The Tal1 Oncoprotein Inhibits E47-mediated Transcription

MECHANISM OF INHIBITION

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Steven T. Park and Xiao-Hong Sun‡
From the Department of Cell Biology, New York University Medical Center, New York, New York 10016

The Tal1 oncogene is a class II basic helix-loop-helix (bHLH) transcription factor, overexpressed in as much as 60% of T cell acute lymphoblastic leukemia cases. Like other class II bHLH proteins, Tal1 can heterodimerize with the class I bHLH proteins, such as E47, and bind to a DNA recognition sequence termed E box. Therefore, it is believed that the oncogenic capacity of Tal1 lies in its ability, as a heterodimer with E47, to activate aberrantly a set of “leukemogenic” genes in T cells. However, compared with E47 homodimers, Tal1/E47 heterodimers are very poor transactivators. Thus the effect of Tal1 is actually to inhibit E47 homodimer activity. Here we propose that the transforming properties of Tal1 are the result of its ability to inhibit E47 activity. We address the mechanism of Tal1 inhibition and demonstrate that Tal1/E47 heterodimers cannot activate transcription because their respective activation domains are incompatible. Furthermore, we present data showing that Tal1 can inhibit E47-mediated activation of the CIP1 gene. Finally, we demonstrate that Tal1 inhibits E47 activity in leukemic T cells.

Members of the basic helix-loop-helix (bHLH) family of transcription factors have been shown to play important roles in cell type determination and differentiation (1, 2). They have been grouped into two classes depending on their expression pattern as either ubiquitous or tissue-specific (3). Class I ubiquitous bHLH proteins can form either homodimers or heterodimers with other class I proteins and with the class II tissue-specific bHLH proteins. Both homodimers and heterodimers have been shown to bind to a canonical DNA sequence, termed E box, located in the regulatory elements of various cell type-specific genes, and concomitantly activate gene transcription. In addition to their roles in cell development, four members of this family, E2A, Lyl1, Tal1, and Tal2, have also been implicated in oncogenesis (4–8).

Tal1, encoding a class II bHLH protein, was originally identified by virtue of its translocation and overexpression in 3% of T cell acute lymphoblastic leukemia cases (4, 5). Subsequent analyses have shown that Tal1 is overexpressed in as much as 60% of T cell acute lymphoblastic leukemia cases through either a specific, interstitial chromosomal deletion event or through other undetectable alterations in DNA (9, 10). However, Tal1 is not normally expressed in T cells; rather it is expressed in the developing hematopoietic system, endothelial cells, and the developing brain (11, 12). Gene knockout experiments in mice have shown that Tal1 plays a crucial role in primitive erythropoiesis (13). Subsequent rescue experiments with chimeras have demonstrated that Tal1 is required for the development of all hematopoietic lineages (14). In conjunction, cell culture experiments have suggested that Tal1 may also play a role in terminal erythroid differentiation, possibly in cooperation with the zinc finger proteins LMO2 and GATA-1 (15, 16).

Recent experiments in transgenic mice have demonstrated the oncogenic potential of Tal1, for when Tal1 expression is directed to the T cell compartment through the promoter of the immunoglobulin and terminal deoxynucleotide transferase genes (22, 23). Obviously in the context of T cell leukemia, these mice develop T cell lymphoma (17, 18). However, the exact mechanism of Tal1-mediated leukemogenesis remains unknown. Because Tal1 is capable of heterodimerizing with the ubiquitous bHLH E proteins (E12, E47, E2-2, HEB) and binding to DNA, it is thought that Tal1 aberrantly activates genes in T cells, whose expression then leads ultimately to leukemogenesis (19, 20). However, Tal1/E47 heterodimers are less than 10% as active as E47 homodimers in their ability to activate a reporter gene driven by E box elements (21). Thus, the effect of Tal1 is actually to inhibit the transactivation potential of E47 homodimers. Therefore, an alternative mechanism may be that Tal1, instead of acting as a transcriptional activator, promotes oncogenesis by acting as an inhibitor of E47 activity.

The purpose of this study was to address mechanistically the following question: If Tal1/E47 heterodimers can bind to DNA with the same or higher affinities than those of E47 homodimers (19), why do they activate transcription so poorly? To address this question, fusion proteins of Tal1 and E47 were constructed. Specifically, we asked which regions of E47 can turn Tal1 into an activator, and which regions of Tal1 can turn E47 into an inhibitor. Furthermore, in addition to artificial reporters, we also tested the effect of Tal1 on the native promoter of an E47-activated gene, which could potentially be a target for Tal1 inhibition. Thus far, E47 has been shown to regulate the expression of B cell-specific genes, such as the immunoglobulin and terminal deoxynucleotide transferase genes (22, 23). Obviously in the context of T cell leukemia, these are not relevant targets for Tal1. Recent experiments have shown that E47 plays a general role in negatively regulating the proliferation of cells (24) and that this effect may be mediated by the ability of E47 to activate expression of the gene for CIP1 (p21, WAF1, Sdi1), an inhibitor of cyclin-dependent kinases (25–28). To determine if CIP1 may be a potential target for negative regulation by Tal1, we therefore asked if Tal1 can inhibit the ability of E47 to transactivate the CIP1 gene.
**Inhibition of E47 by Tal1**

In the present study, we have investigated the interaction between the basic helix-loop-helix (bHLH) transcription factors E47 and Tal1. E47 is a key regulator of cell fate during hematopoiesis, while Tal1 is involved in cell adhesion and morphogenesis. Previous studies have shown that Tal1 can inhibit E47 activity, but the molecular mechanisms behind this inhibition are not fully understood.

To address this question, we constructed a series of plasmids that encode chimeric proteins containing different domains of E47 and Tal1. These plasmids were then transfected into cell lines such as 293T and HeLa, which are commonly used in studies of cellular signaling and gene expression.

**Materials and Methods**

**Plasmids**—The plasmids used in this study include pCMV-LacZ, pE-T/3, and pE-T/1, which contain the basic region of Tal1 and E47, respectively. Other plasmids such as pE-T/4, pE-T/5, and pE-T/6 were used to generate chimeric proteins.

**Construction of pE-T/1 Fusion Plasmids**—To generate the E47-Tal1 fusion plasmids, a PCR product was amplified using primers specific for E47 and Tal1. The PCR product was subsequently cloned into the appropriate vector. The resulting plasmids were then transfected into cells, and luciferase activity was measured using the luciferin substrate (Promega).

**Northern Analyses**—Total cellular RNA was isolated using TRIzol reagent (Life Technologies), and 20 µg of each sample was loaded onto a 1% agarose gel containing 1 × MOPS and 0.68 M formaldehyde. After electrophoresis at 5 V/cm, the gel was rinsed for 45 min in 20 × SSC at room temperature and transferred to a nylon membrane (Zetabind) overnight in 20 × SSC. The blot was hybridized using QUIKHYB (Stratagene) with a CIP1 cDNA probe. The DNA used for the probe was prepared using a kit from Boehringer Mannheim.

**Transfections, Luciferase Assays, and β-Galactosidase Assays**—293T and HeLa cells were transfected by the CaPO4 precipitation method (34). Jurkat cells were transfected by electroporation essentially as described by Hsu et al. (35). Transfected cells were lysed and assayed for β-galactosidase activity using the Galacton kit according to the manufacturer's protocol (Tropix). For both assays, luminescence was determined on a luminometer (Berthold).

**Western Analyses**—Total cellular protein was isolated using 1% SDS-PAGE gel loading buffer. Samples were boiled and separated on 12% SDS-PAGE gels. Monoclonal antibody 9E10 was a gift from P. Cowin (New York University Medical Center). The anti-Tal1 immune serum was described (30). Anti-CIP1 and anti-E47 immune sera were from Transduction Laboratories and Santa Cruz Biotechnology, respectively. Monoclonal antibody 9E10 was a gift from P. Cowin (New York University Medical Center). Transfected 293T or HeLa cells were transfected by electroporation essentially as described by Hsu et al. (35). Transfected cells were lysed and assayed for β-galactosidase activity using the Galacton kit according to the manufacturer's protocol (Tropix). The same cell lysates were used to measure luciferase activity using the luciferin substrate (Promega).

**Antibodies and Western Analyses**—The anti-Tal1 immune serum was as described (30). Anti-CIP1 and anti-E47 immune sera were from Transduction Laboratories and Santa Cruz Biotechnology, respectively. Monoclonal antibody 9E10 was a gift from P. Cowin (New York University Medical Center). Transfected 293T or HeLa cells were transfected by electroporation essentially as described by Hsu et al. (35). Transfected cells were lysed and assayed for β-galactosidase activity using the Galacton kit according to the manufacturer's protocol (Tropix). The same cell lysates were used to measure luciferase activity using the luciferin substrate (Promega).

**Results**—The inhibition of E47 activity by Tal1 was observed in both luciferase and β-galactosidase assays. In the luciferase assay, the luciferase activity was reduced in cells expressing both E47 and Tal1 compared to cells expressing only E47. Similarly, in the β-galactosidase assay, the β-galactosidase activity was also reduced in cells expressing both E47 and Tal1.

**Discussion**—The results of this study suggest that Tal1 inhibits E47 activity by forming a heterodimer with E47, which results in the inhibition of E47-mediated transcription. This inhibition could have important implications for the regulation of transcriptional pathways in hematopoiesis and other cellular processes.

**Conclusion**—In conclusion, our study provides new insights into the mechanisms of Tal1-mediated inhibition of E47 activity. Further studies are needed to understand the molecular basis of this inhibition and to elucidate the role of Tal1 in hematopoiesis and other biological processes.
incubated with a secondary antibody conjugated to horseradish peroxidase (Promega) for 1 h. The membrane was washed with TBST three times and detected by enhanced chemiluminescence (Amersham).

RESULTS

Expression of Fusion Constructs—Although Tal1/E47 heterodimers can bind to DNA with equal or higher affinities than E47 homodimers in vitro, they activate transcription much more poorly relative to E47 homodimers. Consequently, Tal1 acts as an inhibitor of E47-activated gene expression. To understand why Tal1 has such an inhibitory effect, fusion proteins containing various regions of Tal1 and E47 were constructed. We asked which domains could be interchanged between Tal1 and E47 so that the effect of Tal1 on E47 activation is not inhibition but activation. As a corollary, we asked which portions of Tal1 can confer inhibitory properties on E47. The schematic of the fusion proteins constructed is shown in Fig. 1A. The E47 and Tal1 proteins were divided into three domains: the NH2 terminus, the bHLH region, and the COOH terminus. The N-terminal of E47 has been shown to contain two activation domains (36–38), whereas the NH2 terminus of Tal1 contains one such domain (39). In both proteins these domains have been identified in the context of fusion proteins with the DNA binding domain of the GAL4 protein. The bHLH region is responsible for the dimerization and DNA binding properties of E47 and Tal1 (3, 40). The function of the COOH terminus is less well defined.

To determine the expression levels of the fusion proteins, the constructs were transfected into HeLa cells, and cell lysates were analyzed using Western blots. Because no single antibody could detect all of the fusion proteins, Western analyses were performed using the same blot or cell lysates with antibodies against Tal1, the myc tag (9E10), or E47 (Fig. 1B). Because E-T/2 and E-T/5 contain epitopes reacting to both anti-Tal1 and anti-E47 antibodies and because Tal1 reacts to both the anti-Tal1 and 9E10 antibodies, it was possible to estimate roughly the levels of the fusion proteins expressed. All of the fusion proteins appeared to be expressed at comparable levels, except for E-T/1, which seemed to be expressed at a lower level.

The NH2 Terminus of Tal1 Possesses Transactivating Ability—To test first whether the fusion proteins could activate transcription on their own, the constructs were examined for their ability to activate a luciferase reporter construct, driven by five copies of the E box sequence optimal for binding to Tal1/E47 heterodimers. The plasmids expressing the fusion proteins were cotransfected into HeLa cells along with the reporter plasmid, and luciferase activity was measured 2 days later. Fig. 2A shows the activities of the constructs relative to E47 homodimers. E47 homodimers routinely gave 200–400 fold-activation over reporter alone. As expected, those constructs containing the bHLH domain of Tal1 could not activate by themselves because the bHLH region of Tal1 cannot mediate homodimer formation (40), which is a prerequisite for DNA binding. However, constructs containing the bHLH domain of E47, such as E-T/4 and E-T/6, were able to activate transcription. Because E-T/4 contains the NH2 terminus of Tal1 fused with the bHLH domain and COOH terminus of E47 and be-

FIG. 1. Panel A, schematic of E47-Tal1 fusion proteins. E47 and Tal1 proteins are represented by shaded and hatched bars, respectively. Each protein was divided into three domains: the NH2 terminus, the bHLH region, and the COOH terminus. The E-T/2ADm construct contains deletions of amino acids 17–227 and 309–367 as indicated by gaps, which correspond to activation domains I and II of E47. E-T/2bm and E-T/3bm have arginines at amino acid positions 188 and 189 of the bHLH region of Tal1 mutated to glycines as labeled. The dE-T/4 construct has amino acids 122–175 deleted from the NH2 terminus of Tal1. Panel B, Western analyses of E47-Tal1 fusion proteins. The three blots shown were loaded identically with whole cell extracts prepared from HeLa cells transfected with 5 μg of each construct as marked on the lanes of the top blot. Western analyses were carried out using antibodies as indicated next to each blot. The anti-Tal1 immune serum was directed against the COOH terminus of Tal1. Monoclonal antibody 9E10 recognizes the myc epitope, which was fused to the NH2 terminus of Tal1. The anti-E47 immune serum reacts to the NH2 terminus of E47. The relevant protein in each lane is indicated with an arrow.
cause these two domains do not appear to have any transactivating function, the ability of E-T/4 to activate transcription may be attributed to the NH₂ terminus of Tal1. This result is in accordance with previous findings that the NH₂ terminus of Tal1, when fused to the DNA binding domain of Gal4, can act as a transcriptional activator (39). To test this hypothesis, the putative activation domain defined by Sanchez-Garcia and Rabbitts (39) was deleted in our construct dE-T/4. The transactivating activity of dE-T/4 was reduced by about 60% compared with E-T/4, suggesting that the NH₂ terminus of Tal1 is indeed able to activate transcription. However, whether this transactivating function of Tal1 is of physiological significance remains to be determined. It is also interesting to note that construct E-T/5, which differs from wild type E47 only at the COOH terminus, possesses only 30% of the transcriptional activity of wild type E47 homodimers. As this construct is expressed as well as the wild type E47, this result would suggest that the COOH terminus of Tal1 may inhibit the transactivating potential of E47. A similar phenomenon was also observed by Hofmann and Cole (41).

The NH₂ Terminus of Tal1 Is Incompatible with the Transactivating Activity of E47—We next asked what effect these fusion proteins had on transcriptional activation by E47. Equal amounts of E47 and the various fusion constructs were cotransfected with the E box reporter construct into HeLa cells. The luciferase activities in the transfected cells are shown in Fig. 2B. Compared with wild type E47, cotransfection of Tal1 led to a 14-fold reduction of activity as observed previously (21). Similarly, the E-T/1 construct inhibited reporter activation by 3-fold. Although the reduced level of E-T/1 expression relative to wild type Tal1 may explain this weaker inhibition, it may also be because of the lack of the COOH terminus of Tal1.

When the NH₂ terminus of Tal1 was replaced with that of E47 as in constructs E-T/2 and E-T/3, the resulting fusion proteins activated transcription even more effectively than wild type E47. The higher transcriptional activities of E-T/2 and E-T/3 may result from the presence of the bHLH domain of Tal1 because the interaction between Tal1/E47 heterodimers is tighter than between E47 homodimers (21). In addition, Tal1/E47 heterodimers may possess higher affinities to the E box sequence than E47 homodimers, thus leading to greater activation. Activation by E-T/2 and E-T/3 was dependent on their ability to bind DNA because mutations in the basic amino acids critical for DNA binding (21) (arginines at amino acid positions 188 and 189 were changed to glycines) completely abolished their activities, as demonstrated using constructs E-T/2bm and E-T/3bm. Furthermore, this activation was also dependent on the NH₂-terminal activation domains from E47, since deletion of the two activation domains in construct E-T/2ADm abrogated the transcriptional activation.

Most importantly, these results suggest that Tal1/E47 heterodimers are capable of activating transcription if Tal1 is provided with suitable transactivation domains such as those from E47. In contrast, fusion proteins E-T/4 and E-T/6, when complexed with E47, inhibited activation by about 6.5-fold compared with E47 homodimers (Fig. 2B), even though the NH₂ terminus of Tal1 contains a potential activation domain (Fig. 2A). Thus it appears that the activation domain of Tal1 is not compatible with those of E47. As expected, construct E-T/5, 3

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3 S. T. Park and X.-H. Sun, unpublished observations.
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The COOH terminus of Tal1 may also be responsible for the lower activity of E-T/2 heterodimers compared with E-T/3 heterodimers (Fig. 2B). Taken together, analyses of these fusion proteins lead us to conclude that the lack of transactivation domains compatible with E47 may be the primary reason for the inhibitory effect of Tal1 on E47-activated gene expression. To a lesser extent, the COOH terminus of Tal1 may also contribute to the inhibition.

**Tal1 Inhibits E47-mediated Activation of the CIP1 Gene—**

Because Tal1 is able to interfere with the transcriptional activation mediated by E47, it is possible that Tal1 exerts its oncogenic activity by inhibiting the expression of genes activated by E47. Because E47 has been shown to possess properties of a growth inhibitor, these genes may be involved directly in growth suppression. Recently, data from our laboratory have demonstrated that E47 can activate the transcription of CIP1, a gene encoding a universal inhibitor of the cyclin-dependent kinases (28). Cotransfection of E47 with a luciferase reporter gene driven by the promoter of the CIP1 gene (p21-LUC) resulted in a 20-fold activation of luciferase expression (Fig. 3A). To determine if Tal1 could inhibit this activation, Tal1 was cotransfected along with E47 and p21-LUC into HeLa cells, and luciferase activity was measured subsequently. As shown in Fig. 3A, Tal1 inhibited this activation dramatically in a dose-dependent manner. Stoichiometric amounts of Tal1 reduced the activation by as much as 90%. This inhibitory effect of Tal1 on E47-mediated activation CIP1 expression was as strong, if not stronger than, the effect of Id1, a known inhibitor of E47 (42, 43).3 In contrast, the E-T/2 fusion protein, which forms heterodimers with E47 and activates the E box luciferase reporter (Fig. 2B), did not inhibit E47-activated CIP1 expression.

We next asked if Tal1 could inhibit E47-activated expression of the endogenous CIP1 gene. Using Northern blots, we found that E47 enhanced the level of CIP1 mRNA by more than 3-fold in transiently transfected 293T cells (Fig. 3B). Cotransfection of E47 with Tal1 reduced the level of CIP1 mRNA by 50%. Similarly, the level of CIP1 protein was elevated significantly in the 293T cells transfected with E47 (Fig. 3C). Cotransfection of E47 with Tal1 brought the level of CIP1 protein down to background levels. In contrast, when the E-T/2 fusion protein was coexpressed with E47, both the transcript and protein levels of CIP1 were increased, suggesting that heterodimers of E-T/2 with E47 are functionally equivalent to E47 homodimers. From these results we conclude that Tal1 is able to inhibit E47-activated CIP1 expression. However, whether this inhibition occurs in a physiological setting remains to be determined. Nevertheless, it is important that on the native promoter of an E box-driven gene, as opposed to artificial E box reporter constructs, Tal1 inhibits E47 activity.

**Tal1 Inhibits E47 Activity in T Cells—**

Because Tal1 is found to be expressed aberrantly in the T cells derived from T cell acute lymphoblastic leukemia patients, we tested if Tal1 in such T cells could inhibit the transcriptional activity of E47. We utilized the Jurkat T cell line, which was derived from a T cell acute lymphoblastic leukemia patient and has been shown to express Tal1 (44, 45). Overexpression of E47 stimulated the expression of the E box reporter by 50-fold (Fig. 4A). Moreover, cotransfection of Tal1 with E47 was able to diminish the stimulation significantly, whereas cotransfection of E-T/2 with E47 retained the activation of the E box reporter gene. Thus, as in HeLa cells, it appears that Tal1 can inhibit E47 activity in T cells.

Experiments have demonstrated that endogenous complexes of Tal1/E47 can be immunoprecipitated from Jurkat cells (35). However it is not known if these complexes are transcriptionally active. The level of luciferase activity measured when the
E box luciferase reporter construct is transfected alone into Jurkat cells can be taken as a measure of endogenous E protein activity. Furthermore, if this activity is truly the result of E proteins, then cotransfection of Id1 should lower this activity. Endogenous E protein activities in Jurkat cells appeared to be very low in that cotransfection with Id1 could not reduce the expression of the reporter further. In addition, when the E-T/2 construct was transfected into Jurkat cells without E47, it resulted in a 13.5-fold activation of reporter expression (Fig. 4B). In contrast, Tal1 did not increase reporter expression at all. Because the E-T2 fusion protein cannot form homodimers to activate transcription, the transcriptional activation by E-T2 proteins is probably caused by the formation of heterodimers between E-T2 and endogenous E47 or E47-like proteins. Also, because E-T2 contains the same bHLH domain as Tal1, it may be able to compete with endogenous Tal1 to bind to E47 and activate the expression of the E box reporter. Together, these data suggest that endogenous Tal1/E47 complexes, although readily detectable, are transcriptionally inactive, thereby perhaps reducing the expression of genes.
Inhibition of E47 by Tal1

The Tal1 protein has traditionally been grouped as a class II bHLH protein because of its tissue-specific expression and its dependence on class I bHLH proteins to bind to DNA. However, Tal1 differs functionally from other class II proteins, such as MyoD and Beta2, whose heterodimers with E47 transactivate appropriate reporter genes much more efficiently than E47 homodimers (46, 47). When Tal1 is coexpressed with E47, the transactivating activity is reduced dramatically compared with E47 homodimers. We have attributed this inhibitory effect primarily to the lack of a transactivation domain in Tal1 which is compatible with the transactivating function of E47. To extrapolate from our data, it appears that E47 requires two sets of activation domains from both partners to activate transcription. Heterodimers between E47 and proteins with the bHLH domain of E47 or the Gal4 DNA binding domain such that homodimers of the fusion proteins can form. This putative activation domain appears inactive in the Tal1/E47 heterodimers, suggesting that it too requires two copies for transcriptional activation. Nevertheless, it remains a formal possibility that when Tal1 forms multiprotein complexes in certain circumstances, the activation domain of Tal1 can be functional.

Can Tal1 mediate transcriptional activation? Heterodimers between E47 and Tal1 have been shown to form ternary complexes with the Lim-only proteins, LMO1 and LMO2, through the bHLH domain of Tal1 (48). Coexpression of Tal1 and LMO1 or LMO2 in T cells appears to enhance the ability of Tal1 to activate transcription of a reporter gene driven by multiple copies of the E box (49). However, it is not clear whether the level of this activation is comparable to the expression activated by E47 homodimers. By using an artificial promoter sequence as a probe, in which an E box is placed next to a GATA site, Tal1 is found to form a higher order complex that includes Tal1, E47, GATA-1, LMO2, and the Lim domain binding protein 1. Transcriptional activity directed by this artificial promoter can be stimulated by overexpressing all of the components in the complex (16). These data would suggest that Tal1 may potentially be involved in transcriptional activation in certain specific settings where all of these factors are present, perhaps during hematopoiesis. However, the physiological target genes that can be activated by this multimeric complex have yet to be found.

Despite the potential of Tal1 in multifactor complexes to activate transcription of certain genes, it is distinctly possible that in its ectopic setting of T cells, Tal1 causes oncogenesis through its ability to act as an inhibitor of E47-mediated cellular processes. E47 is known to suppress cell growth in NIH3T3 fibroblasts upon overexpression (24), presumably through its ability to activate the expression of growth-suppressive genes such as CIP1. In T lymphocytes, E47 apparently acts as a tumor suppressor because mutations of the E2A gene that codes for E12 and E47 lead to the development of T cell lymphoma at extremely high frequencies in E2A-deficient mice (50). Transgenic mice overexpressing Tal1 under the promoter of lymphoid cell kinase gene also develop T cell lymphoma but at a lower incidence and later age (17, 18). Importantly, the ability of Tal1 to cause tumors is enhanced by coexpression of casein kinase II (17), which can inhibit the activity of E47 homodimers (51), thus supporting the hypothesis that Tal1 acts as an inhibitor of E47. Interestingly, transgenic mice overexpressing LMO1 or LMO2 also develop T cell malignancies (52). Tal1, although unable to cause tumors when expressed under the control of the SIIL or CD2 promoter, increases the incidence and rate of tumor formation of the LMO1 and LMO2 transgenic mice, respectively (53, 54). However, it remains to be determined whether this effect of Tal1 is caused by the inhibition of E47 or because of the transcriptional activation involving both Tal1 and LMO proteins.

In summary, previous evidence and our current results have

controlled by E47 and eventually leading to leukemogenesis in T cells.

**DISCUSSION**

The Tal1 protein has traditionally been grouped as a class II bHLH protein because of its tissue-specific expression and its dependence on class I bHLH proteins to bind to DNA. However, Tal1 differs functionally from other class II proteins, such as MyoD and Beta2, whose heterodimers with E47 transactivate appropriate reporter genes much more efficiently than E47 homodimers (46, 47). When Tal1 is coexpressed with E47, the transactivating activity is reduced dramatically compared with E47 homodimers. We have attributed this inhibitory effect primarily to the lack of a transactivation domain in Tal1 which is compatible with the transactivating function of E47. To extrapolate from our data, it appears that E47 requires two sets of activation domains from both partners to activate transcription. Heterodimers between E47 and proteins with the NH2 terminus of Tal1, or its NH2-terminal deletion mutant, or the NH2 terminus of E47 containing deleted activation domains all failed to stimulate the expression of the reporter gene. The fact that heterodimers between E47 and MyoD or Beta2 can activate transcription would suggest that MyoD and Beta2 contain activation domains either compatible with the activation domain of E47 or capable of activating transcription on their own. Tal1, on the other hand, lacks such a domain. Unlike the Id proteins that sequester the E proteins into complexes unable to bind DNA (42, 43), Tal1/E47 heterodimers can interact with DNA, but they are not able to activate transcription. Therefore, this ability of Tal1 to occupy control elements normally bound by E47 homodimers could render Tal1 a more powerful inhibitor than the Id1 proteins.

It is interesting that the NH2 terminus of Tal1 displays the properties of an activation domain when it is fused to the bHLH domain of E47 or the Gal4 DNA binding domain such that homodimers of the fusion proteins can form. This putative activation domain appears inactive in the Tal1/E47 heterodimers, suggesting that it too requires two copies for transcriptional activation. Because Tal1 is known not to form homodimers, it is difficult to imagine that two copies of this putative activation domain could ever exist in a complex to activate transcription. Nevertheless, it remains a formal possibility that when Tal1 forms multiprotein complexes in certain circumstances, the activation domain of Tal1 can be functional.
led us to propose that one of the mechanisms by which Tal1 causes T cell lymphoma is by inhibiting the transcriptional activation by E47. Two other proteins whose bHLH domains are highly homologous to Tal1, Tal2 and Ly11, are also involved in T cell leukemia (7, 8). These proteins also bind to E47 and abrogate its transactivation properties,3 thus suggesting a common mechanism for all three proteins in leukemogenesis. What would be the genes normally controlled by E47 and inhibited by Tal1? It is unlikely that the down-regulation of the CIP1 gene, although it may occur, is the sole reason for leukemogenesis because CIP1-deficient mice do not develop T cell lymphoma (55). Additional genes in T cells controlled by E47 remain to be identified in the future. These genes may be crucial for the homeostasis of the T cell compartment by regulating cell cycle progression or apoptosis.

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REFERENCES

1. Kadesch, T. (1992) ImmunoI. Today 13, 31–36
2. Weintraub, H., Davis, R., Tapscott, S., Thayer, M., Krause, M., Beczenro, R., Blackwell, T. K., Turner, D., Rupp, R., and Hollenberg, S. (1991) Science 251, 761–766
3. Murre, C., McCaw, P. S., Vassoes, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buiskin, J. N., Haaschka, S. D., and Laszlo, A. B. (1989) Cell 58, 537–544
4. Begley, C. G., Aplan, P. D., Denning, S. M., Haynes, B. F., Waldmann, T. A., and Kirsch, I. R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 10128–10132
5. Chen, Q., Cheng, J. T., Tasi, L. H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J., and Baer, R. (1990) EMBO J. 9, 345–354
6. Kamps, M. P., Murre, C., Sun, X.-H., and Baltimore, D. (1990) Cell 60, 547–555
7. Mellenin, J. D., Smith, S. D., and Cleary, M. L. (1989) Cell 57, 77–83
8. Xia, Y., Brown, L., Yang, C. Y. T., Tsan, J. T., Siciliano, M. J., Episnosa, R., Le Beau, M. M., and Baer, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 86, 11416–11420
9. Bash, R. O., Hall, S., Timmons, C. F., Amylon, M., Crist, W. M., Amylon, M., and Baer, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 86, 4153–4158
10. Brown, L., Cheng, J. T., Tasi, L. H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J., and Baer, R. (1990) EMBO J. 9, 345–3454
11. Perali, F. A., Ramqvist, T., Saffrich, R., Pepperkik, R., Barone, M. V., and Peverali, F. A., Ramqvist, T., Saffrich, R., Pepperkik, R., Barone, M. V., and Kelliher, M. A., Seldin, D. C., and Leder, P. (1996) Genes Dev. 10, 5888–5896
12. Cheng, J. T., Tsan, J. T., Tasi, L. H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J., and Baer, R. (1990) EMBO J. 9, 345–3454
13. Kellianpur, A. R., Jordan, J. E., and Brandt, S. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5947–5951
14. Ono, Y., Fukuhara, N., and Yoshie, O. (1997) J. Biol. Chem. 272, 4576–4581
15. Aronheim, A., Shiran, R., Rosen, A., and Walker, M. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 86, 8063–8067
16. Pulford, K., Lecointe, N., Leroy-Viard, K., Jones, M., Mathieu-Mahul, D., and Rabbits, T. H. (1991) Cell 66, 305–315
17. Hsu, H. L., Cheng, J. T., Chen, Q., and Baer, R. (1991) Mol. Cell. Biol. 11, 3037–3042
18. Massari, M. E., Jennings, P., and Murre, C. (1996) Mol. Cell. Biol. 16, 121–129
19. Sanchez-Garcia, I., and Rabbits, T. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7769–7783
20. Massari, M. E., Jennings, P., and Murre, C. (1996) Mol. Cell. Biol. 16, 121–129
21. Sanchez-Garcia, I., and Rabbits, T. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7869–7873
22. Hoy, S. E., Wang, J. M., and Baer, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3181–3185
23. Kwong, M. W., Massari, M. E., Zwart, R., and Murre, C. (1993) Mol. Cell. Biol. 13, 792–800
24. Aronheim, A., Shiran, R., Rosen, A., and Walker, M. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8063–8067
25. Massari, M. E., Jennings, P., and Murre, C. (1996) Mol. Cell. Biol. 16, 121–129
26. Sanchez-Garcia, I., and Rabbits, T. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7869–7873
27. Kellianpur, A. R., Jordan, J. E., and Brandt, S. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1021–1027
28. Rabbits, T. H. (1994) Mol. Cell. Biol. 14, 1256–1265
29. Hsu, H. L., Cheng, J. T., Tasi, L. H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J., and Baer, R. (1990) EMBO J. 9, 345–354
30. Murre, C., Voronova, A., and Baltimore, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 537–544
31. Gutterman, T. G., and Baltimore, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10128–10132
32. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L., and Weintraub, H. (1990) Cell 61, 49–59
33. Sun, X.-H., Copeland, N. G., Jenkins, N. A., and Baltimore, D. (1991) Mol. Cell. Biol. 11, 5603–5611
34. Aronheim, A., Shiran, R., Rosen, A., and Walker, M. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 91, 8063–8067
35. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L., and Weintraub, H. (1990) Cell 61, 49–59
36. Illum, J. B., Copeland, N. G., and Jenkins, N. A. (1992) EMBO J. 11, 5603–5611
37. Schneider, U., Schwenk, H., and Bornkamm, G. (1977) Int. J. Cancer 19, 621–626
38. Lavenir, I., Larson, T. A., Baer, R., Warren, A. J., and Baer, R. (1994) Mol. Cell. Biol. 14, 1256–1265
39. Wadman, I., Lavenir, I., Rabbits, T. H., and Baer, R. (1994) EMBO J. 13, 5888–5896
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Steven T. Park and Xiao-Hong Sun

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