL/tal protein supports its role as a transcriptional factor. This observation is reinforced by the experimental identification of a nuclear localization domain within the SCL/tal protein. In addition, evidence is presented that the SCL/tal protein undergoes phosphorylation and that some of this phosphorylation may occur before nuclear localization.

MATERIALS AND METHODS

Cell lines. DU.528 (provided by J. Kurtzberg, Duke University) derived from a human stem cell leukemia containing the translocation t(1;14)(p32;q11) as previously described was maintained in RPMI 1640 with 10% fetal bovine serum (FBS) and 10% horse serum. Cos-7 (provided by T. LeBien, University of Minnesota) was maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS.

Cloning of SCL/tal. High molecular weight genomic DNA prepared from DU.528 was used to construct a cosmid library using the Lorist 6 vector. Approximately 200,000 independent clones were screened with a probe derived from the T-cell receptor D-8 locus on chromosome 14q11 (provided by M. Minden, Ontario Cancer Institute). Clones containing the der14 breakpoint were identified and a repeat-free 8.4-kb EcoRI fragment spanning the breakpoint was used to screen a Jurkat cDNA library (Stratagene, La Jolla, CA). Complementary DNA clones of SCL/tal were sequenced and used to screen a human placental genomic DNA λ phage library (Stratagene). A 20-kb genomic DNA λ phage clone, which contained all of the coding exons of SCL/tal, was used to subclone BamHI fragments into pUC 19. B4.4 is a 4.4-kb genomic...
DNA subclone that includes the bHLH and carboxy terminal coding sequences of SCL/tal.

**DNA sequencing.** DNA sequencing was accomplished using single-stranded (M13) or double-stranded (alkali denatured) templates. DNA sequencing reactions used the Sanger dideoxy method with Sequenase T7 polymerase (US Biochemicals, Cleveland, OH). Sequence information was read using a digitizer linked to an Apple Macintosh SE/30 (Apple Computer Inc, Cupertino, CA) and analyzed with DNAStar software (DNASTAR, Inc, Madison, WI).

Prokaryotic expression of SCL/tal and polyclonal antisemur production. DNA containing the coding sequences for the 131 carboxy terminal amino acids of SCL/tal was amplified by polymerase chain reaction (PCR) by using the following primers: 5' GCTCTAGAGCCATGCAAGAATGGTACGGGGCTTT 3' and a 3' mer of 5'AAGGATCCAAAGTCGACCCACGCC-TTGG3'. The template consisted of the B4.4 genomic subclone. The 550-bp PCR product was digested with Xba I and BamHI, blunt ended with the Klenow fragment of DNA polymerase I and ligated into the Sma I site of pBluescript (Stratagene). The SCL/tal fragment was shuttled into the prokaryotic expression vector pGEX-2T (Pharmacia Fine Chemicals, Uppsala, Sweden) with Sequenase T7 polymerase (US Biochemicals, Cleveland, OH). The 550-bp PCR product was digested with Xba I and BamHI, blunt ended with the Klenow fragment and ligated into a unique Sma I site. The recombinant expression construct designated pGEXMB 1#7 includes sequences coding for the 26-Kd carboxy terminus of glutathione S-transferase (GST) under the control of an isopropylthio-β-galactoside (IPTG) inducible promoter. The construct was restricted and sequenced to verify the correct orientation and reading frame. After IPTG induction, GST-SCL/tal fusion protein was purified from crude bacterial lysates by glutathione-Sepharose affinity chromatography. SCL/tal peptide, released from its GST partner by thrombin digestion, was purified by repetition of the glutathione-Sepharose affinity chromatography step. Preparations of the GST-SCL/tal fusion protein and the SCL/tal-thrombin-released peptide were judged as greater than 95% pure by Coomassie brilliant blue staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Primary immunization of New Zealand white rabbits consisted of 50 μg of GST-SCL/tal or GST-SCL/tal peptide emulsified in complete Freund’s adjuvant followed by every other week immunizations of 25 μg protein emulsified in incomplete Freund’s adjuvant. Preimmune serum was obtained from each rabbit before immunization. Three rabbits (A-1, -2, and -3) were immunized with the intact GST-SCL/tal fragment was shuttled into the prokaryotic expression vector pCWB, except with a different 5' mer: 5'GCTCTAGAGCCATGCAAGAATGGTACGGGGCTTT 3'. pCIS consists of an Id-SCL fusion gene encoding amino acids 1 to 58 of Id2 with amino acids 201 to 331 of SCL/tal cloned into pCMV-5. Further cloning of SCL/tal into the prokaryotic expression vector photocloned pCWB, a construct encoding the 131 carboxy terminal amino acids of SCL/tal excluding the basic domain but including the HLH domain, was constructed in the same way as pCWB, except with a different 5' mer: 5'GCTCTAGAGCCATGCAAGAATGGTACGGGGCTTT 3'. s-3, a construct encoding the full-length SCL/tal product was derived by excising a 1.2-kb HindIII-Xba I fragment from pBluescript II-SCL (provided by Dr I.R. Kirsch, Bethesda Naval Hospital, Bethesda, MD) and ligating it into the corresponding sites of pCMV-5. The cNIS consists of an Id-SCL fusion gene encoding amino acids 1 to 58 of Id and amino acids 201 to 331 of SCL/tal cloned into pCMV-5. Further details regarding construction of the Id-SCL fusion gene will be published elsewhere. The inserts of all PCR-built constructs were sequenced in their entirety to rule out spontaneous mutations introduced by Taq polymerase into the open reading frames.

**Transfections and immunofluorescent staining.** Cos-7 cells on 100-mm plastic dishes or glass cover slips were grown to ~80% confluence. Cells were repeatedly washed with phosphate-buffered saline (PBS) then incubated with 30 μg Lipofectin (GIBCO BRL Life Technologies, Inc, Gaithersburg, MD) plus 7 μg plasmid in serum-free DMEM overnight at 37°C, 5% CO2. Cells were then allowed to recover 24 hours in DMEM with 10% fbs before harvesting or fixation. Cells grown on coverslips were fixed with methanol at −20°C followed by 5% in acetone at −20°C. Coverslips were washed in PBS and then blocked with PBS/0.2% Tween 20/5% normal goat serum, incubating 30 minutes at room temperature. After repeated washes in PBS/0.2% Tween 20, coverslips were overlaid with secondary antibody, fluorescein isothiocyanate goat antirabbit, diluted 1/2,000 in PBS/0.2% Tween 20/5% normal goat serum, incubating 30 minutes at room temperature. Coverslips were then repeatedly washed first in PBS/0.2% Tween 20 and then in PBS alone.

**Immunoblotting.** Cells grown to ~90% confluency on 100-mm plastic dishes were washed once with PBS and then scraped in PBS/5 mmol/L EDTA. Pelleted cells were resuspended in 100 μL of NP-40 extraction buffer (0.5% NP-40, 50 mmol/L Tris HCl, pH 7.6, 100 mmol/L NaCl, 5 mmol/L MgCl2, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL pepstatin, 1 μg/mL leupeptin, 0.1% aprotinin) and incubated on ice 10 minutes with gentle agitation. Debris was then removed by centrifugation at 13,000 rpm for 10 minutes at 4°C. Supernatants were fractionated by standard SDS-PAGE using 12% gels. Proteins were electroblotted onto nitrocellulose membranes. Membranes were blocked with 10% normal goat serum in TBS (20 mmol/L Tris HCl, pH 7.5, 500 mmol/L NaCl) with 5% nonfat dried milk for 2 hours at room temperature. Primary antibody (adsorbed rabbit anti-GST-SCL/tal) was diluted 1:350 in TTBS (TBS/0.2% Tween 20) with 1% nonfat dried milk and incubated on membranes for 2 hours at room temperature. After washing membranes repeatedly in TTBS, secondary antibody, alkaline phosphatase-conjugated goat anti-rabbit, was diluted 1:1,000 in TTBS with 1% nonfat dried milk and applied to membranes for 1 hour at room temperature. Membranes were developed using 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/Nitro blue tetrazolium (NBT) as chromogenic substrate.

**Metabolic labeling and immunoprecipitation.** At 60 hours post-transfection, Cos cells were washed extensively with serum-free deficient media for (35S)-methionine labeling, methionine-deficient RPMI 1640 [Sigma Chemical Co, St Louis, MO]; for 32P-labeling labeling, phosphate-deficient DMEM, [Sigma]). Cells were then incubated for 2 hours at 37°C, 5% CO2 in labeling media (methionine-deficient RPMI 1640 with 0.5 μCi/mL Tran-35S Label [ICN
with 0.5 mCi/mL carrier-free 32P-orthophosphate [Amersham, Arlington Heights, IL]. Each labeling consisted of 0.5 x 10⁶ cells in 5 mL labeling media. After metabolic labeling, cells were extensively washed with PBS and scraped in PBS/0.5 mmol/L EDTA. To the cell pellet was added 500 µL extract buffer: 0.5% NP-40, 50 mmol/L Tris HCl, pH 7.8, 100 mmol/L NaCl, 5 mmol/L MgCl₂, 5 mmol/L EDTA, 1 mmol/L PMSF, 0.1% aprotinin, 1 µg/mL leupeptin, 1 µg/mL pepstatin, 10 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 2 mmol/L sodium orthovanadate, 0.1 mmol/L sodium metavanadate. Cells were incubated on ice with extract buffer for 20 minutes and nuclear debris was removed by centrifugation. Cellular extracts were preclerared by the addition of 20 µL preimmune rabbit sera followed by 50 µL Pansorbin (Calbiochem Inc, San Diego, CA). Following preclearing, 20 µL of immune or control serum was added to extracts which were then incubated for 2 hours on ice. Immune complexes were collected on protein-A agarose (Calbiochem) and subjected to sequential washes with extract buffer, triple detergent radioummunoprecipitation buffer (RIPA buffer) (containing 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS), TBS (20 mmol/L Tris HCl, pH 7.5, 500 mmol/L NaCl), and TE (10 mmol/L Tris HCl, pH 7.5, 1 mmol/L EDTA). Immune complexes were then dissociated by boiling in SDS-PAGE loading buffer for 5 minutes and subjected to SDS-PAGE followed by autoradiography.

Phosphoamino acid analysis. 32P-metabolically labeled, immunoprecipitated SCL/tal protein was subjected to phosphoamino acid analysis exactly as described by Sorenson et al. In brief, protein was hydrolyzed in 6 mol/L HCl at 100°C for 2 hours under nitrogen. Hydrolysate was then diluted with a vast excess of deionized water and lyophilized. The pellet was redissolved in thin-layer chromatography (TLC) buffer containing phosphoamino acid standards and spotted onto Whatman 3M paper (Whatman Lab Sales, Hillsboro, OR). Amino acids were resolved in one dimension by thin-layer chromatography. Standards were visualized by ninhydrin staining and 32P-phosphoamino acids were visualized by autoradiography.

RESULTS

Sequence, genomic organization, and prokaryotic expression of SCL/tal. The portion of SCL/tal coding sequence that includes the bHLH motif is found on a 4.4-kb BamH1 genomic DNA fragment containing exon VI (following the exon nomenclature used by Aplan et al). The open reading frame of SCL/tal is extended through two upstream exons with a putative initiation codon residing in exon IV. Published reports describe various additional 5’ exons and at least two 5’ cap sites as well as variable patterns of splicing of 5’ untranslated exons within different hematopoietic cell lines. To raise antibodies against the SCL/tal protein product, we designed a prokaryotic expression construct for production of vaccine. DNA sequences corresponding to the 131 carboxy terminal amino acids of SCL/tal were cloned in-frame into the pGEX-2T expression vector. Production of the GST-SCL/tal fusion protein was induced by IPTG treatment of Escherichia coli transformed with the expression construct. Fusion protein synthesis was shown by SDS-PAGE analysis of crude bacterial lysates and verified by purification of the induced fusion protein by glutathione-sepharose affinity chromatography.
Fig 3. Indirect immunofluorescence of Cos cell transfectants. (A) Untransfected cells stained with polyclonal rabbit anti-SCL/tal. (B) Cells transfected with pS-3 stained with preimmune rabbit serum. (C) Cells transfected with pS-3 stained with polyclonal rabbit anti-SCL/tal. (D) Cells transfected with pCIS stained with polyclonal rabbit anti-SCL/tal (original magnification ×630).

Fig 4. Indirect immunofluorescence of Cos cell transfectants continued. Cells transfected with pCIS stained with polyclonal rabbit anti-SCL/tal. (original magnification ×630).
Fig 5. Indirect immunofluorescence of Cos cell transfectants continued. (A and B) Cells transfected with pCWB stained with polyclonal rabbit anti-SCL/tal. (C and D) Cells transfected with pCMB stained with polyclonal rabbit anti-SCL/tal (original magnification x630).

Antisera were purified by either adsorption with GST protein or by affinity selection on a GST-SCL/tal–Affigel column. Both modes of purification yielded similar results on immunofluorescence and immunoblot analysis.

Expression of SCL/tal protein in Cos cells. The constructs depicted in Fig 1 were transiently transfected into Cos-7 cells, which were then subjected to standard immunoblot analysis. As seen in Fig 2, the full-length SCL/tal

Fig 6. Indirect immunofluorescence of NIH-3T3 transfectants. (Top two panels) Cells transfected with pS-3 stained with polyclonal rabbit anti-SCL/tal. (Bottom panel) Cells transfected with control vector pCMV5 stained with polyclonal rabbit anti-SCL/tal (original magnification x630).
cDNA yielded a protein product of 42 Kd (lane c); close inspection showed this 42-Kd species to be a tight multiplet. In addition, a minor species of 37-Kd (not clearly visible in Fig 2 but clearly evident in Figs 7 and 8) also derived from the full-length SCL/tal cDNA. Two amino terminal deletion mutants pCWB and pCMB yielded products of 24 Kd and 22 Kd, respectively (Fig 2, lanes d and e). As with the full-length SCL/tal products, the proteins encoded by each of the deletion mutants also consisted of multiple species. A domain swap mutant in which the amino terminal of SCL/tal including the basic domain is replaced by the corresponding region of the HLH protein Id, yielded a protein product of 28 Kd (Fig 2 lane f); this product likewise consisted of multiple species.

Indirect immunofluorescence microscopy and subcellular localization of SCL/tal protein. To determine the subcellular localization of the SCL/tal protein product, Cos cells transiently transfected with the constructs depicted in Fig 1 were fixed and subjected to immunofluorescent staining using purified rabbit anti-SCL/tal polyclonal antibody. Representative results of these studies are shown in Figs 3A through D, 4, and 5A through D. Cells expressing the full-length SCL/tal product showed a clear, strong nuclear staining pattern with faint or (in most cells) absent cytoplasmic staining (Fig 3C). The pattern of nuclear staining in these cells, diffuse and granular with an absence of nucleolar staining, is consistent with that of a nucleoplasmic or DNA-associated protein rather than a nucleoskeleton-associated protein. Cells transfected with pCWB, a deletion construct that retains the bHLH domain of SCL/tal, similarly showed efficient nuclear localization (Fig 5A and B). Cells transfected with pCMB, a deletion construct similar to pCWB except lacking the basic domain, showed absence of nuclear localization by the encoded protein (Fig 5C and D). In fact, the pCMB-encoded protein seemed to be excluded from the nucleus, with the vast majority of the product located in the cytoplasm. Cells transfected with pCIS, the Id-SCL fusion construct in which the basic domain of SCL/tal has been replaced by the corresponding neutral domain of Id3, showed relatively inefficient nuclear localization of the encoded product (Figs 3D and 4). Significant quantities of the Id-SCL protein product were clearly detectable in the cytoplasm, extending along the cytoplasmic processes of cells. Control cells transfected with the parent vector and stained with the anti-SCL/tal antibody showed no background fluorescence (Fig 3A). Likewise, control cells transfected with all of the constructs depicted in Fig 1 and stained with preimmune rabbit serum showed no background fluorescence (representative field in Fig 3B). To insure that the nuclear localization of the intact SCL/tal protein was not an artifact of expression in Cos cells, stably transfected NIH-3T3 cells expressing SCL/tal were also analyzed by immunofluorescence. Control NIH-3T3 cells, stably transfected with pCMV5, and stained with the anti-SCL/tal antibody showed no detectable fluorescence (Fig 6, bottom panel). By comparison, antibody staining of NIH-3T3 cells expressing the SCL/tal protein resulted in a clear pattern of nuclear fluorescence (Fig 6, top two panels). (The heterogeneity of SCL/tal expression observed in the NIH-3T3 transfectants has occurred because the cells are not a pure clonal population). In summary, these experiments show that SCL/tal is a nuclear protein and that the basic domain is critical in directing the proper subcellular localization.

Extractability of the SCL/tal protein. Hann and Eisenman have shown that the c-Myc protein is avidly complexed with the nucleus and cannot, even when present in high abundance, be extracted with nonionic detergent.24 In addition, Mittnacht and Weinberg have shown that specific isoforms of the Rb protein show preferential “tethering,” ie, high affinity binding to the nucleus.25 To explore the association of the SCL/tal protein with the nuclear compartment, transiently transfected Cos cells were subjected to sequential rounds of extraction with buffer containing 0.5% NP-40 and increasing concentrations of NaCl (0 mmol/L, 250 mmol/L, and 500 mmol/L). After extraction with 500 mmol/L NaCl, residual nuclei were solubilized by boiling in SDS-PAGE buffer. As shown in Fig 7, the vast majority of

![Fig 7. Immunoblot analysis of Cos cell extracts with polyclonal rabbit anti-SCL/tal. (A) Cos cells were transiently transfected with pS-3 and then subjected to extraction with NP-40 extraction buffer as described in Materials and Methods except that the NaCl concentrations were altered as indicated. Thus, cells were initially extracted with 100 μL of extraction buffer with 0 mmol/L NaCl then washed three times with 1 mL of the same buffer. After washing cells were extracted with 100 μL of extraction buffer with 250 mmol/L NaCl and washed with this buffer. Cells were then subjected to a similar round of extraction in 500 mmol/L NaCl followed by washing. The residual nuclear debris was then solubilized by boiling in SDS-PAGE buffer. (B) Results from the same type of experiment performed on Cos cells transfected with pCMB are shown.](#)
the intact SCL/tal protein within the cells (> 99%) was extractable with nonionic detergent and either no salt or low salt (250 mmol/L NaCl) conditions. As expected, the cytoplasmic amino terminal truncation mutant, MB, was virtually completely extracted under no salt conditions. Therefore, the intact SCL/tal protein in contrast to c-Myc is primarily located in the detergent-extractable nucleoplasmic compartment and in contrast to Rb does not show any detectable isoform-specific nuclear “tethering.”

**Phosphorylation of the SCL/tal protein.** Cos cells expressing the intact SCL/tal protein and the cytoplasmic amino terminal truncation mutant, MB, were metabolically labeled either with 35S-methionine or with 32P-orthophosphate and subjected to immunoprecipitation followed by SDS-PAGE (Fig 8). For the intact SCL/tal protein, 35S-methionine labeling with immunoprecipitation yielded similar results to those observed with immunoblotting: a predominant 42-Kd species and a minor 37-Kd species. With 32P-orthophosphate metabolic labeling, only the 42-Kd species was observed, suggesting that the 37-Kd product may represent a hypophosphorylated species of SCL/tal. Alternatively, 32P metabolic labeling in these experiments may simply not provide sufficient isotope incorporation to permit detection of the minor 37-Kd product. For the cytoplasmic truncation mutant, MB, 35S-labelling yielded a single species of 22-Kd, whereas 32P-labelling yielded, in addition to the major 22-Kd species, a minor species of 24-Kd. This 24-Kd species most likely represents a low abundance, hyperphosphorylated form of MB.

Phosphoamino acid analysis of 32P-labeled intact SCL/tal showed that the vast majority of phosphorylation of SCL/tal (> 99%) within nonsynchronized, basally proliferating Cos 7 cells occurs on serine residues (Fig 9). Under these circumstances there was no detectable threonine or tyrosine phosphorylation. Antiphosphotyrosine immunoblot analysis of immunoprecipitated intact SCL/tal protein also showed no evidence of tyrosine phosphorylation in the Cos cells (data not shown).

**DISCUSSION**

The SCL/tal gene encodes a putative transcriptional factor that was cloned by virtue of its physical association with a chromosomal translocation breakpoint in a leukemic cell line. Its bHLH motif implies a functional similarity to other proteins in the family of bHLH transcriptional regulators. In this report we characterize the SCL/tal protein product primarily as a 42-Kd phosphoprotein with a minor species at 37 Kd. The 37-Kd species may represent a hypophosphorylated form of SCL/tal. Our studies have not ruled out the additional possibilities that the 37-Kd isoform may arise from limited proteolysis or from use of a downstream initiation codon. Additional causes for the discrepancy between predicted (34 Kd) and observed (37 to 42 Kd) sizes of SCL/tal may include other forms of posttranslational modification or simply anomalous migration on SDS-PAGE as has been observed for C-myc. 44

The nuclear localization of the SCL/tal product is consistent with its bHLH motif and putative function as a transcriptional factor. The identification of the basic domain within the bHLH motif as an element important in nuclear localization of the SCL/tal product is of interest. Because the T-ALL putative oncogenes lyl-1 and tal-2 show marked homology to SCL/tal within this region, it is likely that their protein products use a similar if not identical mechanism for nuclear localization. The colocalization of
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However, of the two nuclear localization sequences in C-myc, the one coinciding with the basic domain shows much less efficiency in conferring nuclear localization. In the bHLH protein MyoD deletion mutagenesis has identified only the basic domain as playing a role in nuclear localization. Id, a member of the HLH protein family, displays nuclear localization despite lacking a basic domain. However, an Id-SCL fusion protein containing the amino terminal half of Id joined to the carboxy terminal half of SCL/tai displayed relatively inefficient nuclear localization. Thus, even within the HLH family of proteins divergent mechanisms have probably evolved to promote nuclear localization, diminishing the likelihood that some shared, universal nuclear localizing sequence exists within this large family of proteins. Furthermore, heterogeneity exists among bHLH proteins with regard to the compartment occupied within the nucleus. Whereas C-myc occupies a detergent-insoluble structural compartment within the nucleus, SCL/tai exists in a detergent-extractable nucleoplasmic fraction.

The phosphorylation of SCL/tai is not unexpected because other bHLH proteins such as C-myc and MyoD are phosphoproteins. The phosphorylation of the cytoplasmic truncation mutant of SCL/tai, MB, indicates that a fair portion of the phosphorylation of SCL/tai occurs on serine residues in the carboxy terminal region of the protein. Of relevance, multiple casein kinase II serine recognition sites are observed in the carboxy terminal region of SCL/tai. In addition, our laboratory has recently shown in vitro phosphorylation by purified casein kinase II of a recombinant SCL/tai peptide containing the carboxy terminal 130 amino acids (data not shown). The phosphorylation of the cytoplasmic truncation mutant of SCL/tai, MB, also indicates that phosphorylation of this portion of SCL/tai may occur in the cytoplasm before nuclear localization. Further studies to address the exact sites of phosphorylation in SCL/tai, the responsible kinases, and the functional consequences of phosphorylation are being performed in our laboratory.

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