Target-dependent Effect of Phosphorylation on the DNA Binding Activity of the TAL1/SCL Oncoprotein*

(Received for publication, November 12, 1996, and in revised form, February 5, 1997)

K. S. Srinivas Prasad‡ and Stephen J. Brandt¶§

From the Departments of \‡ Medicine and \¶ Cell Biology, Vanderbilt University Medical Center and the \§ Department of Veterans Affairs Medical Center, Nashville, Tennessee 37232

Activity of the TAL1 (or SCL) gene, initially identified through its involvement by a recurrent chromosomal translocation, is the most frequent gain-of-function mutation recognized in T-cell acute lymphoblastic leukemia. The translational products of this gene contain the basic domain helix-loop-helix motif characteristic of a family of transcription factors that bind to a consensus nucleotide sequence termed the E-box. Previous work established that the TAL1 proteins are phosphorylated exclusively on serine and identified Ser122 as a substrate for the mitogen-activated protein kinase ERK-1. We provide evidence that an additional serine residue located proximal to the basic region could be juxtaposed to the promoter of the upstream LYL1, can be phosphorylated in vitro and in vivo by the catalytic subunit of cAMP-dependent protein kinase. Phosphorylation was found to alter TAL1 DNA binding activity in a target-dependent manner that was influenced by both the specific CANNTG E-box core motif and its flanking sequences. In contrast, the ability of TAL1 to interact with the E2A gene product E12 and its subcellular localization in transfected COS cells were unaffected by Ser172 phosphorylation. These results suggest this serine residue has a regulatory function and indicate a mechanism by which phosphorylation could affect DNA binding site discrimination.

The TAL1 (or SCL) gene was initially identified as a result of its involvement in a recurrent chromosomal translocation with the T-cell receptor α/δ locus in T-cell acute lymphoblastic leukemia (1–3). It was recognized subsequently that its coding region could be juxtaposed to the promoter of the upstream SIL locus (for SCL interrupting locus) gene as a result of certain interstitial deletions apparently cytogenetically (4–6). In still other patients, the gene can be misexpressed in the apparent absence of chromosomal rearrangement (7). In aggregate, ectopic TAL1 expression characterizes up to 60% of individuals with T-cell acute lymphoblastic leukemia, making it the most frequent gain-of-function mutation recognized in this disorder. TAL1 belongs to the helix-loop-helix (HLH) family of transcription factors, so-named for the conformation adopted by its defining motif of two amphipathic helices with an intervening loop (10–12). The HLH domain functions in the formation of protein hetero- and homodimers, whereas an adjacent basic region found in many of the members of this gene family mediates sequence-specific DNA binding (13–15). As with other tissue-restricted basic domain-HLH (bHLH) proteins, TAL1 binds DNA as a heterodimer with the products of the widely expressed E2A gene (16, 17). These complexes recognize a motif with the hexanucleotide core sequence CANNTG termed the E-box (18, 19) and function as transcriptional regulators (20).

Within this larger family, distinct subgroups of HLH genes can be recognized on the basis of sequence relatedness, expression pattern, and function. In the best characterized example, the four bHLH genes expressed in developing skeletal muscle share the ability to induce myogenic differentiation when introduced into certain cell lines (21, 22) and appear capable of substituting for each other's functions in vivo (23). TAL1 shows greater than 80% sequence identity over its basic and HLH domains with two other genes, LYL1 and TAL2, also involved by chromosomal translocation in T-cell acute lymphoblastic leukemia (24).

The protein products of the TAL1 locus are expressed in a number of cell types during development, becoming essentially restricted to hematopoietic and endothelial cells postnataally (25, 26). A requirement for TAL1 in embryonic hematopoiesis was recently demonstrated by targeted mutation of the murine TAL1 coding region (27, 28), and evidence also points to its involvement in the terminal, erythropoietin-regulated stages of red cell production (29, 30). TAL1 and LYL1 show overlapping expression in several hematopoietic lineages (31) and may, by analogy to the myogenic HLH genes, share some similarities in function.

Up to three proteins can be translated from TAL1 transcripts (32–34), with two potentially made from the full-length message (amino acids 1–330 and 26–330, numbered according to the mouse protein). The shorter of these, whose synthesis in cells has not been rigorously demonstrated, likely arises by alternative translational initiation (35). A significantly smaller protein (amino acids 176–330) lacking the amino-terminal half of the molecule but retaining the basic and HLH domains is translated from a series of spliced RNAs that exclude the first coding exon. This species has been detected in leukemia cell lines (33) and differentiating erythroblasts (30).

The TAL1 polypeptides are serine phosphorylated (32, 33), with phosphorylation of Ser122 by the mitogen-activated protein kinase ERK-1 (34) found to increase the activity of a transcriptional activation domain (36). We provide evidence that an additional serine residue located proximal to the basic region can be phosphorylated in vitro and in vivo by cAMP-de-

* This work was supported in part by United States Public Health Service Grant R29 HL49118 (to S. J. B.) and by the Lucille P. Markey Charitable Trust (to S. J. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Division of Hematology, Rm. 547 MRB II, Vanderbilt University Medical Center, Nashville, TN 37232. Tel.: 615-936-1809; Fax: 615-936-1812.

¶ The abbreviations used are: HLH, helix-loop-helix; bHLH, basic domain helix-loop-helix; PKA, cAMP-dependent protein kinase; MBP, maltose binding protein; PAGE, polyacrylamide gel electrophoresis; EMSA, electrophoretic mobility shift assay; PVDF, polyvinylidene difluoride.
Phosphorylation and TAL1/SCL Function

Experimental Procedures

TAL1 Fusion Proteins—A 640-base pair murine TAL1 cDNA derived by the polymerase chain reaction (25) was subcloned into the EcoRI site of vector pMALc2 (New England Biolabs) and the encoded maltose-binding protein (MBP)-murine TAL1 fusion (MBP-TAL1)164–330 expressed in the DH5a strain of Escherichia coli. To express the corresponding Ser172 to Ala mutant (MBP-TAL1172–330), oligonucleotides TATGGGCAAGGAGGCTTCCCTGCGGTTT and AATTCACAACACGGGGAAGAGGAGGTTCC were annealed and ligated into a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.

MBP-TAL1 fusion proteins were purified on composite amylose-agarose beads (New England Biolabs) and eluted with maltose as described (37). Purified MBP was provided by Paul Riggs.

Site-directed Mutagenesis—A 1.0-kilobase full-length mouse TAL1 cDNA was subcloned into the BamHI site of vector pALTER-1 (Promega), and oligonucleotide-mediated mutagenesis of Ser172 to Ala was carried out according to the supplier’s instructions and verified by nucleotide sequencing. The mutated cDNA was released from pALTER-1 and subcloned into the BamHI site of vector pcdNA I (Invitrogen) for cell transfection.

In Vitro Phosphorylation—Affinity purified fusion proteins (500 ng) were phosphorylated by the addition of 5 units of purified PKA catalytic subunit (25) provided by Sharron Francis-West, and the radioactivity incorporation into a gel slice containing the fusion protein was quantitated in a liquid scintillation counter. The mean number of mol of phosphate incorporated per mol of protein ± S.D. was determined for three independent reactions, taking into account the specific activity of the γ-32PATP and the efficiency of the counter.

Electrophoretic Mobility Shift Assay—Electrophoretic mobility shift assays (EMSA) of DNA binding activity were carried out with an amino-terminally truncated E12 protein produced by coupled transcription/translation (TntT system, Promega) of plasmid E12R (10) in rabbit reticulocyte lysates and MBP-TAL1 fusion proteins purified from bacterial sonicates. The indicated proteins were incubated in a buffer containing 20 mM Hepes, pH 7.8, 20% glycerol, 100 mM KCl, 0.5 mM dithiothreitol, 0.2 mM EDTA, and 20 mM MgCl2 for 10 min. In some cases as noted, anti-TAL1 antibody was added for an additional 5 min. Double-stranded oligonucleotides were labeled with 32P by T4 polynucleotide kinase and incubated with transcription factor complexes in 30 μl of binding buffer containing 100 mM Hepes, pH 7.8, 2.5 mM dithiothreitol, 100 mM KCl, 60 mM MgCl2, 1 mM EDTA, and 1 μg of poly(dI- dC) for 20 min at room temperature. Protein-DNA complexes were resolved on 5% formaldehyde gels containing 3% glycerol. Dried gels were exposed to film, and laser scanning densitometry was used to quantify the relevant retarded complexes on the resulting autoradiographs. The significance of differences in the DNA binding of complexes was determined by a two-tailed t test. Mean values ± S.D. were calculated from four independent experiments.

The sequences of the upper strand of each pair of oligonucleotides were used, with the core E-box sequences underlined, as are follows: μE2 enhancer, CCTGCAGGACCTGGACAGA; μE5 enhancer, GAACAGGAAACCTCGACCA; preferred binding site, ACCTGAAGATGGTCGCT; chimeric site with core sequences from preferred binding site and flanking sequences from μE2 enhancer, CCTGCAGGACCTGGACAGA; and ligated in a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.

The sequences of the upper strand of each pair of oligonucleotides were used, with the core E-box sequences underlined, as are follows: μE2 enhancer, CCTGCAGGACCTGGACAGA; μE5 enhancer, GAACAGGAAACCTCGACCA; preferred binding site, ACCTGAAGATGGTCGCT; chimeric site with core sequences from preferred binding site and flanking sequences from μE2 enhancer, CCTGCAGGACCTGGACAGA; and ligated in a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.

The sequences of the upper strand of each pair of oligonucleotides were used, with the core E-box sequences underlined, as are follows: μE2 enhancer, CCTGCAGGACCTGGACAGA; μE5 enhancer, GAACAGGAAACCTCGACCA; preferred binding site, ACCTGAAGATGGTCGCT; chimeric site with core sequences from preferred binding site and flanking sequences from μE2 enhancer, CCTGCAGGACCTGGACAGA; and ligated in a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.

The sequences of the upper strand of each pair of oligonucleotides were used, with the core E-box sequences underlined, as are follows: μE2 enhancer, CCTGCAGGACCTGGACAGA; μE5 enhancer, GAACAGGAAACCTCGACCA; preferred binding site, ACCTGAAGATGGTCGCT; chimeric site with core sequences from preferred binding site and flanking sequences from μE2 enhancer, CCTGCAGGACCTGGACAGA; and ligated in a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.

The sequences of the upper strand of each pair of oligonucleotides were used, with the core E-box sequences underlined, as are follows: μE2 enhancer, CCTGCAGGACCTGGACAGA; μE5 enhancer, GAACAGGAAACCTCGACCA; preferred binding site, ACCTGAAGATGGTCGCT; chimeric site with core sequences from preferred binding site and flanking sequences from μE2 enhancer, CCTGCAGGACCTGGACAGA; and ligated in a trimolecular reaction to EcoRI- and BamHI-digested pMALc2 and an NdeI-BamHI murine TAL1 cDNA extending from bases 519 to 1003. For preparation of an MBP-TAL1164–330 fusion protein, a 1.0-kilobase NotI- and BamHI-digested fragment of a full-length murine TAL1 cDNA (provided by Glenn Begley) was subcloned into EcoRI and BamHI-digested pPLR166 (provided by Paul Riggs) after blunting of the NotI site of the insert and the EcoRI site of the vector with the Klenow fragment of DNA polymerase I. Automated nucleotide sequencing of fusion constructs was carried out using dye-labeled deoxyxterminators (Applied Biosystems) to confirm that the nalaE and TAL1 coding regions were in the same reading frame.
RESULTS

TAL1 Is a Substrate for Phosphorylation by PKA in Vitro: Identification of Ser^{172} as the Site of Phosphorylation—To identify potential sites of TAL1 protein phosphorylation, bacterial expressed MBP-TAL1 fusion proteins were incubated with purified protein kinases and radiolabeled ATP in in vitro kinase assays. TAL1, but not MBP, sequences were efficiently phosphorylated by the catalytic subunit of PKA, and the site(s) of phosphorylation was localized using a series of amino-terminally truncated TAL1 fusion proteins as substrates to a region encompassing amino acids 164–255 (not shown). Phosphoamino acid analysis indicated further that PKA-stimulated TAL1 phosphorylation occurred only on serine (not shown). By inspection, a potential PKA recognition sequence (RVRKRPSPY) resembling, especially, that of the β chain of phosphorylase kinase (41) was noted that contains Ser^{172}. This sequence is completely conserved between the chicken (42), mouse (1), and human (2, 43) TAL1 proteins and shows considerable homology to one found in a similar context in another HLH oncoprotein human (2, 43) TAL1 proteins and shows considerable homology to one found in a similar context in another HLH oncoprotein.

To determine specifically whether Ser^{172} was the target of PKA-stimulated phosphorylation, an MBP-TAL1 fusion protein containing this site (MBP-TAL1164–330) and its corresponding Ser^{172} to Ala mutant (MBP-TAL1164–330(S172A)) were incubated with purified catalytic subunit of PKA in the presence of [γ-32P]ATP. Under the conditions described, the MBP-TAL1164–330 fusion was phosphorylated to a stoichiometry of 0.78 ± 0.17 mol of phosphate/mol of protein. The alanine substitution mutant, in contrast, showed no measurable phosphorylation by PKA (Fig. 2), demonstrating the ability of Ser^{172} to function as a phosphoacceptor for this kinase in vitro.

Phosphorylation of Ser^{172} Has a Target-dependent Effect on TAL1 DNA Binding Activity—Given its proximity to the basic domain, we investigated whether phosphorylation of Ser^{172} would affect the binding of TAL1-containing complexes to DNA. Previous work had demonstrated sequence-specific DNA binding activity for hetero-oligomers of TAL1 with E12, E47, and HEB (18), and the ability of the MBP-TAL1164–330 fusion protein to bind with E12 to E-box sequences from the μE2 and μE5 immunoglobulin enhancers and one identified by a polymerase chain reaction-assisted site-selection assay (19) was tested by EMSA. In addition to a complex attributable to the binding of E12 homodimers, particularly prominent with the μE5 probe, a less retarded complex dependent on the presence of both TAL1 and E12 protein and specifically disrupted by anti-TAL1 antibody was identified (Fig. 3). Significantly greater binding of this TAL1-E12 heterodimeric complex was found to the preferred sequence, consistent with it being a high affinity site (19) (not shown). No DNA binding was noted with either purified MBP or unprogrammed reticulocyte lysate.

The effect of PKA-mediated TAL1 phosphorylation on the DNA binding activity of TAL1-E12 complexes was further investigated using MBP-TAL1164–330 protein as substrate. Phosphorylation resulted in greater than 50% inhibition of binding of MBP-TAL1164–330-E12 complexes to the μE2 and μE5 enhancers, whereas it had no effect with the preferred site as probe (Figs. 4 and 5A). Site-specific mutagenesis abrogated the effect of phosphorylation on DNA binding activity concomitant with its inhibition of radiophosphate incorporation (Figs. 4 and 5A), demonstrating the action of PKA to be a direct one requiring Ser^{172} as phosphoacceptor.

Although it demonstrated the same binding preferences as
Ser172 Phosphorylation Has No Effect on TAL1 Protein—Given the importance of the basic region for nuclear uptake in addition to DNA binding (44), we also investigated whether Ser172 phosphorylation could affect the subcellular localization of TAL1 protein. To that end, an expression vector containing a full-length murine TAL1 cDNA was transfected into COS cells with and without one encoding the catalytic subunit of PKA, and the distribution of TAL1 protein was analyzed by indirect immunofluorescence. Consistent with its location in tissue sections (25, 26) and with previously published results in this cell line (25, 32, 44), immunoreactive TAL1 was restricted to the nuclei of transfected cells, where it appeared to be excluded from nucleoli. This pattern was not altered by coexpression of PKA catalytic subunit (Fig. 7), indicating that phosphorylation of Ser172, verified by phosphopeptide mapping (see below), has no effect on the protein's intracellular localization. The nuclear uptake of full-length TAL1 proteins containing either Ser172 to Ala or Ser172 to Glu substitutions was similarly unaffected (not shown).

Ser172 Acts as a Phosphoacceptor in Vivo—To determine whether Ser172 could also function as a phosphoacceptor in cells, two-dimensional phosphopeptide mapping was carried out with MBP-TAL1 164–330 fusion containing untreated TAL1 protein and subjected to EMSA with each of the four binding sites illustrated schematically below. Probes consisted of the μE2 enhancer, the preferred binding site, a chimeric site containing the core E-box sequences from the preferred site with flanking sequences from the μE2 oligonucleotide, and the reciprocal oligonucleotide containing the core E-box sequences of the μE2 enhancer with flanking sequences from the preferred binding site. Binding was quantitated by densitometric analysis of the retarded TAL1 protein complex on autoradiograms, and the relative binding for complexes containing PKA-treated versus those containing untreated TAL1 protein is expressed as a percentage for each fusion protein and binding site. For the MBP-TAL1 164–330 fusion, these results provide a graphical representation of the data in Fig. 4. Plotted is the mean percent binding ± S.D. from four independent experiments. The statistical significance of the difference in the means of adjacent conditions is denoted by the symbol, * (p < 0.001). B, MBP-TAL1 164–330 protein incubated with or without PKA was mixed with in vitro translated E12 protein and subjected to EMSA with each of the indicated binding sites. Binding was quantitated by densitometric analysis of the retarded TAL1–E12 complex on autoradiograms, and the relative binding for complexes containing PKA-treated versus those containing untreated TAL1 protein is expressed as a percentage for each fusion protein and binding site. For the MBP-TAL1 164–330 fusion, these results provide a graphical representation of the data in Fig. 4. Plotted is the mean percent binding ± S.D. from four independent experiments. The statistical significance of the difference in the means of adjacent conditions is denoted by the symbol, * (p < 0.05) and ** (p < 0.0001).
The effect of Ser172 phosphorylation on the interaction of TAL1 with E12. [3H]Leucine-labeled E12 protein was incubated with MBP-TAL164–330 fusion protein that had been incubated with (denoted P) and without (denoted C) catalytic subunit of PKA. Bound protein was collected by the addition of composite amyloseagarose beads and quantitated by fluorography following SDS-PAGE.

![Image](https://example.com/fig6.png)

**FIG. 6.** Effect of Ser172 phosphorylation on the interaction of TAL1 with E12. [3H]Leucine-labeled E12 protein was incubated with MBP-TAL164–330 fusion protein that had been incubated with (denoted P) and without (denoted C) catalytic subunit of PKA. Bound protein was collected by the addition of composite amyloseagarose beads and quantitated by fluorography following SDS-PAGE.

![Image](https://example.com/fig7.png)

**FIG. 7.** Effect of Ser172 phosphorylation on the subcellular location of TAL1. COS cells cultured on coverslips were mock-transfected (top) and transfected with an expression vector for full-length mouse TAL1 cDNA without (middle) and with (bottom) one encoding the catalytic subunit of PKA. Cells were fixed 48 h later with 2% formaldehyde, permeabilized with 0.1% dimethyl sulfoxide, and stained with rabbit anti-TAL1 antibody. TAL1 protein localization was determined by indirect immunofluorescence using fluorescein-conjugated goat anti-rabbit antibody. Fluorescence photomicroscopic images are shown on the left and corresponding phase contrast images on the right. Original magnification, 500 ×.

Although the ability of phosphorylation to modify the binding of a variety of transcription factors to their cognate DNA sequences is well known (reviewed in Ref. 49), it has only recently been appreciated that this action may be target-dependent. For p53 (50, 51) and the POU homeodomain protein MyoD homodimers (45), and the crystal structures of these bHLH proteins bound to DNA indicate their basic regions can make contact with them (11, 12). Although Ser172 would not be expected to interact with DNA directly, transfer of a charged phosphate group to this residue potentially could induce a conformational change that alters the strength of such interactions.

Site selection analysis has demonstrated a preference of TAL1-containing complexes for specific sequences flanking, in addition to those comprising, the central CANNTG motif of the E-box, and these same bases appear also to be important for the effect of PKA on TAL1 DNA binding activity. Discrimination of E-box-flanking bases has been similarly noted for E47 and MyoD homodimers (45), and the crystal structures of these bHLH proteins bound to DNA indicate their basic regions can make contact with them (11, 12). Although Ser172 would not be expected to interact with DNA directly, transfer of a charged phosphate group to this residue potentially could induce a conformational change that alters the strength of such interactions.

Our studies suggest that a region of extended homology between TAL1 and another member of the subgroup of HLH oncoproteins activated by chromosomal rearrangement, LYL1,
Phosphorylation and TAL1/SCL Function

represents a conserved regulatory site through which phosphorylation could alter protein function. Although not specifically tested, given the extent of homology over these sequences and the similarity in their location, phosphorylation of the corresponding serine residue in LYL1 (Fig. 1) would be predicted to have the same template-dependent effect on DNA binding. In view of the considerable homology in their basic and HLH domains, near-identical preference in DNA binding sites (19, 54), and overlapping patterns of expression (31), it is likely the biological consequences of this modification would be similar as well.

Although a requirement for TAL1 in embryonic hematopoiesis was demonstrated by targeted mutation of the mouse gene (27, 28) and a role in erythroid differentiation postnatally is suggested by antisense studies (29), little is actually known about the specific functions of this transcription factor, the mechanism by which the affinity of TAL1-containing complexes and, potentially, the sets of target genes activated could be subject to regulation.

Acknowledgments—We thank Liying Yang for assistance in preparing TAL1 cDNA, Paul Riggs for providing purified MBP and MBP expression vectors, Sharron Francis and Jackie Corbin for supplying purified PKA and its regulatory components, and Gough, N. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 80–84.

REFERENCES

1. Begley, C. G., Visvader, J., Green, A. R., Aplan, P. D., Metcalfe, D., Kirsch, I. R., and Gough, N. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 869–873
2. Chen, Q., Cheng, J.-T., Tsai, L.-H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J., and Baer, R. (1990) EMBO J. 9, 415–424
3. Finger, L. R., Kagan, J., Christopher, G., Kurzberg, J., Herskowitz, I., Newell, P. C., and Croce, C. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5039–5043
4. Aplan, P. D., Lombardi, D. P., Ginsberg, A. M., Cossman, J., Bertness, V. L., and Kirsch, I. R. (1989) Science 250, 1426–1429
5. Brown, L., Cheng, J.-T., Chen, Q., Siciliano, M. J., Crist, W., Buchanan, G., and Baer, R. (1990) EMBO J. 9, 3543–3551
6. Bernard, O., Guglielmi, P., Jonveaux, P., Cherif, D., Gisselbrecht, S., Mauchalle, M., Berger, R., Larsen, C. J., and Mathieu-Mahul, D. (1991) Oncogene 6, 1477–1488
7. Wadman, I. A., Hsu, H.-L., Cobb, M. H., and Baer, R. (1991) Oncogene 9, 3711–3716
8. Guan, C., Li, P., Riggs, P. D., and Inouye, H. (1988) Gene (Amst.) 67, 21–30
9. Graham, F. L., and van der Eb, A. J. (1979) Virology 86, 456–467
10. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149
11. Maitland, F., and Hanks, S. K. (1988) Nucleic Acids Res. 16, 8189
12. Zetterqvist, O., Ragnarsson, U., and Engstrom, L. (1990) in Peptides and Protein Phosphorylation (Kemp, B. E., ed) pp. 171–187, CRC Press, Inc., Boca Raton, FL
13. Goodwin, G., MacGregor, A., Zhu, J., and Crompton, M. R. (1992) Nucleic Acids Res. 20, 368
14. 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
15. Goldfarb, A. N., Goueli, S., Mickelson, D., and Greenberg, J. M. (1992) EMBO J. 11, 4073–4081
16. Prasad, K. S. S., Jordan, J. E., Koury, M. J., Bondurant, M. C., and Brandt, S. M. (1995) J. Biol. Chem. 270, 11603–11611
17. Visvader, J., Begley, C. G., and Adams, J. M. (1991) Oncogene 6, 187–194
18. Garrell, J., and Campuzano, S. (1991) Science 253, 786–789
19. Segal, N., Roberts, S. B., and Heintz, N. (1991) Science 254, 1814–1816
20. Miyamoto, A., Cui, X., Naumovski, L., and Cleavey, M. L. (1996) Mol. Cell. Biol. 16, 2394–2401
21. Pilz, R. B., Eigenather, M., and Boss, G. R. (1992) J. Biol. Chem. 267, 16161–16167
22. Pilz, R. B. (1993) J. Biol. Chem. 268, 20252–20258
23. Sherman, M. L., Shafman, T. D., and Kufe, D. W. (1986) Biochem. Biophys. Res. Commun. 134, 845–851