**The EMBO Journal** Vol.16 No.9 pp.2408–2419, 1997

# An *scl* gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice

**Peter D.Aplan[1–4], Craig A.Jones[5], David S.Chervinsky[1,2], XianFeng Zhao[1,2], MaryKay Ellsworth[5], Chuanzen Wu[5], Elizabeth A.McGuire[6] and Kenneth W.Gross[5]**

Departments of [1]Pediatrics, [5]Cellular and Molecular Biology and [2]Molecular Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, [3]Department of Pediatrics, State University of New York at Buffalo, Buffalo, NY 14222 and [6]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

[4]Corresponding author
e-mail: paplan@sc3101.med.buffalo.edu

**The product of the *scl* (also called *tal-1* or *TCL5*) gene is a basic domain, helix–loop–helix (bHLH) transcription factor required for the development of hematopoietic cells. Additionally, *scl* gene disruption and dysregulation, by either chromosomal translocations or a site-specific interstitial deletion whereby 5′ regulatory elements of the *sil* gene become juxtaposed to the body of the *scl* gene, is associated with T-cell acute lymphoblastic leukemia (ALL) and T-cell lymphoblastic lymphoma. Here we show that an inappropriately expressed scl protein, driven by *sil* regulatory elements, can cause aggressive T-cell malignancies in collaboration with a misexpressed LMO1 protein, thus recapitulating the situation seen in a subset of human T-cell ALL. Moreover, we show that inappropriately expressed *scl* can interfere with the development of other tissues derived from mesoderm. Lastly, we show that an scl construct lacking the scl transactivation domain collaborates with misexpressed *LMO1*, demonstrating that the scl transactivation domain is dispensable for oncogenesis, and supporting the hypothesis that the *scl* gene product exerts its oncogenic action through a dominant-negative mechanism.**

*Keywords*: acute lymphoblastic leukemia/LMO1/scl/T cells/transgene

## Introduction

The *scl* gene (also known as *TCL5* or *tal-1*) was first identified by virtue of its involvement in a t(1;14)(p33;q11) translocation present in the multipotential DU528 stem cell leukemia cell line (Begley *et al.*, 1989; Finger *et al.*, 1989; Chen *et al.*, 1990). The *scl* gene product contains the 'bHLH' (basic domain, amphipathic α-helix, loop, amphipathic α-helix) motif, conserved in a wide array of eukaryotic transcription factors in organisms ranging from baker's yeast to man (Murre *et al.*, 1989; Olson and Klein, 1994). A transcription activation domain has been identified at the amino-terminal portion of the scl protein (Hsu *et al.*, 1994b; Sanchez-Garcia and Rabbitts, 1994; Wadman *et al.*, 1994a), and a preferred DNA-binding sequence (CAGATG) has been identified (Hsu *et al.*, 1994a), although no natural target genes for scl have been firmly established. Several forms of the scl protein have been detected in mammalian cells; a 43 kDa full-length protein and shorter proteins of 39 and 22 kDa (Cheng *et al.*, 1993; Pulford *et al.*, 1995). The 22 kDa protein can be produced by translation of an alternately spliced transcript which lacks exons 2, 3 and 4; this shorter protein lacks the scl transcription activation domain (Bernard *et al.*, 1991; Cheng *et al.*, 1993). Similarly to other bHLH proteins, scl has been shown to bind to the E-proteins E2-2 and E2A (Hsu *et al.*, 1991; Voronova and Lee, 1994; Goldfarb *et al.*, 1996). By analogy with the myogenic bHLH proteins, it has been speculated that scl exerts its biochemical function by binding DNA in a sequence-specific fashion and activating transcription of target genes (Green, 1996; Porcher *et al.*, 1996; Robb *et al.*, 1996).

Several reports have demonstrated that *scl* is expressed primarily in hematopoietic and vascular endothelial cells (Green *et al.*, 1992; Hwang *et al.*, 1993; Kallianpur *et al.*, 1994; Pulford *et al.*, 1995), although fetal brain tissue also expresses easily detectable levels of *scl* mRNA (Green *et al.*, 1992). Recent experiments with mice harboring a targeted deletion of *scl* have demonstrated that the *scl* gene product is needed for all hematopoietic development, including T and B lymphocytes (Robb *et al.*, 1995b; Shivdasani *et al.*, 1995; Porcher *et al.*, 1996). These findings have led to the speculation that *scl* expression is required for commitment of ventral mesoderm to hematopoietic progenitor cells (Porcher *et al.*, 1996).

Non-random chromosomal translocations are a recurrent theme in hematopoietic malignancies; in T-cell acute lymphoblastic leukemia (T-cell ALL) and lymphoblastic lymphoma of T-cell phenotype, the translocations often serve to activate transcription factors inappropriately through the juxtaposition of these transcription factors with the enhancer elements of the T-cell receptor (TCR) loci (for review, see Rabbitts 1994). Several of the most common translocations associated with T-cell ALL activate either *HOX11* (Hatano *et al.*, 1991), *LMO1* (formerly known as *TTG1* or *RBTN1*) (McGuire *et al.*, 1989; Boehm *et al.*, 1991), *LMO2* (formerly known as *TTG2* or *RBTN2*) (Boehm *et al.*, 1991; Royer-Pokora *et al.*, 1991) or *scl* (Begley *et al.*, 1989; Chen *et al.*, 1990; Finger *et al.*, 1990). In general, these genes are not highly expressed in normal T cells, but T cells which have undergone a translocation involving one of these genes express the translocated gene at relatively high levels (Rabbitts, 1994). It has been proposed that inappropriate expression of these transcription factors, in a T-cell millieu, leads to malignant transformation, possibly through direct transcriptional activation of target genes. While *scl* translocations are present in only 3% of T-cell ALL patients (Carroll *et al.*,


© Oxford University Press

1990), ~25% of T-cell ALL patients activate *scl* by a site-specific interstitial deletion which replaces the *scl* 5′ regulatory sequences with those of an upstream gene, *sil* (Aplan *et al.*, 1990b; Brown *et al.*, 1990). In addition, T-cell ALL cell lines and patient samples with no detectable *scl* gene rearrangments often express high levels of *scl* mRNA; one report has demonstrated ectopic *scl* mRNA expression in a majority (60%) of T-cell ALL patients (Bash *et al.*, 1995).

Despite the striking association of *scl* gene activation with T-cell ALL, until recently, there has been little evidence presented to demonstrate direct proof that *scl* gene activation leads to malignant transformation. Two groups demonstrated that transgenic mice overexpressing *scl* did not develop T-cell malignancies (Robb *et al.*, 1995a, Larson *et al.*, 1996), nor did lethally irradiated mice reconstituted with bone marrow engineered to overexpress *scl* (Elwood and Begley, 1995). However, when *scl* transgenic mice were crossed with mice engineered to overexpress *LMO2*, the offspring positive for both *scl* and *LMO2* developed T-cell malignancies 3 months earlier than did mice transgenic only for *LMO2*, indicating a collaborative effect of *scl* and *LMO2* (Larson *et al.*, 1996). More recently, 28% of mice transgenic for a construct which expressed a full-length scl protein driven by an *lck* promoter developed T-cell malignancies within 350 days, producing clear evidence that *scl* was a proto-oncogene (Kelliher *et al.*, 1996). The onset and penetrance of disease was significantly increased when these mice were crossed with transgenic mice which overexpress casein kinase IIα (Kelliher *et al.*, 1996). Several investigators have speculated that scl may exert its oncogenic action either by inappropriately activating target genes through its transactivation domain, or through a dominant-negative mechanism, where scl may bind and sequester other HLH proteins, making them unavailable to form complexes with additional HLH proteins (Goldfarb and Greenberg, 1994; Green, 1996; Kelliher *et al.*, 1996; Porcher *et al.*, 1996).

Here we describe generation of transgenic mice using constructs which express *scl* mRNA driven by a *sil* promoter, thus mimicking the most common type of *scl* gene dysregulation associated with human T-cell ALL. We demonstrate that mice transgenic for either a full-length or an amino-terminal truncated *scl* driven by a *sil* promoter surprisingly display bony abnormalities and growth retardation, but do not develop T-cell malignancies. However, when crossed with mice that overexpress *LMO1* in the thymus, enforced expression of an amino-terminal truncated scl, which lacks the scl transactivation domain, leads to aggressive T-cell malignancies at an early age. These experiments demonstrate that the transactivation domain of scl is dispensible for leukemogenesis, and suggest that scl is not acting by activating transcription of its normal target genes, but instead is more likely to act through a dominant-negative mechanism.

## Results

### *Generation of constructs recapitulating sil and scl recombination*

Since we were aware of reports demonstrating a lack of oncogenicity in transgenic mouse models using the CD2 promoter to target *scl* expression to thymocytes (Robb *et al.*, 1995a), we decided to use *sil* regulatory elements to drive *scl* expression, thus recapitulating the most common situation seen in human T-cell ALL patients. In order to accomplish this goal, we isolated a human *sil* promoter, demonstrated it was functional when integrated in the mouse genome, and searched for alternate forms of *sil–scl* fusion mRNA species in malignant T cells which had undergone a *sil–scl* rearrangement. A 2.4 kb human genomic *sil* fragment which encompassed the predicted *sil* transcript initiation site (Aplan *et al.*, 1991) was isolated and shown to activate a CAT reporter gene in transient transfection assays using human and murine cell lines. This promoter fragment was then used to produce the pSIL/SCL and pSIL/TSCL vectors (Figure 1A). Since this promoter had not been used previously in transgenic experiments, we verifed that the *sil* promoter fragment

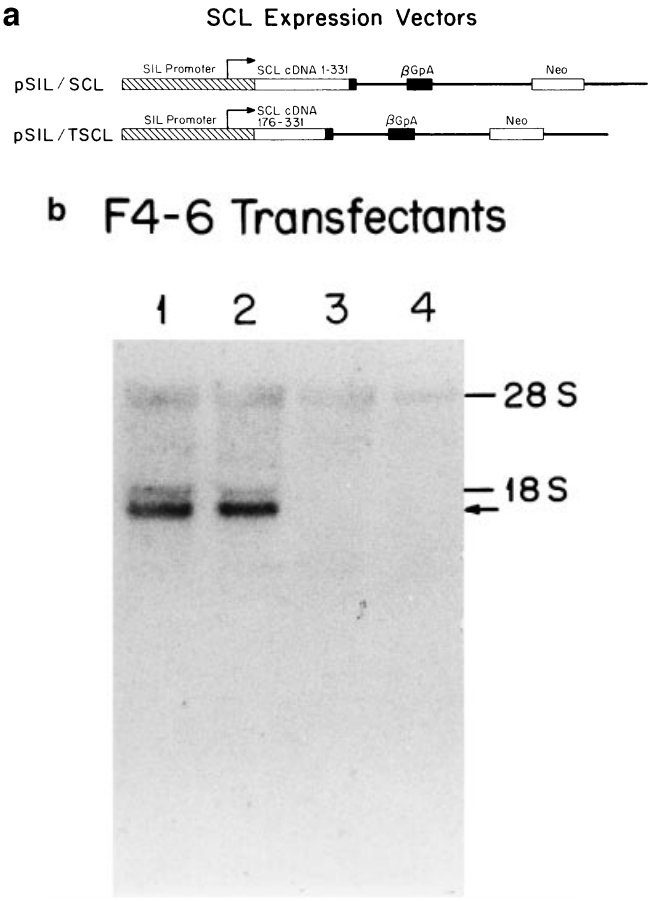



**Fig. 1.** SIL/SCL vectors. (**a**) Diagram of the SIL/SCL vectors. The pSIL/SCL vector contains human *sil* promoter sequences, an *scl* cDNA encoding a full-length human scl protein, a human β-globin genomic fragment encompassing exon 2 and 3 sequences including the polyadenylation site, and a neomycin resistance cassette. The pSIL/TSCL contains human *sil* promoter sequences and *sil* exon 1 sequences fused to *scl* exon 5 and 6 sequences, encoding an amino-terminal truncated scl protein. (**b**) Expression of the transfected *scl*. Northern blot analysis of four independent stable transfectants of the F4-6 murine erythroleukemia cell line hybridized to a human *scl* cDNA probe (1.0SX). The 28S and 18S rRNA bands are indicated; exogenous *scl* mRNA is indicated with an arrow. The human *scl* probe cross-hybridizes with the endogenous murine *scl*, and is seen as a band which co-migrates with the 28S rRNA. The transfectants in lanes 1 and 2 express transfected *scl*; those in lanes 3 and 4 express little, if any, exogenous *scl*.

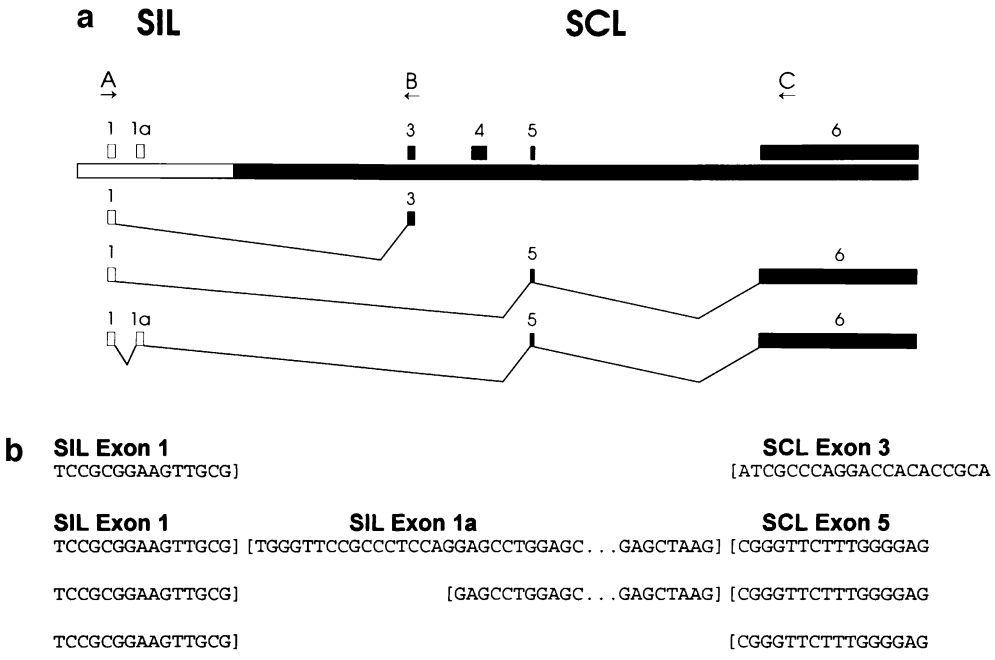



**Fig. 2.** Cells with *sil–scl* rearrangements generate several related mRNA species. (**a**) Schematic representation of *sil–scl* fusion mRNA species. The *sil* locus is indicated by open boxes (□), the *scl* locus by filled boxes (■). Exon numbers are indicated; *sil* exon 1 (A), *scl* exon 3 (B) and *scl* exon 6 (C) primers are shown. Three alternately spliced transcripts are shown. (**b**) Nucleotide sequence at splice junctions (brackets) of four different *sil–scl* fusion mRNA species.

remained functional when incorporated into the mouse genome by transfecting the pSIL/SCL vector into murine erythroleukemia cells and selecting stable transfectants. Fourteen of 20 randomly selected clones expressed easily detectable amounts of exogenous *scl* mRNA (Figure 1b), demonstrating that the human *sil* promoter was functional when integrated into the mouse genome.

Our initial experiments used the pSIL/SCL construct, which produced the full-length scl protein. While the initial cohort of mice generated with this construct was under observation, we investigated the possibility that alternately spliced forms of the fusion *sil–scl* mRNA may exist. We were especially interested in this possibility since the intact *scl* locus normally generates a complex pattern of alternately spliced transcripts, including one which produces an amino-terminal truncated form of the scl protein (Aplan *et al.*, 1990a). We had previously used RNase protection probes to demonstrate the production of *sil* exon 1–*scl* exon 3 fusion mRNA species in leukemic cells which displayed a *sil–scl* recombination (Aplan *et al.*, 1990b). Since this fusion occurs in the 5′-untranslated region (UTR) of both genes, the net effect of the *sil* exon 1–*scl* exon 3 fusion is the production of a fusion mRNA encoding a full-length scl protein.

We employed an RT–PCR assay that utilized an *scl* exon 6 primer and a *sil* exon 1 primer. Several amplification products were obtained as depicted in Figure 2; the predominant amplification product corresponded to a *sil* exon 1–*scl* exon 5 fusion. Although a *sil* exon 1–*scl* exon 3 product could theoretically have been amplified with these primers, we could not detect an amplification product corresponding to a *sil* exon 1–*scl* exon 3 fusion using these primers. This may be due to competition with the smaller *sil–scl* fusion mRNAs, or to difficulty in reverse transcribing through GC-rich *scl* exon 4 sequences, since amplification of the same mRNA templates used in Figure 2 with *sil* exon 1 and *scl* exon 3 primers generated quantities of a *sil* exon 1–*scl* exon 3 PCR product that were roughly equivalent to the amount of *sil* exon 1—*scl* exon 5 PCR products generated by *sil* exon 1 and *scl* exon 6 primers. The pSIL/TSCL vector (Figure 1a) recapitulates the *sil* exon1–*scl* exon 5 fusion, and encodes a truncated 22 kDa scl protein that lacks the scl transactivation domain and inititiates transcription at scl residue 176, similar to the protein product produced by *scl* exon1a–exon5 transcripts in normal cells (Bernard *et al.*, 1991; Cheng *et al.*, 1993).

### *Phenotype of mice generated with the pSIL/SCL vector*

Four founder animals were obtained by injection of the pSIL/SCL construct. Founder A1 and A4 both incorporated one copy of the transgene and expressed relatively low levels of the transgene in thymus, liver and spleen (Figure 3). Founder A2 incorporated two copies of the transgene and expressed 5-fold higher levels of exogenous *scl* mRNA. Founder A3 incorporated three copies of the transgene, but never transmitted the transgene, and mRNA expression was never assayed. Only one of 180 positive progeny of these three founders developed a T-cell malignancy over an observation period lasting 16 months (see below).

Continued observation of these mice revealed an unexpected phenotype. Nine (3/34) and eight (5/62) percent of the *scl*-positive progeny from lines A1 and A4 had obviously kinked tails (Figure 4a), while being otherwise healthy. All but one (5/6) of the positive progeny of line A2, the line with the highest *scl* mRNA expression, displayed kinked tails in conjunction with more severe abnormalities, including growth retardation, sparse hair

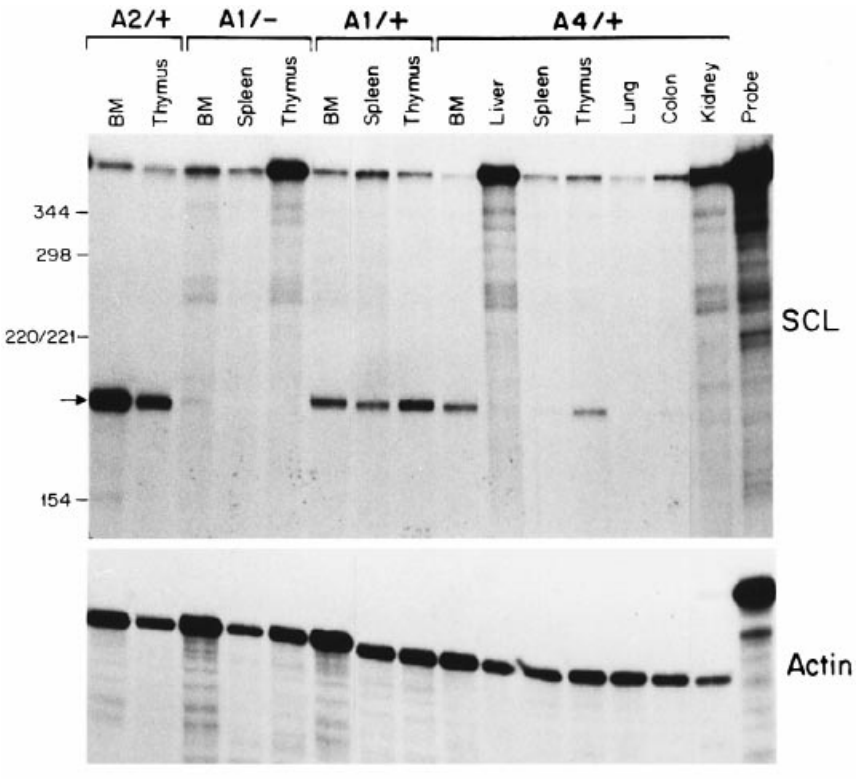


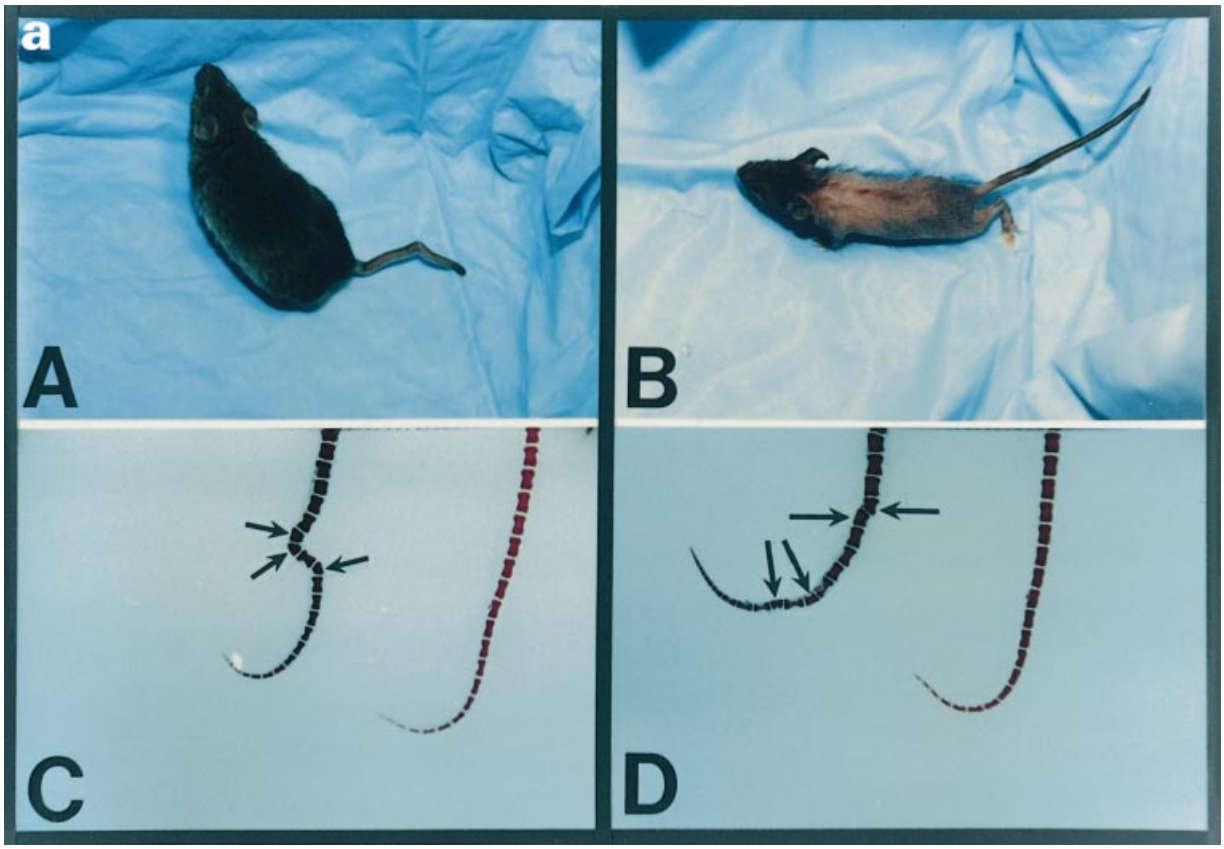

**Fig. 3.** RNase protection assay demonstrating expression of the *scl* transgene. An antisense human *scl* probe was hybridized to total RNA extracted from the indicated tissues. Transgenic offspring from three founder lines (A2/+, A1/+, A4/+) demonstrate expression in bone marrow and thymus (indicated by an arrow); a non-transgenic littermate (A1/-) is negative. Parallel samples were hybridized to a murine β-actin probe as a control. A faint band seen in bone marrow from the non-transgenic animal is due to cross-hybridization with the endogenous murine *scl*.

and infertility (Figure 4a). The females all had an atretic vaginal opening that could not be probed, while the only male was unable to impregnate any females despite numerous attempts at mating. It is of note that Southern blot analysis of genomic DNA from the only transgenic offspring of line A2 which did not display this unusual phenotype demonstrated that the transgene had been rearranged and largely deleted. None of >300 non-transgenic offspring observed during this period had tail kinks. Alizarin red/alcian blue staining showed the only obvious bony abnormality of these animals to be within the tail vertebrae (Figure 4a). In addition, while lines A1 and A4 transmitted the transgenic allele in expected Mendelian fashion, line A2, which expressed the highest level of exogenous *scl* mRNA, transmitted the transgene to only six of 45 progeny.

To evaluate a potential dosage effect of *scl* transgene expression on the generation of this phenotype, we crossed two line A4 animals. Ten of 11, seven of 39, and none of 18 animals with two, one or zero copies of the transgene, respectively, displayed tail kinks. Additionally, four of the animals with two copies of the transgene were growth retarded and had sparse hair (Figure 4b). None of these animals developed a T-cell malignancy over a 16 month observation period. Some of these double-positive (A4+/A4+) animals were fertile; one of these was crossed with a line A1-positive animal (A1+/A1-). Of 46 offspring from this cross, 31 were A4+/A1-; two of these had kinked tails; 15 offspring (only half of the expected number) were A4+/A1+, nine of these had kinked tails; one of these 15 developed an agressive T-cell malignancy at age 15 months. Taken together, these data suggest that ectopic *scl* expression causes bony abnormalities in a dose-dependent fashion, and that higher levels of ectopic *scl* expression are associated with embryonic lethality.

### *Phenotype of mice generated with an scl construct lacking the transactivation domain*

The fact that mice transgenic for a full-length scl were not developing T-cell malignancies led us to investigate the possibility that the amino-terminal truncated p22 scl isoform was the oncogenic form. In addition to being formed by a *sil* exon1–*scl* exon 5 fusion mRNA, the p22 scl protein can also be produced from T-cell ALL cells through the use of an alternate *scl* promoter, which initiates transcription from within *scl* exon 4 (Aplan *et al*., 1990a; Bernard *et al.*, 1992, 1996). Therefore, we generated four additional founder lines using the pSIL/TSCL construct. Line A5(3) expressed the highest level of exogenous *scl* and had incorporated 5–7 copies of the transgene. Similar to the pSIL/SCL founders, 0/33, 3/29, 4/18 and 2/27 positive offspring of lines A5(1), A5(2), A5(3) and A5(4), respectively, demonstrated kinked tails. Also, while lines A5(1), A5(2) and A5(4) transmitted the transgene within the range expected for Mendelian inheritance, only 18/86 (21%) of the A5(3) offspring were transgenic, again suggesting the possibility of embryonic lethality. None of the 107 positive progeny of these founders have developed T-cell malignancies over a 12 month observation period.



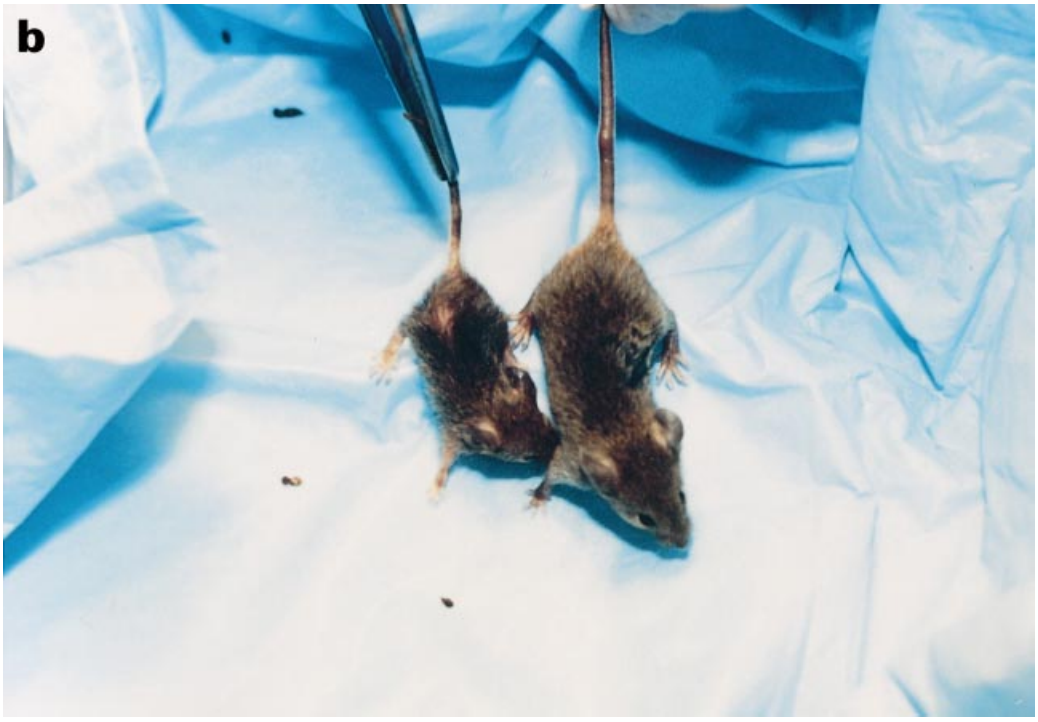




**Fig. 4.** Phenotype of *sil–scl* transgenic mice. (**a**) *sil–scl* transgenic mice show kinked tails and hair loss. (A) Shows a line A4-positive animal with tail kink, (B) shows a line A2-positive animal with tail kink and hair loss, (C) and (D) each show alizarin stains of kinked tails on the left, with normal controls on the right. The misformed vertebrae are indicated with arrows; note that the tail in (D) has defects in opposite orientations, leading to a less marked kink. (**b**) Dose effect of the *sil–scl* transgene. Two offspring from a line A4×A4 cross are shown. The animal on the left has two copies of the transgene (A4+/A4+), his littermate on the right has one copy (A4+/A4-). Both animals have kinked tails; note the growth retardation and sparse hair of the animal on the left.

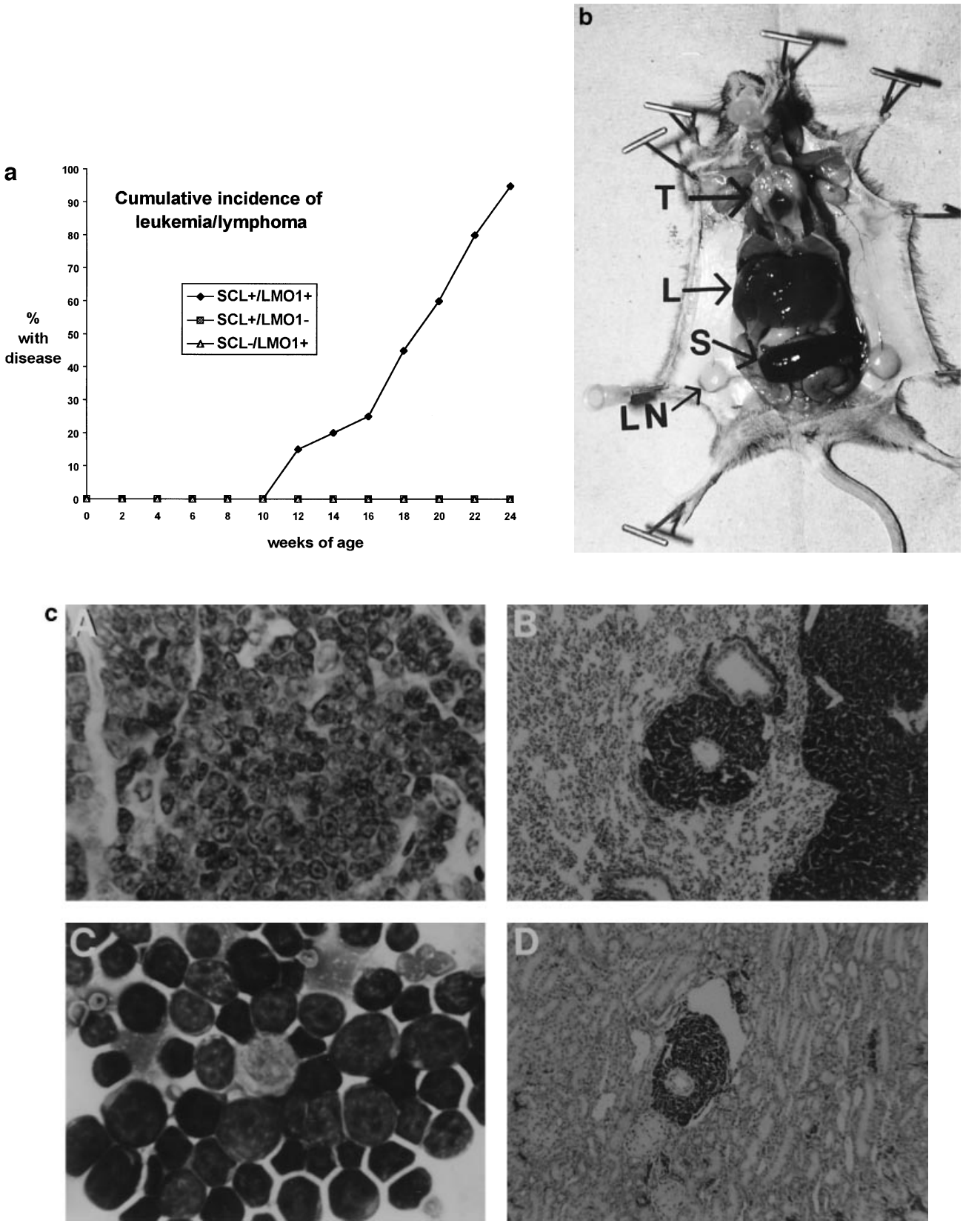



**Fig. 5.** Mice transgenic for both a *sil–scl* construct and an *lck–LMO1* construct develop T-cell leukemia/lymphoma at a young age. (**a**) Cumulative incidence of leukemia/lymphoma in *scl*/*LMO1*-positive mice. Nine litters from an *scl*×*LMO1* cross were observed for clinical signs of lymphoma/leukemia over a 6 month study period. Genotypes were *scl*+/*LMO1*+ ($n = 20$), *scl*+/*LMO1*- ($n = 8$), *scl*-/*LMO1*+ ($n = 14$). (**b**) Gross appearance of tumors in a double transgenic mouse. Note the enlarged thymus (T), liver (L), spleen (S) and lymph nodes (LN). (**c**) Microscopic appearance of leukemia/lymphoma. (A) Thymus (×720); (B) lung (×120); (C) bone marrow (×1200); (D) kidney (×120). Note the appearance of typical lymphoblasts with a high nuclear/cytoplasm ratio, and the perivascular infiltration of the lung and kidney.

**Table I.** Clinical and immunophenotypic characteristics

| Mouse | Sex | Age (weeks) | Organ enlargement | | | | Immunophenotype | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Thy | Liv | Spl | LN | CD3 | CD4 | CD8 |
| 6384/4 | M | 17 | + | − | + | + | 8 | 38 | 50 |
| 6386/3 | M | 18 | + | − | + | + | 68 | 26 | 61 |
| 6469/2 | M | 13 | − | + | + | + | 54 | <1 | 22 |
| 6466/2 | M | 12 | + | + | + | + | 56 | 52 | 57 |
| 6471/4 | M | 14 | + | + | + | + | 11 | 60 | 86 |
| 6472/2 | M | 13 | + | − | + | + | 77 | 43 | 72 |
| 6385/4 | F | 23 | + | − | + | + | 70 | 47 | 68 |
| 6385/1 | F | 24 | − | − | + | + | 63 | 59 | 63 |
| 6466/1 | M | 22 | + | + | + | + | 94 | 88 | 90 |
| 6465/2 | M | 23 | + | − | + | + | 93 | 82 | 98 |
| 6385/3 | F | 24 | + | + | + | + | 47 | 70 | 91 |
| 6466/4 | M | 19 | + | + | + | + | 43 | 66 | 81 |
| 6467/2 | M | 21 | + | + | + | + | 34 | 32 | 84 |
| 6600/1 | M | 18 | + | + | + | + | 81 | 40 | 95 |
| 6472/3 | M | 21 | + | + | + | + | 89 | 43 | 92 |
| 6600/2 | M | 19 | + | + | + | + | 89 | 63 | 86 |
| 6464/2 | F | 25 | + | + | + | + | 95 | 78 | 99 |
| 6600/3 | M | 20 | + | − | + | + | 95 | 86 | 79 |
| 6603/5 | M | 22 | + | + | + | + | 95 | 86 | 96 |

Abbreviations: M, male sex; F, female sex; Thy, thymus; Liv, liver; Spl, spleen; LN, lymph nodes. Age reflects the age in weeks when the animal was sacrificed. Immunophenotype is expressed as percent of malignant cells positive for the indicated antigen.

### *scl does not collaborate with pim-1 to induce T-cell malignancies*

In order to determine if *scl* was able to cooperate with other known transforming genes, we crossed offspring of the A4 line with mice transgenic for an Eμ-*pim-1* construct. *pim-1* was chosen as a candidate gene since it was identified initially by virtue of its frequent activation at MuLV insertion sites in murine T-cell lymphomas (Cuypers *et al.*, 1984), and has been shown to accelerate dramatically lymphoid malignancies produced by the bHLH proteins c-Myc and N-Myc (van Lohuizen *et al.*, 1989). We have now followed a cohort of mice positive for both *scl* and *pim-1* for >1 year, and compared them with control groups positive for either *scl* or *pim-1* only, or negative for both transgenes. Two of eight *scl+/pim-1+* animals have developed non-T-cell malignancies over a 15 month observation period; three of 17 *scl-/pim-1+* mice have developed malignancies (one spindle cell sarcoma of unknown primary, two T-cell lymphomas), and none of 15 *scl+/pim-1-* mice have developed T-cell malignancies. We conclude that ectopically expressed *scl* does not accelerate the rate at which *pim-1* transgenic mice develop malignancies.

### *The p22 isoform of scl collaborates with LMO1 to induce an aggressive T-cell leukemia/lymphoma*

In order to determine if *scl* could cooperate with *LMO1* to induce T-cell ALL, we crossed the A5(3) line with mice engineered to express *LMO1* from an *lck* promoter (McGuire *et al.*, 1992). *LMO1* was chosen as a candidate since a subset of T-cell ALL patients and cell lines have activated both *scl* and *LMO1* (Wadman *et al.*, 1994b). In a previous report, 50% of the progeny of *lck–LMO1* line 11 developed T-cell leukemia/lymphoma at ages ranging from 5 to 16 months; the incomplete penetrance and relatively long latency period led to the speculation that additional genetic events were required to produce a frank malignancy (McGuire *et al.*, 1992). We chose to cross the *lck–LMO1* mice with a line expressing the truncated form of scl to investigate whether the scl transactivation domain would be required for malignant transformation. As seen in Figure 5a, 19/20 (95%) of mice positive for both *scl* and *LMO1* developed an aggressive T-cell leukemia/lymphoma by age 6 months. In contrast, none of the mice positive for *scl* or *LMO1* alone developed T-cell malignancies during the 6 month observation period.

The mice who developed leukemia/lymphoma typically displayed labored breathing, lethargy, peripheral lymphadenopathy, thymic enlargement, splenomegaly and hepatomegaly (Figure 5b). Microscopic exam showed widespread organ infiltration, including liver, kidney, lung and bone marrow; the infiltration of solid tissues such as kidney and liver was typically in a perivascular pattern (Figure 5c). Bone marrow samples were largely replaced by lymphoblasts; complete blood counts generally showed a low red blood cell count, a low platelet count and a normal or increased white blood cell count. The immunophenotypes were typical of immature T-cell leukemia/lymphoma and are presented in Table I. The malignant cells expressed both *scl* and *LMO1* (Figure 6a) mRNA. Western blot analysis using a monoclonal scl antibody (Pulford *et al.*, 1995) demonstrated the presence of a 22 kDa scl protein in tumor tissue (data not shown); lymphomas from the *lck–LMO1* mice have been shown previously to express LMO1 by immunohistochemistry (McGuire *et al.*, 1992). The presence of TCRβ gene rearrangments was evaluated on a subset of the tumors and demonstrated clonal patterns of TCRβ rearragements (Figure 6b). As further evidence of their malignant nature, cells from a subset of these tumors have been cultured *in vitro* for several months without the addition of hematopoietic growth factors.

Since the T-cell leukemia/lymphomas were not evident until 12 weeks at the earliest, it is conceivable that additional mutational events are required for malignant transformation of the *scl*/*LMO1+* cells. Given the frequent

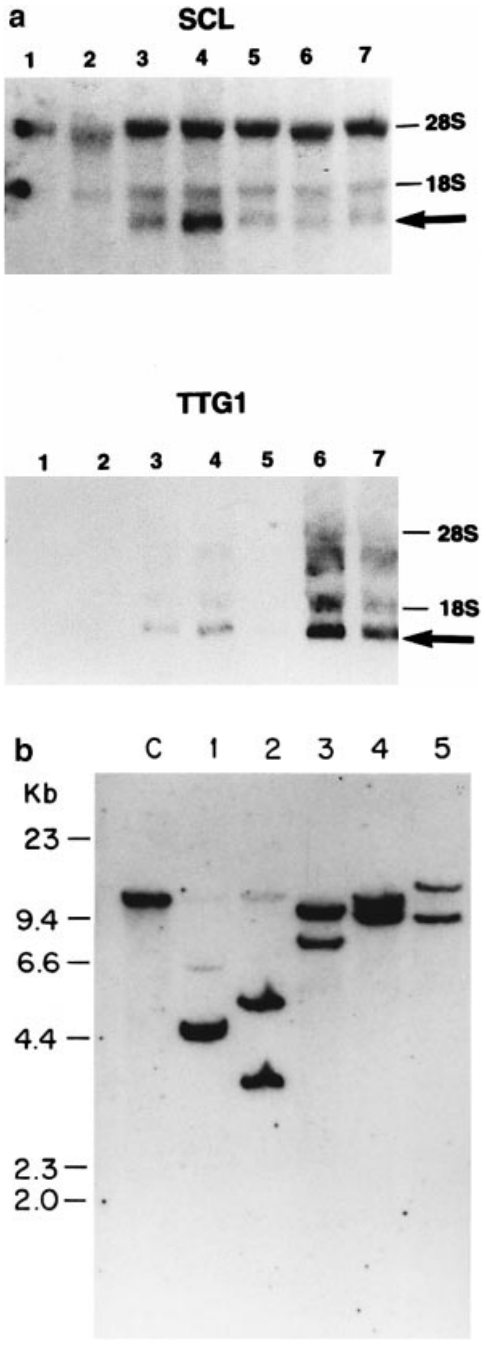



**Fig. 6.** Molecular analysis of tumor tissue. (**a**) Expression of exogenous *scl* and *LMO1* mRNA in tumor tissue. Upper panel: Northern blot hybridized to *scl* cDNA probe (67HX). Lane 1, non-transgenic bone marrow; lane 2, non-transgenic liver; lanes 3–7, five representative tumors (tissue from bone marrow, thymus or lymph node). An arrow indicates a signal from the exogenous *scl*. Bottom panel: a duplicate blot was hybridized to a *LMO1* (TTG1) cDNA probe. (**b**) Clonal TCRβ gene rearrangements in tumor tissue. Southern blot of *Sst*I-digested tumor tissue hybridized to a human TCRβ Cβ2 probe. Lane 1, tail DNA from non-transgenic mouse used as a germline control, lanes 2–6, DNA from five different tumor samples. Clonal rearranged bands from one or both alleles are seen in all five tumor samples. Size standards are in kb.

homozygous deletions of p16 in human T-cell ALL and lymphoblastic lymphoma (Hirama and Koeffler, 1995), we probed Southern blots of tumor tissue with a PCR-generated p16 probe. No samples showed evidence of a homozygous deletion of p16 (data not shown).

## Discussion

In this study, we demonstrate a role for *scl* deregulation in generating aggressive T-cell malignancies. The immunophenotype and clinical manifestations of disease in the *scl*/*LMO1* double positive mice is quite similar to that recognized in human patients with T-cell ALL. The fact that all but one of a cohort of 20 double-positive mice developed T-cell leukemia/lymphoma within a relatively short time span indicates that *scl* and *LMO1* can cooperate quite effectively to induce T-cell malignancies. Moreover, given that both of these genes were originally identified at chromosome translocation breakpoints, and that both of these genes are activated in human T-cell leukemias, this would seem to be a valid, useful murine model of the human disease.

In order to simulate the situation seen in human T-cell ALL, we generated transgenic mice which ectopically expressed either a full-length or an amino-terminal truncated scl protein under the control of *sil* regulatory elements. We focused our later experiments on the *sil* exon1–*scl* exon 5 fusion splice form, since it was one of the more abundant forms, and encoded a truncated *scl* gene product which lacked the scl transactivation domain. This finding, together with the observation that some T-cell ALL patients produce a truncated *scl* transcript encoding a protein which lacks the scl transactivation domain (Bernard *et al.*, 1992; Goldfarb and Greenberg, 1994), suggested that the scl transactivation domain may be dispensable for oncogenesis.

An unexpected finding was the observation that mice transgenic for either the full-length or truncated form of the *scl* gene product developed bony abnormalities and growth retardation, in what seemed to be a dose-dependent fashion. We initially would not have expected the *sil* promoter to be functional in non-hematopoietic tissues, based on reports (Aplan *et al.*, 1991) that suggested *sil* expression was limited to hematopoietic tissues. However, additional studies have demonstrated that *sil* mRNA is ubiquitously expressed in proliferating cells, and seems to be an immediate early response gene (Collazo-Garcia *et al.*, 1995; Izraeli *et al.*, 1995). Seen in this light, one would expect the *sil* promoter to be active, and generate ectopic *scl* production, in all proliferating tissues of the transgenic mouse. The most consistent and obvious phenotypic effect of ectopic *scl* expression was the incomplete formation of tail vertebrae. Numerous animals from several different founder lines produced from both *scl* constructs displayed this finding, which made the possibility that it was due to an insertional mutation highly unlikely. Although the molecular events which generate this phenotype have not been defined, it is worth noting that, since the phenotype is observed in mice which express a truncated scl form, the scl transactivation domain is not required to generate the phenotype. However, since the truncated scl contains the HLH protein dimerization domain, it retains the potential to bind and sequester those proteins which normally bind scl through its HLH domain.

As mentioned above, the fact that some animals did not develop clinical signs of disease until 6 months of age was consistent with the possibility that additional genetic events were required for leukemic transformation. Given the likely importance of the interactions between

p16, CDK4, D-type cyclins and pRB in T-cell leukemia/lymphoma (Hirama and Koeffler, 1995), these proteins would seem to be reasonable candidates for the proposed additional events. However, a preliminary analysis of the genes coding for p16 and pRB did not reveal any gross abnormality (deletion or rearrangement) at the Southern blot level. A more thorough analysis of these proteins as potential candidates is currently underway.

Several investigators (Green, 1996; Kelliher *et al.*, 1996; Porcher *et al.*, 1996) have speculated that the oncogenic potential of dysregulated scl may be conferred either through inappropriate activation of physiologic scl target genes, or through a dominant-negative mechanism, where misexpressed scl binds to and functionally inactivates those proteins to which it normally binds (Figure 7). Our data suggests that ***scl*** is unlikely to exert its oncogenic potential through inappropriate activation of target genes, since mice transgenic for a *sil–scl* fusion mRNA lacking the scl transactivation domain developed aggressive T-cell leukemia/lymphomas. This finding supports the notion that scl is likely to be oncogenic through a dominant-negative mechanism.

If one supports the concept that scl is oncogenic through a dominant-negative mechanism, the next obvious question focuses on binding partners for scl. The bHLH 'E-proteins' E2-2, E2A and HEB (Hsu *et al.*, 1991; Voronova and Lee, 1994; Goldfarb *et al.*, 1996), the LIM domain proteins LMO1 and LMO2 (Wadman *et al.*, 1994) and the putative tumor suppressor DRG (Mahajan *et al.*, 1996) have all been shown to bind to scl protein. DRG, a GTP-binding protein originally isolated through a subtractive hybridization approach designed to identifiy potential tumor suppressor genes (Schenker *et al.*, 1994), has been recovered recently as an scl-binding protein in a yeast two-hybrid screen for scl-binding proteins (Mahajan *et al.*, 1996). The LIM domain proteins LMO1 and LMO2 have been shown to bind scl using both co-immunoprecipitation and two-hybrid assays (Wadman *et al.*, 1994), and mice transgenic for both *scl* and either *LMO1* (this report) or *LMO2* (Larson *et al.*, 1996) develop aggressive T-cell leukemia/lymphomas. However, although *scl* and *LMO1* clearly cooperate in the genesis of T-cell malignancies, it remains possible that this cooperativity does not need to take place through a direct interaction, but that *scl* and *LMO1* may instead be acting through different pathways.

Scl binding with E-proteins through HLH domain interactions has been demonstrated by several laboratories; a recent report (Zhuang *et al.*, 1996) has studied mice lacking either E2-2, E2A or HEB (Hu *et al.*, 1992). Mice lacking E2-2 appeared to have normal T-cell development, while mice lacking HEB demonstrated abnormal fetal and neonatal T-cell development, primarily manifested by a lack of CD4 and CD5 expression in thymocytes. Interestingly, whereas CD4 and CD8 expression on thymocytes from 5-week-old mice positive for either an *scl* or *LMO1* transgene is similar to that seen with negative control animals, thymocytes from *scl*/*LMO1* double-positive mice also show decreased numbers of CD4-positive cells (D.S.Chervinsky and P.D.Aplan, unpublished data). It is not known whether mice lacking HEB are prone to developing T-cell malignancies as they generally die of undefined causes before weaning (Zhuang *et al.*, 1996). In addition, a region within the scl carboxy-terminus that appears to repress the transactivation domain of the E-proteins (Hofmann and Cole, 1996) has been identified, making the E-proteins likely candidates for scl sequestration and functional inactivation in a dominant-negative model.

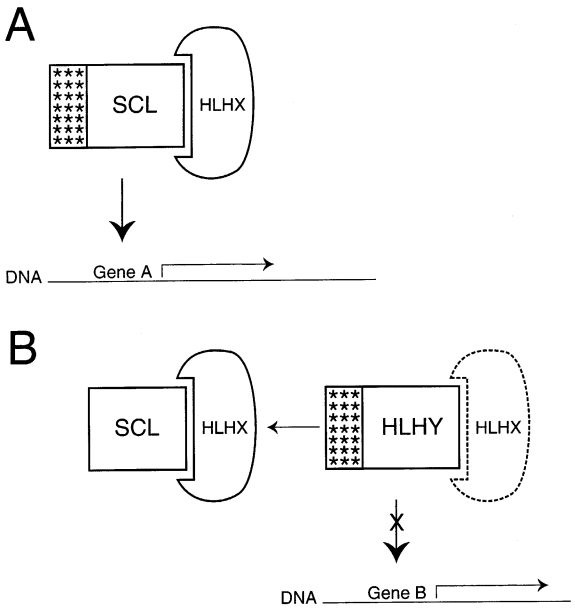



**Fig. 7.** Two potential models for scl-induced leukemogenesis. In (**A**), aberrantly expressed scl binds another HLH protein (HLHX), and activates transcription of gene A (a gene that is normally a target for scl activation) through the scl transactivation domain (indicated by asterisks). In (**B**), a dominant-negative model is presented. An aberrantly expressed scl protein that lacks the scl transactivation domain binds the HLHX protein and effectively sequesters the HLHX protein, preventing it from binding to the HLHY protein. The HLHX protein is then unavailable to form heterodimers with the HLHY protein, and the HLHY protein therefore is prevented from activating transcription of its normal targets (gene B in this model). In this model, the primary effect of ectopic scl expression is not the inappropriate activation of gene A, but the functional inactivation of HLHX, and protein complexes dependent of the presence of HLHX. Note that although this model depicts scl binding to a HLH protein, scl has been shown to bind non-HLH proteins and could exert a dominant-negative action by binding one of these non-HLH proteins (see text). Also note that in this model, an ectopically expressed full-length scl protein (which retained the transactivation domain) could still act in a dominant-negative fashion with respect to the HLHX protein. Preliminary results suggest that mice transgenic for both a full-length scl protein and LMO1 develop T-cell leukemia/lymphoma at approximately the same rate as mice transgenic for the truncated scl and LMO1 (D.S.Chervinsky and P.D.Aplan, unpublished results).

In summary, we have demonstrated that *scl* and *LMO1* can cooperate to generate aggressive T-cell malignancies in a mouse model of human T-cell ALL. The oncogenic effect of scl can be produced by an amino-terminal truncated form of the scl protein which lacks the scl transactivation domain and is commonly found in T-cell ALL, demonstrating that the scl transactivation domain is dispensable for its oncogenic action. Furthermore, mice transgenic for either the full-length or truncated scl display developmental abnormalities in additional mesoderm-derived tissues. Although other models cannot be excluded, the most likely model that accounts for both malignancies and bony abnormalities using constructs which lack the scl transactivation domain is one which asserts that scl acts in a dominant-negative fashion.

## Materials and methods

### Plasmid construction

The pSIL/SCL plasmid was generated as follows. A 2.4 kb *Sst*II–*Sst*II human *sil* genomic fragment encompassing the *sil* transcript initiation site was isolated from a genomic *sil* clone (Aplan *et al.*, 1991); the 5′ *Sst*II site is derived from the pBS II (Stratagene) polylinker, the 3′ *Sst*II site is located within *sil* exon 1. This *Sst*II fragment was then cloned into the *Sst*II site of pBSII, and a *Hin*dIII–*Hin*dIII fragment from the pMSCL-WT vector (Aplan *et al.*, 1992) containing (i) an *scl* cDNA encoding the full-length human scl protein; (ii) a human β-globin fragment encompassing 18 bp of β-globin exon 2 and all of exon 3 (including the polyadenylation signal); and (iii) a neomycin resistance cassette, was cloned into the *Hin*dIII site of pBSII, immediately downstream of the *sil* promoter fragment. The vector was sequenced to verify its orientation. The pSIL/TSCL was constructed in three steps. First, RT–PCR was used to amplify a *sil* exon 1–*scl* exon5 fusion from a cell line (CEM) known to contain a *sil–scl* fusion (Aplan *et al.*, 1990b), using oligonucleotides A and B (see below). This PCR product was cloned into the PCRII (Invitrogen) vector and sequenced to verify that no PCR artefacts had been introduced. Next, a 0.3 kb insert from this plasmid containing *sil* exon 1, *scl* exon 5 and a portion of *scl* exon 6 was excised with *Sst*I [the 5′ *Sst*I site was derived from the PCRII polylinker, the 3′ *Sst*I site is within *scl* exon 6 (nucleotide 354 of DDBJ/EMBL/GenBank accession No. M29038)] and ligated in-frame to a 9 kb *Sst*I fragment derived from the pSIL/SCL vector containing (i) human *scl* exon 6 sequences, (ii) human β-globin exon 2 and 3 as above, (iii) a neomycin resistance cassette, and (iv) the pBSII plasmid backbone. Lastly, the 2.4 kb *Sst*II–*Sst*II *sil* promoter fragment was then cloned into the *Sst*II site (located within *sil* exon 1) of this plasmid, generating the pSIL/TSCL plasmid. All cloning junctions were sequenced to verify the construct.

### Nucleic acid manipulations

Genomic DNA was isolated from tumors and tail biopsies using conventional techniques, Southern blots were performed as described previously (Aplan *et al.*, 1990a). Total RNA was isolated using Trizol (BRL); Northern blots were performed as described previously (Collazo-Garcia *et al.*, 1995). Probes used in this study included a 1.2 kb *Hin*dIII–*Xba*I human *scl* cDNA fragment (67HX, Aplan *et al.*, 1992), a 1.0 kb *Sst*I–*Xba*I *scl* cDNA probe (1.0SX, Begley *et al.*, 1989), a PCR-amplified human *LMO1* cDNA fragment (nucleotides 544–957 of DDBJ/EMBL/GenBank accession No. M26682), a PCR-amplified murine p16 cDNA fragment (nucleotides 206–458 of DDBJ/EMBL/GenBank accession No. L76150) and a 0.2 kb human TCR Cβ2 probe (gift of Dr Ilan Kirsch). The *sil–scl* fusion mRNA RT–PCR assay was accomplished using rTth DNA polymerase (Perkin Elmer Cetus). Briefly, 0.5 μg of total RNA from CEM or HSB2 (T-cell lines known to have undergone a *sil–scl* recombination) or HL60 (a myeloid cell line used as a negative control) was reverse transcribed using an *scl* exon 6 antisense oligonucleotide (primer C in Figure 1; 5′-ATGTGTGGGGATCAGCTTGC-3′) for 15 min at 70°C. A *sil* exon 1 sense oligonucleotide (primer A in Figure 1; 5′-GCTCCTACCCTGCAAACAGA-3′) was added followed by 35 PCR cycles of 60 s at 95°C, 60 s at 51°C and 90 s at 72°C, and a terminal extension of 7 min at 72°C. Amplification of the *sil* exon1–*scl* exon 3 fusion mRNA was performed using an identical protocol, except an *scl* exon 3 antisense oligonucleotide (primer B in Figure 1; 5′-GGCATATT-TAGAGAGACCG-3′) was used in place of primer C. RNase protection assays were carried out using a RPAII kit (Ambion) and the manufacturer's recommended protocol. The hybridization conditions were 50°C for 16 h; the probe used was a human *scl* cDNA fragment encompassing *scl* exons 1a, 5 and 6 (Aplan *et al.*, 1990a). Nucleotide sequencing was carried out using Sequenase (USB) enzyme and reagents.

### Transfections

The F4-6 murine erythroleukemia cell line was transfected with the pSIL/SCL vector, using Lipofectin (BRL), as described previously (Aplan *et al.*, 1992). Clonal stable transfectants were selected with 800 μg/ml of G418 (BRL). The stable transfectants were expanded over a 2 week period, and $5\times10^6$ cells were harvested for mRNA isolation.

### Generation of transgenic lines

The pSIL/SCL and pSIL/TSCL inserts were purified away from plasmid sequences by agarose gel electrophoresis and glass beads (Bio101). Transgenic mice were produced by pronuclear microinjection using established methods (Hogan *et al.*, 1986). Constructs were microinjected into zygotes derived from a C57 Bl/10Ros-$_p^d$×C3H/HeRos cross (BCF1). Founders were identified by Southern blot analysis of DNA from tail biopsy and lines established by mating with BCF1 animals. Transgenic mice were maintained on Harlan Teklad Laboratory Rodent Diet. Mice transgenic for an Eμ-*pim-1* construct (van Lohuizen *et al.*, 1989) were obtained from GenPharm International. Mice transgenic for a *lck–LMO1* construct (line 11 of McGuire *et al.*, 1992) have been described previously.

### Alizarin staining

Mouse skeletons were stained using a modification of previously described techniques (Kimmel and Trammell, 1981). Briefly, animals were skinned and eviscerated; the skeletons dehydrated in 95% ethanol for 24 h, defatted in acetone for 5 days, and stained for 6 h at 37°C using a 70% ethanol/acetic acid/saturated alizarin red/0.14% alcian blue solution. The skeletons were cleared in 1% KOH for 2–5 days and fixed using stepwise incubations with 20, 50 and 80% glycerol in 1% KOH. The skeletons were stored in 100% glycerol.

### Immunophenotype and Western blot analysis

Cells were immunophenotyped using monoclonal antibody conjugates and standard techniques. Briefly, $10^6$ cells were blocked with 1 μg of rat IgG for 15 min at 4°C followed by the addition of 1 μg of the indicated monoclonal antibody conjugate for 45 min at 4°C. Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and resuspended in 1% buffered formalin until analysis. Data was scored and analyzed using a FACScan analyzer (Beckton-Dickinson). Five thousand events were acquired for each sample. The antibodies used were anti-mouse CD3R-PE, CD4-FITC and CD8-Red613 (GIBCO/BRL). For Western blots, ~$10^7$ cultured cells were harvested, washed once in ice-cold PBS, and resuspended in RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing a cocktail of proteinase inhibitors (1 mg/ml Pefabloc SC, 1 mM EDTA, 25 μg/ml leupeptin, 10 μg/ml pepstatin, 2.5 μg/ml aprotinin). Cells and organelles were disrupted in a hand-held glass Dounce homogenizer, incubated on ice for 30 min and centrifuged at 14 000 r.p.m. for 30 min in a 4°C microcentrifuge. The precipitates were discarded and the supernatants aliquoted and stored at –80°C. Protein concentrations were determined by the Bradford method. Cell lysates from tissues were prepared as above except that individual cells were first released and separated from tissues by brief homogenization in ice-cold PBS before they were precipitated and resuspended in RIPA buffer. For Western blots, 100 μg of cell lysate protein was resolved by 15% SDS–PAGE (4% stacking gel) and transferred electrophoretically (Towbin *et al.*, 1979) to a Hybond nitrocellulose membrane (Amersham). Blots were incubated at 4°C overnight in 5% non-fat dry skim milk in TBS (50 mM Tris–HCl, pH 7.6, 150 mM NaCl) containing 0.05% Tween-20 (TBS-T). The blots were rinsed briefly with TBS-T and incubated at room temperature for 1 h with a human scl monoclonal antibody (antibody BTL73, kind gift of Dr Karen Pulford). Immunoreactive proteins were revealed using horseradish peroxidase-labeled anti-mouse IgG and ECL Western blotting detection reagents (Amersham) by exposure to Kodak X-ray film.

## Acknowledgements

We would like to acknowledge Ms Elaina Greco for help with illustrations, Mr Al Cairo for oligonucleotide synthesis, Dr Stanley Korsmeyer for the *lck–LMO1* mice, and Drs Ilan Kirsch, Paul Soloway and Adam Goldfarb for helpful conversations. This work was supported in part by grants from the Leukemia Research Foundation, American Cancer Society and National Institutes of Health to P.D.A., grants from the National Institutes of Health to K.G. and grants from the American Cancer Society, The Council for Tobacco Research-USA and the Lauri Strauss Leukemia Foundation to E.A.M.

## References

Aplan,P.D., Begley,C.G., Bertness,V.L., Nussmeier,M., Ezquerra,A., Coligan,J. and Kirsch,I.R. (1990a) The *SCL* gene is formed from a transcriptionally complex locus. *Mol. Cell. Biol.*, **10**, 6426–6435.

Aplan,P.D., Lombardi,D.P., Ginsberg,A.M., Cossman,J., Bertness,V.L. and Kirsch,I.R. (1990b) Disruption of the human *SCL* locus by 'illegitimate' V(D)J recombinase activity. *Science*, **250**, 1426–1429.

Aplan,P.D., Lombardi,D.P. and Kirsch,I.R. (1991) Structural chracterization of *SIL*, a gene frequently disrupted in T-cell acute lymphoblastic leukemia. *Mol. Cell. Biol.*, **11**, 5462–5469.

Aplan,P.D., Nakahara,K., Orkin,S.H. and Kirsch,I.R. (1992) The *SCL* gene product: a positive regulator of erythroid differentiation. *EMBO J.*, **11**, 4073–4082.

Bash,R., Hall,S., Timmons,C., Crist,W., Amylon,M., Smith,R. and Baer,R. (1995) Does activation of the *TAL1* gene occur in a majority of patients with T-cell acute lymphoblastic leukemia? *Blood*, **86**, 666–676.

Begley,C.G., Aplan,P.D., Denning,S.M., Haynes,B.F., Waldmann,T.A. and Kirsch,I.R. (1989) The gene *SCL* is expressed during early hematopoiesis and encodes a differentiation-related binding motif. *Proc. Natl Acad. Sci. USA*, **86**, 10128–10132.

Bernard,M., Smit,L., Macintyre,E., Matthieu-Mahul,D. and Pulford,K. (1996) Nuclear localization of the SCL/TAL1 basic helix–loop–helix protein is not dependent on the presence of the basic domain. *Blood*, **84**, 3356–3357.

Bernard,O., Lecointe,N., Jonveaux,P., Suoyri,M., Mauchauffe,M., Berger,R., Larsen,C.J. and Mathieu-Mahul,D. (1991) Two site-specific deletions and t(1;14) translocation restricted to human T-cell acute leukemias disrupt the 5′ part of the *tal-1* gene. *Oncogene*, **6**, 1477–1488.

Bernard,O., Azogui,O., Lecointe,N., Mugneret,F., Berger,R., Larsen,C.J. and Mathieu-Mahul,D. (1992) A third tal-1 promoter is specifically used in human T-cell leukemias. *J. Exp. Med.*, **176**, 919–925.

Boehm,T., Foroni,L., Kaneko,Y., Perutz,M.F. and Rabbitts,T.H. (1991) The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. *Proc. Natl Acad. Sci. USA*, **88**, 4367–4371.

Brown,L., Cheng,J.-T., Chen,Q., Siciliano,M.J., Crist,W., Buchanan,G. and Baer,R. (1990) Site-specific recombination of the *tal-1* gene is a common occurrence in human T-cell leukemia. *EMBO J.*, **9**, 3343–3351.

Carroll,A.J., Crist,W.M., Link,M.P., Amylon,M.D., Pullen,D.J., Ragab, A.H., Buchanan,G.R., Wimmer,R.S. and Vietti,T.J. (1990) The t(1;14)(p34;q11) is nonrandom and restricted to T-cell acute lymphoblastic leukemia. A POG study. *Blood*, **76**, 1220–1224.

Chen,Q. *et al*. (1990) The *tal* gene undergoes chromosome translocation in T-cell leukemia and potentially encodes a helix–loop–helix protein. *EMBO J.*, **9**, 415–424.

Cheng,J.-T., Hsu,H.-L., Hwang,L.-Y. and Baer,R. (1993) Products of the *TAL1* oncogene: basic helix–loop–helix proteins phosphorylated at serine residues. *Oncogene*, **8**, 677–683.

Collazo-Garcia,N., Scherer,P. and Aplan,P.D. (1995) Cloning and characterization of a murine SIL gene. *Genomics*, **30**, 506–513.

Cuypers,H.T., Selten,G., Quint,W., Zijlstra,M., Maandag,E.R., Boelens,W., van Wezenbeek,P., Melief,C. and Berns,A. (1984) Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell*, **37**, 141–150.

Elwood,N.J. and Begley,C.G. (1995) Reconstitution of mice with bone marrow cells expressing the *scl* gene is insufficient to cause leukemia. *Cell Growth Diff.*, **6**, 19–25.

Finger,L.R., Kagan,J., Christopher,G., Kurtzberg,J., Herschfield,M.S., Nowell,P.C. and Croce,C.M. (1989) Involvement of the *TCL5* gene on human chromosome 1 in T-cell leukemia and melanoma. *Proc. Natl Acad. Sci. USA*, **86**, 5039–5043.

Goldfarb,A.N. and Greenberg,J.M. (1994) T-cell acute lymphoblastic leukemia and the associated basic helix–loop–helix gene SCL/tal. *Leuk. Lymphoma*, **12**, 157–166.

Goldfarb,A.N., Lewandowska,K. and Shoham,M. (1996) Determinants of helix–loop–helix dimerization affinity. *J. Biol. Chem.*, **271**, 2683–2688.

Green,A.R., Lints,T., Visvader,J. and Begley,C.G. (1992) SCL is coexpressed with GATA-1 in hematopoietic cells but is also expressed in developing brain. *Oncogene*, **7**, 653–660.

Green,T. (1996) Master regulator unmasked. *Nature*, **383**, 575–576.

Hatano,M., Roberts,C., Minden,M., Crist,W. and Korsmeyer,S. (1991) Deregulation of a homeobox gene, *HOX11*, by the t(10;14) in T cell leukemia. *Science*, **253**, 79–82.

Hirama,T. and Koeffler,H.P. (1995) Role of the cyclin-dependent kinase inhibitors in the development of cancer. *Blood*, **86**, 841–854.

Hofmann,T.J. and Cole,M.D. (1996) The *TAL1/Scl* basic helix–loop–helix protein blocks myogenic diferentiation and E-box dependent transactivation. *Oncogene*, **13**, 617–624.

Hogan,B., Constantini,F. and Lacy,E. (1986) *Manipulating the Mouse Embryo*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hsu,H.-L., Cheng,J.-T., Chen,Q. and Baer,R. (1991) Enhancer binding activity of the tal-1 oncoprotein in association with the E47/E12 helix–loop–helix proteins. *Mol. Cell. Biol.*, **11**, 3037–3042.

Hsu,H.-L., Huang,L., Tsan,T., Funk,W., Wright,W.E., Hu,J.-S., Kingston,R.E. and Baer,R. (1994a) Preferred sequences for DNA recognition by the TAL1 helix–loop–helix proteins. *Mol. Cell. Biol.*, **14**, 1256–1265.

Hsu,H.-L., Wadman,I., Tsan,J.T. and Baer,R. (1994b) Positive and negative transcriptional control by the TAL1 helix–loop–helix protein. *Proc. Natl Acad. Sci. USA*, **91**, 5947–5951.

Hu,J.-S., Olson,E.N. and Kingston,R.E. (1992) HEB, a helix–loop–helix protein related to E2A and ITF2 that can modulate the DNA-binding ability of myogenic regulatory factors. *Mol. Cell. Biol.*, **12**, 1031–1042.

Hwang,L.-Y., Siegelman,M., Davis,L., Oppenheimer-Marks,N. and Baer,R. (1993) Expression of the *TAL*1 proto-oncogene in cultured endothelial cells and blood vessels of the spleen. *Oncogene*, **8**, 3043–3046.

Izraeli,S., Bertness,V.L., Mani,K.M., Aplan,P.D. and Kirsch,I.R. (1995) *SIL*, the most frequent 'dysregulator' of *SCL* (*tal-1*) in T-cell acute lymphoblastic leukemia (T-ALL) is an immediate early response gene expressed in proliferating cells. *Blood*, **86**, 34a.

Kallianpur,A.R., Jordan,J.E. and Brandt,S.J. (1994) The *SCL/TAL1* gene is expressed in progenitors of both the hematopoietic and vascular systems during embryogenesis. *Blood*, **83**, 1200–1208.

Kelliher,M.A., Seldin,D.C. and Leder,P. (1996) Tal-1 induces T-cell acute lymphoblastic leukemia accelerated by casein kinase IIα. *EMBO J.*, **15**, 5160–5166.

Kimmel,C.A. and Trammell,C. (1981) A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. *Stain Technol.*, **56**, 271–273.

Larson,R.C., Lavenir,I., Larson,T.A., Baer,R., Warren,A.J., Wadman,I., Nottage,K. and Rabbitts,T.H. (1996) Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. *EMBO J.*, **15**, 1021–1027.

Lowsky,R., DeCoteau,J.F., Reitmair,A.H., Ichinohasama,R., Dong,W.F., Xu,Y., Mak,T.W., Kadin,M.E. and Minden,M.D. (1996) Mutations of *hMSH2* in human lymphoblastic lymphomas. *Blood*, **88**, 353a.

Mahajan,M.A., Park,S.T. and Sun,X.-H. (1996) Association of a novel GTP binding protein, DRG, with TAL oncogenic proteins. *Oncogene*, **12**, 2343–2350.

McGuire,E.A., Hockett,R.D., Pollock,K.M., Bartholdi,M.F., O'Brien,S.J. and Korsmeyer,S.J. (1989) The t(11;14)(p15;q11) in a T cell acute lymhoblastic leukemia cell line activates multiple transcripts, including *Ttg-1*, a gene encoding a potential zinc finger protein. *Mol. Cell. Biol.*, **9**, 2124–2132.

McGuire,E.A., Rintoul,C.E., Sclar,G.M. and Korsmeyer,S.J. (1992) Thymic overexpression of *Ttg-1* in transgenic mice results in T-cell acute lymphoblastic leukemia/lymphoma. *Mol. Cell. Biol.*, **12**, 4186–4196.

Murre,C., McCaw,P.S. and Baltimore,D. (1989) A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD and myc proteins. *Cell*, **56**, 777–783.

Olson,E.N. and Klein,W.H. (1994) bHLH factors in muscle development: dead lines and commitments, what to leave in and what to leave out. *Genes Dev.*, **8**, 1–8.

Porcher,C., Swat,W., Rockwell,K., Fujiwara,Y., Alt,F.W. and Orkin,S.H. (1996) The T-cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell*, **86**, 47–57.

Pulford,K., Lecointe,N., Leroy-Viard,K., Jones,M., Mathieu-Mahul,D. and Mason,D.Y. (1995) Expression of TAL-1 proteins in human tissues. *Blood*, **85**, 675–684.

Rabbitts,T.H. (1994) Chromosomal translocations in human cancer. *Nature*, **372**, 143–148.

Robb,L., Lyons,I., Li,R., Hartley,L., Kontgen,F., Harvey,R.P., Metcalf,D. and Begley,C.G. (1995b) Absence of yolk sac hematopoiesis from mice with a targeted disruption of the *scl* gene. *Proc. Natl Acad. Sci. USA*, **92**, 7075–7079.

Robb,L., Rasko,J.E., Bath,M., Strasser,A. and Begley,C.G. (1995a) *scl*, a gene frequently activated in human T cell leukaemia, does not induce lymphomas in transgenic mice. *Oncogene*, **10**, 205–209.

Robb,L., Elwood,N.J., Elefanty,A.G., Kontgen,F., Li,R., Barnett,L.D. and Begley,C.G. (1996) The *scl* gene product is required for the generation of all hematopoietic lineages in the adult mouse. *EMBO J.*, **15**, 4123–4129.

Royer-Pokora,B., Loos,U. and Ludwig,W.-D. (1991) *TTG-2*, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)(p13;q11). *Oncogene*, **6**, 1887–1893.

Sanchez-Garcia,I. and Rabbitts,T.H. (1994) Transcriptional activation by TAL-1 and FUS-CHOP proteins expressed in acute malignancies as a result of chromosomal abnormalities. *Proc. Natl Acad. Sci. USA*, **91**, 7869–7873.

Schenker,T., Lach,C., Kessler,B., Calderara,S. and Trueb,B. (1994) A novel GTP-binding protein which is selectively repressed in SV40 transformed fibroblasts. *J. Biol. Chem.*, **268**, 25447–25453.

Shivdasani,R.A., Mayer,E.L. and Orkin,S.H. (1995) Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature*, **373**, 432–434.

Towbin,H., Staehelin,T. and Gordon,J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl Acad. Sci. USA*, **76**, 4350–4354.

van Lohuizen,M., Verbeek,S., Krimpendorf,P., Domen,J., Saris,C., Radaszkiewicz,T. and Berns,A. (1989) Predisposition to lymphomagenesis in *pim-1* transgenic mice: cooperation with c-*myc* and N-*myc* in murine leukemia virus-induced tumors. *Cell*, **56**, 673–682.

Voronova,A.F. and Lee,F. (1994) The E2A and tal-1 helix–loop–helix proteins associate *in vivo* and are modulated by Id proteins during interleukin 6-induced myeloid differentiation. *Proc. Natl Acad. Sci. USA*, **91**, 5952–5956.

Wadman,I.A., Hsu,H.-L., Cobb,M.H. and Baer,R. (1994a) The MAP kinase phosphorylation site of TAL1 occurs within a transcriptional activation domain. *Oncogene*, **9**, 3713–3716.

Wadman,I., Li,J., Bash,R.O., Forster,A., Osada,H., Rabbitts,T.H. and Baer,R. (1994b) Specific *in vivo* association between the bHLH and LIM proteins implicated in human T cell leukemia. *EMBO J.*, **13**, 4831–4839.

Zhuang,Y., Cheng,P. and Weintraub,H. (1996) B-lymphocyte development is regulated by the combined dosage of three basic helix–loop–helix genes, E2A, E2–2 and HEB. *Mol. Cell. Biol.*, **16**, 2898–2905.

*Received on December 17, 1996; revised on January 23, 1997*