*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 5947–5951, June 1994
Biochemistry

# Positive and negative transcriptional control by the TAL1 helix–loop–helix protein

**(T-cell leukemia/transcription factor/Id polypeptide)**

HAI-LING HSU, ISOBEL WADMAN, JULIA TSOU TSAN, AND RICHARD BAER

Department of Microbiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235

*Communicated by Steven L. McKnight, March 14, 1994*

ABSTRACT **Tumor-specific activation of the *TAL1* gene is the most common genetic defect associated with T-cell acute lymphoblastic leukemia. The *TAL1* gene products possess a basic helix–loop–helix (bHLH) motif, a protein-dimerization and DNA-binding domain found in several transcription factors. TAL1 polypeptides interact, *in vitro* and *in vivo*, with class A bHLH proteins (e.g., E47) to form heterodimers with sequence-specific DNA-binding activity. In this study, we show that TAL1 can regulate the transcription of an artificial reporter gene that contains binding sites for bHLH heterodimers involving TAL1. Transcription of the reporter is strongly induced by E47–E47 homodimers and moderately induced by TAL1–E47 heterodimers. Thus, in a cellular environment that allows formation of E47–E47 homodimers (e.g., in the absence of Id regulatory proteins) TAL1 can repress transcription by recruiting E47 into bHLH complexes with less transcriptional activity (i.e., TAL1–E47 heterodimers). However, in other settings TAL1 can activate transcription because TAL1–E47 heterodimers are more resistant to negative regulation by Id proteins. Hence, TAL1 can potentially regulate transcription in either a positive or negative fashion.**

Tumor-specific alteration of the *TAL1* gene occurs in nearly 25% of patients with T-cell acute lymphoblastic leukemia (T-ALL), and as such it represents the most common genetic defect associated with this disease (1–10). These alterations are mediated by chromosomal rearrangements that serve to activate transcription of the affected *TAL1* allele. During normal development, *TAL1* expression is observed in several hematopoietic lineages with the apparent exception of T cells. Thus, the gene alterations associated with T-ALL may promote leukemogenesis by inducing inappropriate *TAL1* expression in T-lineage cells.

The *TAL1* gene (also called *TCL5* or *SCL*) encodes at least two protein products: a full-length species containing amino acid residues 1–331 ($pp42^{TAL1}$) and a truncated species containing residues 176–331 ($pp22^{TAL1}$) (11). Both products contain the basic helix–loop–helix (bHLH) motif, a protein-dimerization and DNA-binding domain found in several transcription factors (12–14). An etiologic role for *TAL1* in leukemia is supported by its relationship with *TAL2* and *LYL1*, distinct bHLH genes that were also identified on the basis of chromosomal rearrangement in T-ALL patients (15, 16). Although >60 bHLH proteins have been identified to date, the bHLH domain of TAL1 is most closely related to those encoded by *TAL2* and *LYL1*. Thus, TAL1, TAL2, and LYL1 constitute a unique subgroup of bHLH proteins, each of which is a potential mediator of T-cell leukemogenesis (9).

Protein dimerization is required for DNA recognition by many transcription factors, including those that harbor the bHLH motif (12, 17). TAL1 polypeptides do not have intrinsic DNA-binding activity, presumably because they cannot self-associate to form bHLH homodimers (18–20). However, they do have the potential to interact in a stable manner with certain other bHLH proteins. For example, TAL1 polypeptides have been shown to associate with each of the known "class A" bHLH proteins, including both products (E12 and E47) of the *E2A* gene (19). The resultant heterodimers (TAL1–E12 and TAL1–E47) specifically recognize DNA sequences that contain the E-box motif (CANNTG), a cis-acting element found in a variety of eukaryotic transcription enhancers (19).

TAL1–E2A complexes with DNA-binding activity have been detected in leukemic T cells derived from T-ALL patients (19, 20). Therefore, it is conceivable that the leukemic, and perhaps the normal, properties of TAL1 are mediated through its putative function as a transcription factor. However, transcriptional regulation by TAL1 has not as yet been demonstrated. In this study, we show that TAL1 polypeptides can potentially modulate transcription of a single target gene in either a positive or negative fashion.

## MATERIALS AND METHODS

**Plasmid Clones.** Complementary oligonucleotides that contain two copies of the preferred sequence for DNA recognition by TAL1–E2A heterodimers (TCGAACAGATGTTCA-CACGACCATCTGGTGGG and TCGACCCACCAGATG-GTCGTGTGAACATCTGT) were annealed and ligated into the *Sal* I site of the E1bTATA-CAT reporter plasmid (21); E1bTATA-CAT derivatives with one (E1bCAT-E2), two (E1bCAT-E4), or three (E1bCAT-E6) copies of the oligonucleotide insert were used in subsequent studies. The E47 expression vector (pCDM8-E47) (22) was kindly provided by Anna Voronova (DNAX). An expression plasmid encoding Id1 (Id1/pCMV4) was generated by transferring a 0.9-kbp *Sma* I fragment from pE:Id(S) into the pCMV4 vector (23, 24). The TAL1 expression plasmid (TAL1/pCMV4) has been described (11). An expression plasmid (TAL1-B2mut/pCMV4) encoding the mutant TAL1 (R188G;R189G) polypeptide was generated by site-directed mutagenesis of TAL1/pCMV4 (25).

**DNA Transfections and Chloramphenicol Acetyltransferase (CAT) Assays.** Approximately $3 \times 10^5$ C3H/10T½ fibroblasts were seeded onto each 100-mm plate and cultured in 10 ml of growth medium. After 2 days, the adherent cells were treated with the calcium phosphate transfection system (GIBCO/BRL). Unless otherwise indicated (i.e., see Fig. 2), each 100-mm culture was transfected with 5 μg of the E1bCAT-E6 reporter plasmid, 5 μg of the pRSV-βgal control plasmid, and expression plasmids encoding E47 (1 μg), TAL1 (10 μg), or Id1 (10 μg); where necessary pCMV4 plasmid DNA was added to provide a constant DNA mass (31 μg) for transfec-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: bHLH, basic helix–loop–helix; T-ALL, T-cell acute lymphoblastic leukemia; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay.

tion of each 100-mm culture. After 24 h, the adherent cells were washed twice in TBS buffer (11) and cultured in fresh growth medium for an additional 24 h. Nuclear extracts were prepared from each culture as described by Lee *et al.* (26); the postnuclear supernatant ("cytoplasmic extract") obtained by this procedure was normalized for $\beta$-galactosidase activity, and equivalent aliquots were examined for CAT activity by using [$^{14}$C]chloramphenicol and *n*-butyryl-coenzyme A. Each reaction mixture was then fractionated by thin-layer chromatography, and the conversion of [$^{14}$C]chloramphenicol into its butyrylated forms was measured using a Betascope 603 (Betagen, Waltham, MA).

**Western Blot Analysis and Electrophoretic Mobility Shift Assays (EMSAs).** The protein content of each nuclear lysate was determined by the Bradford assay (Bio-Rad), and equivalent amounts (25 $\mu$g) were subject to Western blot analysis, which was performed by enhanced chemiluminescence (Amersham) with a rabbit serum (no. 1080) specific for the N-terminal 121 residues of TAL1 (19). EMSAs were performed with a $^{32}$P-labeled double-stranded oligonucleotide probe containing the preferred sequence for DNA-binding by TAL1–E47 heterodimers (underlined; sense strand, ACCTGAACAGATGGTCGGCT) (19). Each EMSA shown in Figs. 1*B* and 4*C* was conducted with 25 $\mu$g of nuclear extract. EMSAs of *in vitro*-translated polypeptides (see Fig. 3) were conducted with reticulocyte lysates containing E47S (2 $\mu$l), TAL1 (5 $\mu$l), and Id1 (2–8 $\mu$l); where necessary, unprogrammed reticulocyte lysate was added to provide a constant volume (15 $\mu$l) of lysate in each EMSA. The plasmid templates for *in vitro* transcription/translation of E47 (pE47S) and TAL1 (talT7pGEM) polypeptides have been described (12, 18); the template for Id1 (Id0.9-T7/pGEM4) was generated by transferring a 0.9-kbp *Sma* I fragment from pE:Id(S) into the pGEM-4 vector (23).

## RESULTS

**Transcriptional Activity of E47–E47 Homodimers and TAL1–E47 Heterodimers.** Natural target genes for transcriptional regulation by TAL1 have not as yet been identified. Therefore, an artificial reporter was constructed from ElbTATA-CAT, a plasmid vector that contains the bacterial CAT gene under the control of a promoter from the adenovirus *Elb* gene (21). Previous experiments had shown that TAL1–E2A heterodimers preferentially bind a consensus DNA sequence (AACAGATGGT) that includes the E-box motif (CANNTG) (underlined) commonly recognized by bHLH proteins (19). Therefore, oligonucleotides containing this sequence were inserted immediately upstream of the *Elb* promoter in ElbTATA-CAT to generate reporter constructs with multiple copies of the preferred TAL1–E2A-binding site. CAT reporters containing zero, two, four, or six copies of the binding site were then cotransfected into C3H10T½ fibroblasts along with expression plasmids encoding either full-length TAL1 or full-length E47 polypeptides. The cells were harvested 48 h after transfection, and cell lysates were evaluated for CAT activity. As expected, TAL1 polypeptides, which do not have intrinsic DNA-binding activity (18, 19), did not induce expression of CAT from any of the reporter constructs. In contrast, E47 readily induced CAT expression from the ElbTATA-CAT derivatives containing two, four, or six copies of the TAL1–E2A-binding site but not from the parental ElbTATA-CAT reporter (data not shown).

Further studies were conducted with ElbCAT-E6, the derivative of ElbTATA-CAT that harbors six copies of the TAL1–E2A-binding site. C3H10T½ fibroblasts were cotransfected with the ElbCAT-E6 reporter and various combinations of expression plasmids encoding E47 and TAL1. After 48 h, the cells of each transfected culture were harvested for the preparation of cytoplasmic and nuclear extracts. Fig. 1*A*

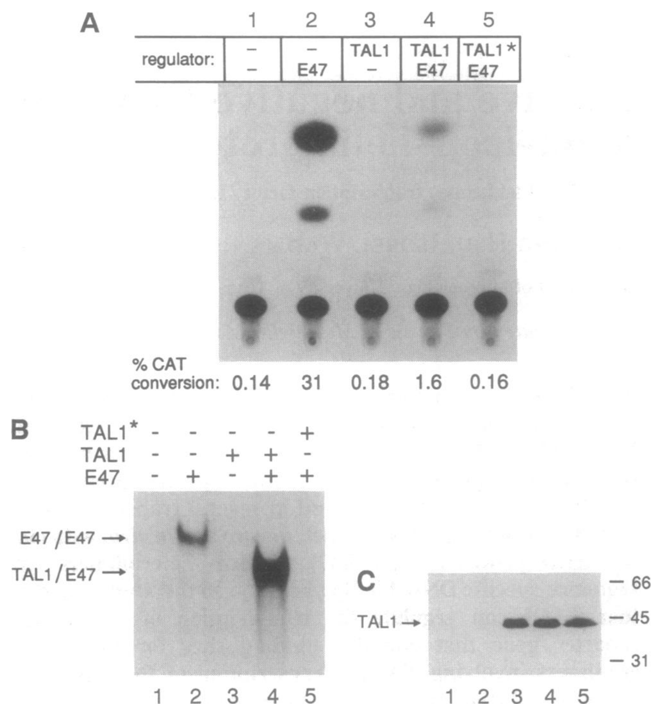


FIG. 1. Transcriptional activation by E47–E47 homodimers and TAL1–E47 heterodimers. (*A*) Trans-activation of ElbCAT-E6, a CAT reporter gene with multiple binding sites for TAL1–E47. C3H10T½ fibroblasts were transfected with the ElbCAT-E6 reporter, a $\beta$-galactosidase expression plasmid (RSV-$\beta$gal), and plasmids encoding E47, wild-type TAL1, or mutant TAL1 (denoted as TAL1*). Cytoplasmic and nuclear extracts were prepared from each cell culture 48 h after transfection. The cytoplasmic extracts were evaluated for $\beta$-galactosidase activity, and CAT enzyme assays were then performed using equivalent amounts ($0.5 \times 10^{-4}$ $\beta$-galactosidase unit) of each extract. (*B*) EMSAs of the corresponding nuclear extracts. Each extract was subjected to EMSA after incubation with a $^{32}$P-labeled double-stranded oligonucleotide containing the preferred recognition sequence for TAL1–E47. Protein–DNA complexes representing E47–E47 homodimers and TAL1–E47 heterodimers are marked with arrows. (*C*) Western blot analysis of the corresponding nuclear extracts (lanes 1–5 correspond to lanes 1–5 in *B*). Equivalent aliquots of each extract were fractionated by SDS/PAGE, transferred to a nylon filter, and examined by immunoblot analysis with a TAL1-specific rabbit antiserum.

illustrates that transcription of the ElbCAT-E6 reporter, as measured by CAT activity in the cytoplasmic extracts, is induced by E47 (lane 2) but not by TAL1 (lane 3). Transcription of ElbCAT-E6 was also apparent upon coexpression of E47 and TAL1 (lane 4); in this case, the CAT levels obtained were nearly 20-fold lower than those induced by E47 alone. Significantly, however, coexpression of E47 and TAL1 did not induce detectable CAT production from ElbTATA-CAT, the parental reporter that is devoid of TAL1–E2A-binding sites (data not shown).

To identify the bHLH complexes responsible for transcription of ElbCAT-E6, the corresponding nuclear extracts were evaluated in an EMSA with a radiolabeled oligonucleotide probe containing the preferred TAL1–E47-binding sequence. As illustrated in Fig. 1*B*, protein complexes that bound the radiolabeled probe were observed in nuclear extracts from cells containing E47 alone (lane 2) and both E47 and TAL1 (lane 4) but not TAL1 alone (lane 3). The electrophoretic mobilities of these complexes are consistent with the formation of E47–E47 homodimers (lane 2) and TAL1–E47 heterodimers (lane 4). Moreover, the presumptive TAL1–E47 complex is disrupted by rabbit antisera specific for either TAL1 or E47, whereas the E47–E47 complex is only disrupted by E47-specific antisera (data not shown).

It is noteworthy that E47–E47 complexes are not observed in cells that express both E47 and TAL1 (e.g., Fig. 1*B*, lane 4). This is consistent with *in vitro* studies that show preferential formation of TAL1–E47 heterodimers over E47–E47 homodimers (18, 19). Hence, the transcription of E1bCAT-E6 observed in cells expressing both TAL1 and E47 (Fig. 1*A*, lane 4) is likely to be mediated by the abundant TAL1–E47 complexes present in these cells (Fig. 1*B*, lane 4), rather than by residual E47–E47 homodimers. Additional evidence to support this conclusion was obtained by evaluating E1bCAT-E6 transcription in response to TAL1(R188G;R189G), a mutant TAL1 polypeptide with two amino acid substitutions in the basic region of the bHLH domain. Myogenic bHLH factors (e.g., MyoD1 and myogenin) bearing similar mutations have been shown to interact with E2A proteins to form aberrant bHLH heterodimers that no longer bind DNA (27, 28). Likewise, cells expressing both E47 and mutant TAL1 lack bHLH heterodimers with DNA-binding activity (Fig. 1*B*, lane 5). Homodimeric E47–E47 complexes are also absent from these cells, indicating that the E47 polypeptides are assembled into aberrant bHLH heterodimers with mutant TAL1. As shown in Fig. 1*C*, equivalent levels of wild-type (lanes 3 and 4) and mutant (lane 5) TAL1 polypeptides are generated upon transfection of C3H10T½ cells with their respective expression plasmids. Nevertheless, transcription of the E1bCAT-E6 reporter is not detected in cells expressing both E47 and mutant TAL1 (Fig. 1*A*, lane 5). This result confirms that the E1bCAT-E6 transcription observed in cells expressing both E47 and wild-type TAL1 (Fig. 1*A*, lane 4) is mediated by TAL1–E47 heterodimers.

The experiment presented in Fig. 1 demonstrates that transcription of the E1bCAT-E6 reporter can be induced by either E47–E47 homodimers or TAL1–E47 heterodimers. However, the latter are clearly less potent activators of E1bCAT-E6 transcription. Since heterodimer formation is favored over homodimer formation, cells containing both E47 and TAL1 produce CAT levels significantly lower than cells containing E47 alone; a mean 15.2-fold reduction was observed in four experiments (SEM = 3.1). Moreover, as shown in Fig. 2, TAL1 polypeptides with proline substitutions in helix 2 of the bHLH region (A232P or K234P) do not reduce transactivation by E47 (lanes 2–5). These mutations eliminate heterodimer formation between TAL1 and E47 (data not shown), presumably by destabilizing the TAL1 bHLH domain. Thus, the ability of TAL1 to influence transcriptional activation by E47–E47 homodimers appears to be dependent upon its dimerization potential.

The data indicate that TAL1 can repress transcription of a target gene by reducing the formation of E47–E47 homodimers. It should be noted, however, that the degree of TAL1-mediated repression is influenced by the relative levels of E47 and TAL1. The experiments presented above were conducted under conditions in which the cellular quantities of E47 were limited with respect to TAL1; that is, cotransfections were performed with 1 μg of the E47 expression plasmid and 10 μg of the TAL1 expression plasmid. Since the recruitment of E47 polypeptides into heteromeric TAL1–E47 complexes occurs stoichiometrically, the degree of TAL1-mediated repression is diminished at higher ratios of E47 to TAL1 (Fig. 2, lanes 6–8). Nevertheless, in a cellular environment that allows stable assembly of E2A homodimers, expression of TAL1 can potentially modulate transcription in a negative fashion.

**The Effect of Id on Formation of E47–E47 Homodimers and TAL1–E47 Heterodimers.** The transcriptional activities of bHLH proteins are often subject to regulation by the Id polypeptides, a small family of HLH proteins that lack the basic region of the bHLH domain (23, 29–34). Changes in the cellular levels of Id polypeptides are associated with various physiologic events, including cell differentiation and growth factor induction. When Id levels are high, the transcriptional activity of some bHLH polypeptides is repressed because they preferentially associate with Id to form heterodimers that lack DNA-binding activity (23). Previous studies have shown that the Id1 and Id2 polypeptides interact avidly with the *E2A* gene products but not with TAL1 (23, 31). In light of the evidence that TAL1–E47 heterodimers are more stable than E47–E47 homodimers, we sought to determine whether the formation of these complexes is influenced differentially by the presence of Id polypeptides. Therefore, bHLH complexes assembled from *in vitro*-translated polypeptides were evaluated by EMSA with a radiolabeled probe containing the preferred TAL1–E2A-binding sequence. As illustrated in Fig. 3, E47–E47 homodimers with DNA-binding activity are present in reticulocyte lysates programmed to produce a truncated E47 polypeptide (lane 2) but not in unprogrammed lysates (lane 1) (12). However, formation of the E47–E47 complex is completely disrupted by the addition of *in vitro*-translated Id1 (lane 3). In contrast, the formation of TAL1–

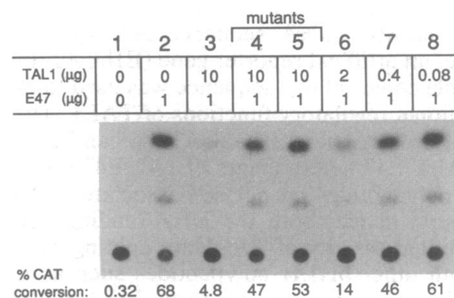


FIG. 2. Effect of TAL1 on the transcriptional activity of E47–E47 homodimers. C3H10T½ cells were transfected with the E1bCAT-E6 reporter (5 μg), the RSV-βgal control plasmid (5 μg), and expression plasmids encoding E47 plus either wild-type (lanes 3 and 6–8) or mutant (lane 4, K234P mutant; lane 5, A232P mutant) TAL1. The transfections were conducted using 10 μg (lanes 3–5), 2 μg (lane 6), 0.4 μg (lane 7), or 0.08 μg (lane 8) of TAL1 expression plasmid. Cytoplasmic and nuclear extracts were prepared from each cell culture 48 h after transfection. Cytoplasmic extracts were evaluated for β-galactosidase activity, and CAT assays were performed using equivalent amounts ($0.5 \times 10^{-4}$ β-galactosidase unit) of each extract. The corresponding nuclear extracts were evaluated by immunoblot analysis as described in Fig. 1 (data not shown).

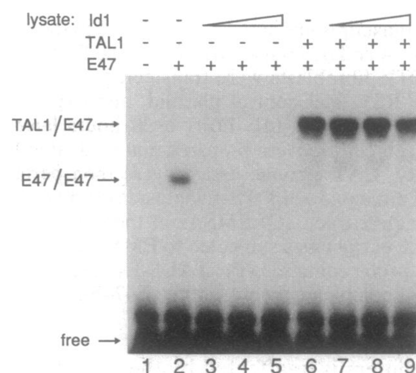


FIG. 3. Effect of Id1 on DNA binding by E47–E47 homodimers and TAL1–E2A heterodimers. A $^{32}$P-labeled probe containing the preferred recognition sequence for TAL1–E47 was incubated with an unprogrammed reticulocyte lysate (lane 1), lysates containing *in vitro*-translated E47S (lanes 2–5), or lysates containing *in vitro*-translated E47S and TAL1 (lanes 6–9). Some binding reaction mixtures were supplemented with 2 μl (lanes 3 and 7), 4 μl (lanes 4 and 8), or 8 μl (lanes 5 and 9) of a lysate containing *in vitro*-translated Id1. Reaction products were then analyzed by EMSA. Protein–DNA complexes representing E47–E47 homodimers and TAL1–E47 heterodimers are indicated.

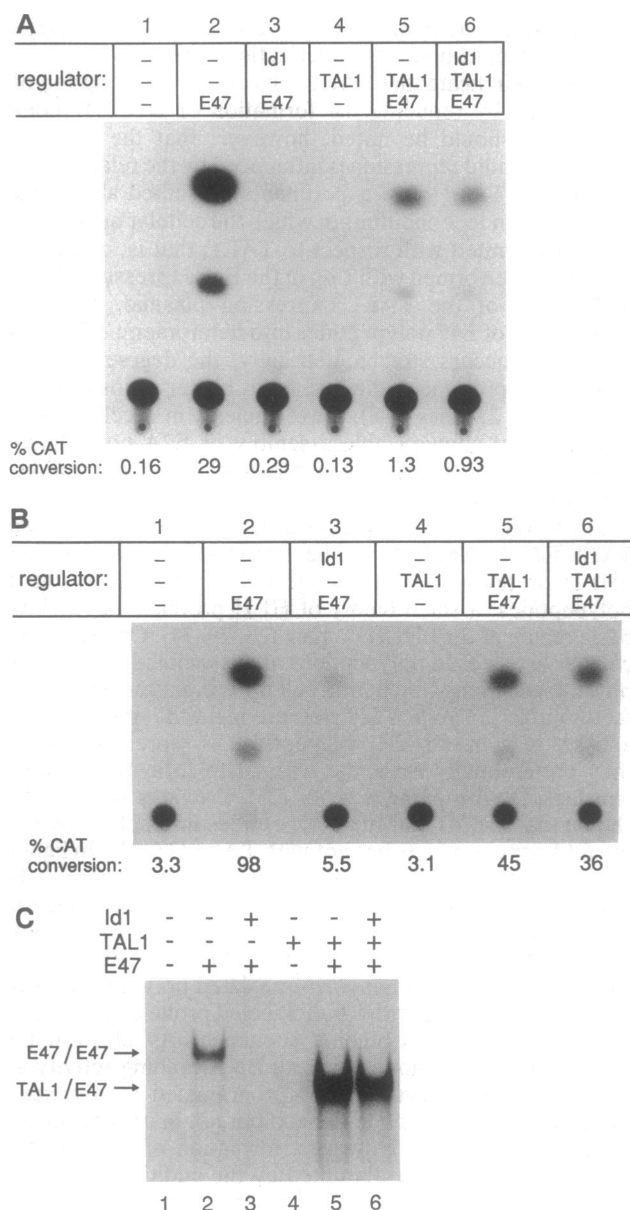


FIG. 4. Transcriptional activation by TAL1 in the presence of Id1 polypeptides. (*A* and *B*) Trans-activation of the E1bCAT-E6 reporter gene. C3H10T½ fibroblasts were transfected with the E1bCAT-E6 reporter, the RSV-βgal control plasmid, and expression plasmids encoding E47, TAL1, or Id1. Forty-eight hours later cytoplasmic extracts of each culture were prepared and evaluated for β-galactosidase activity; CAT enzyme assays were then performed on the equivalent of either $0.5 \times 10^{-4}$ β-galactosidase unit (*A*) or $2.5 \times 10^{-4}$ unit (*B*) of each extract. (*C*) EMSAs of the corresponding nuclear extracts. Each extract was subjected to EMSA after incubation with a $^{32}$P-labeled probe containing the TAL1–E47 recognition sequence. Protein–DNA complexes representing E47–E47 homodimers and TAL1–E47 heterodimers are indicated.

E47 heterodimers is unaffected by the presence of an equivalent level of Id1 (lane 7), and it is only modestly diminished by 4-fold higher levels (lane 9). This result suggests that the transcriptional activity of E47–E47 homodimers may be more susceptible to down-regulation by Id1 than that of TAL1–E47 heterodimers.

**The Effect of Id on the Transcriptional Activities of E47–E47 Homodimers and TAL1–E47 Heterodimers.** To ascertain whether Id polypeptides have a differential effect on the transcriptional activities of E47–E47 homodimers and TAL1–E47 heterodimers, C3H10T½ cells were cotransfected with the E1bCAT-E6 reporter and various combinations of expression plasmids encoding E47, TAL1, and Id1. As shown in Fig. 4*C*, Id1 expression results in the complete elimination of E47–E47 complexes from the nuclear extracts of transfected C3H10T½ cells (compare lanes 2 and 3). This loss is accompanied by a dramatic 100-fold decrease in the transcription of E1bCAT-E6, as represented by CAT levels in the corresponding cytoplasmic extracts (Fig. 4*A*, lanes 2 and 3). In contrast, Id1 expression only produces a modest diminution in both the formation of TAL1–E47 complexes (Fig. 4*C*, lanes 5 and 6) and the activation of E1bCAT-E6 transcription by these complexes (Fig. 4*A*, lanes 5 and 6). Therefore, the transcriptional activity of TAL1–E47 heterodimers is relatively resistant to repression by Id1. To better assess the effect of Id1 on E1bCAT-E6 transcription, CAT assays were also performed using larger quantities of each cytoplasmic extract (Fig. 4*B*). Although these assays are beyond the linear range of CAT conversion for extracts containing E47–E47 homodimers (Fig. 4*B*, lane 2), they clearly demonstrate that Id1 expression has only a marginal effect on the transcriptional activity of TAL1–E47 heterodimers (lanes 5 and 6). Moreover, given the presence of Id1 polypeptides, expression of TAL1 results in a net increase in E1bCAT-E6 transcription (Fig. 4*B*, compare lanes 3 and 6); three separate experiments yielded a mean increase of 4.53-fold (SEM = 1.01). Thus, in the presence of Id polypeptides TAL1 can potentially modulate transcription in a positive fashion.

## DISCUSSION

Tumor-specific rearrangement of the *TAL1* gene is the most common genetic lesion observed in T-ALL patients (1–10). These rearrangements result in what appears to be inappropriate expression of *TAL1* in T-lineage cells. It is not understood why the unscheduled appearance of TAL1 should promote T-cell leukemogenesis. Nevertheless, TAL1 polypeptides are believed to function as transcriptional regulatory factors based on two criteria: the presence of a bHLH domain and their ability to form protein complexes (e.g., TAL1–E2A heterodimers) that bind DNA in a sequence-specific manner (18–20). However, evidence of transcriptional activity by TAL1 polypeptides has not been reported, perhaps in part because natural target genes subject to TAL1 regulation have yet to be identified.

Binding-site selection studies show that bHLH heterodimers involving TAL1 (e.g., TAL1–E47) preferentially recognize a subset of E-box elements that can be represented by the consensus DNA sequence AACAGATGGT (19). Therefore, an artificial reporter gene (E1bCAT-E6) containing multiple copies of this sequence was used to evaluate the transcriptional regulatory functions of TAL1. These studies show that transcription of E1bCAT-E6 can be activated by either TAL1–E47 heterodimers of E47–E47 homodimers.

TAL1 polypeptides do not self-associate to form bHLH homodimers; hence, both the DNA-binding potential and transcriptional activity of TAL1 are contingent upon interaction with other bHLH polypeptides such as E47. Three additional factors influence whether TAL1 modulates transcription in a positive or negative manner. (*i*) E47–E47 homodimers are far more potent activators of E1bCAT-E6 transcription than are TAL1–E47 heterodimers. (*ii*) In the presence of TAL1, E47 polypeptides preferentially form heteromeric TAL1–E47 complexes rather than E47–E47 homodimers. (*iii*) E47–E47 homodimers are more readily dissociated by the presence of Id polypeptides than are TAL1–E47 heterodimers. As a consequence of these considerations, the effect of TAL1 on transcription of a given target gene (e.g., E1bCAT-E6) will depend on the cellular environment. In a setting that permits formation of E47–E47 homodimers (e.g., in the absence of Id polypeptides), expression of TAL1

can repress transcription by recruiting E47 polypeptides into bHLH complexes with less transcriptional activity (i.e., TAL1-E47 heterodimers). Since the recruitment of E47 into TAL1-E47 complexes occurs stoichiometrically, the degree of TAL1-mediated repression will be influenced by the relative quantities of E47 and TAL1 polypeptides. Conversely, in the presence of Id proteins TAL1 expression can activate transcription because the resultant TAL1-E47 heterodimers, albeit less potent activators than E47-E47 homodimers, are relatively resistant to dissociation by moderate levels of Id. Thus, TAL1 may potentially exert either a positive or negative effect on transcription of a given target gene depending on the presence or absence of Id.

At least three distinct genes encoding Id polypeptides have been identified in mammals (23, 31-33). Although the expression patterns of these genes have not been fully defined, changes in the levels of Id polypeptides have been observed during cell differentiation and in response to growth factor induction (23, 31, 32, 34). Indeed, fluctuations of Id levels occur in developmental settings that may be relevant to TAL1 function. In particular, the levels of Id1 and Id2 transcripts rapidly decrease as erythroid cells, the sites of normal TAL1 expression, are induced to differentiate (23, 31). In light of the influence that Id polypeptides exercise over TAL1-mediated transcriptional activity, it will be important to also establish the status of Id expression during normal T-cell development and in the leukemic cells of T-ALL patients.

We thank Yi Xu for technical assistance, Susan Campbell for preparing the manuscript, and Richard Gaynor for his critical comments. We are also especially grateful to Anna Voronova for providing the E47 expression plasmid (pCDM8-E47), Harold Weintraub for the pE:Id(S) plasmid, and Kathy Martin for the CAT reporter plasmid (E1bTATA-CAT). This work was supported by a grant from the National Cancer Institute (CA46593). R.B. is a member of the Simmons Arthritis Research Center and the recipient of a Faculty Research Award from the American Cancer Society (FRA-421). I.W. was supported by a fellowship from the Lady Tata Memorial Trust.


1. Brown, L., Cheng, J.-T., Chen, Q., Siciliano, M. J., Crist, W., Buchanan, G. & Baer, R. (1990) *EMBO J.* **9**, 3343-3351.
2. Bernard, O., Lecointe, N., Jonveaux, P., Souyri, M., Mauchauffe, M., Berger, R., Larsen, C. J. & Mathieu-Mahul, D. (1991) *Oncogene* **6**, 1477-1488.
3. Aplan, P. D., Lombardi, D. P., Ginsberg, A. M., Cossman, J., Bertness, V. L. & Kirsch, I. R. (1990) *Science* **250**, 1426-1429.
4. Chen, Q., Cheng, J.-T., Tsai, L.-H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J. & Baer, R. (1990) *EMBO J.* **9**, 415-424.
5. Bernard, O., Guglielmi, P., Jonveaux, P., Cherif, D., Gisselbrecht, S., Mauchauffe, M., Berger, R., Larsen, C.-J. & Mathieu-Mahul, D. (1990) *Genes Chromosome Cancer* **1**, 194-208.
6. Begley, C. G., Aplan, P. D., Denning, S. M., Haynes, B. F., Waldmann, T. A. & Kirsch, I. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 10128-10132.
7. Finger, L. R., Kagan, J., Christopher, G., Kurtzberg, J., Hershfield, M. S., Nowell, P. C. & Croce, C. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5039-5043.
8. Bash, R. O., Crist, W. M., Shuster, J. J., Link, M. P., Amylon, M., Pullen, J., Carroll, A. J., Buchanan, G. R., Smith, R. G. & Baer, R. (1993) *Blood* **81**, 2110-2117.
9. Baer, R. (1993) *Semin. Cancer Biol.* **4**, 341-347.
10. Mouthon, M.-A., Bernard, O., Mitjavila, M.-T., Romeo, P.-H., Vainchenker, W. & Mathieu-Mahul, D. (1993) *Blood* **81**, 647-655.
11. Cheng, J.-T., Hsu, H.-L., Hwang, L.-Y. & Baer, R. (1993) *Oncogene* **8**, 677-683.
12. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56**, 777-783.
13. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58**, 537-544.
14. Kadesch, T. (1993) *Cell Growth Differ.* **4**, 49-55.
15. Mellentin, J. D., Smith, S. D. & Cleary, M. L. (1989) *Cell* **58**, 77-83.
16. Xia, Y., Brown, L., Yang, C. Y.-C., Tsan, J. T., Siciliano, M. J., Espinosa, R., III, Le Beau, M. M. & Baer, R. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11416-11420.
17. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240**, 1759-1764.
18. Hsu, H.-L., Cheng, J.-T., Chen, Q. & Baer, R. (1991) *Mol. Cell. Biol.* **11**, 3037-3042.
19. Hsu, H.-L., Huang, L., Tsan, J. T., Funk, W., Wright, W. E., Hu, J.-S., Kingston, R. E. & Baer, R. (1994) *Mol. Cell. Biol.* **14**, 1256-1265.
20. Hsu, H.-L., Wadman, I. & Baer, R. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 3181-3185.
21. Lillie, J. W. & Green, M. R. (1989) *Nature (London)* **338**, 39-44.
22. Murre, C., Voronova, A. & Baltimore, D. (1991) *Mol. Cell. Biol.* **11**, 1156-1160.
23. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61**, 49-59.
24. Andersson, S., Davis, D. N., Dahlback, H., Jornvall, H. & Russell, D. W. (1989) *J. Biol. Chem.* **264**, 8222-8229.
25. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488-492.
26. Lee, K. A. W., Bindereif, A. & Green, M. R. (1988) *Genet. Anal. Tech. Appl.* **5**, 22-31.
27. Davis, R. L., Cheng, P.-F., Lassar, A. B. & Weintraub, H. (1990) *Cell* **60**, 733-746.
28. Brennan, T. J., Chakraborty, T. & Olson, E. N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5675-5679.
29. Ellis, H. M., Spann, D. R. & Posakony, J. W. (1990) *Cell* **61**, 27-38.
30. Garrell, J. & Modolell, J. (1990) *Cell* **61**, 39-48.
31. Sun, X.-H., Copeland, N. G., Jenkins, N. A. & Baltimore, D. (1991) *Mol. Cell. Biol.* **11**, 5603-5611.
32. Christy, B. A., Sanders, L. K., Lau, L. F., Copeland, N. G., Jenkins, N. A. & Nathans, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1815-1819.
33. Biggs, J., Murphy, E. V. & Israel, M. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1512-1516.
34. Kreider, B. L., Benezra, R., Rovera, G. & Kadesch, T. (1992) *Science* **255**, 1700-1702.