TEL Is a Sequence-specific Transcriptional Repressor*

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TEL is a gene frequently involved in specific chromosomal translocations in human leukemia and sarcoma that encodes a member of the ETS family of transcriptional regulators. TEL is unusual among other ETS proteins by its ability to self-associate in vivo, a property that is essential to the oncogenic activation of TEL-derived fusion proteins. We show here that TEL is a sequence-specific transcriptional repressor of ETS-binding site-driven transcription of model and natural promoters. Deletion of the oligomerization domain of TEL or its substitution by the homologous region of monomeric ETS1 impaired the ability of TEL to repress. In contrast, substitution of the oligomerization domain of TEL by unrelated oligomerization domains resulted in an active repressor, showing that the ability of TEL to repress depends on its ability to self-associate. The study of the properties of TEL fusions to the heterologous DNA binding domain of Gal4 identified two autonomous repression domains in TEL, distinct from its oligomerization domain, that are essential to the ability of TEL to repress ETS-binding site-containing promoters. These results have implications for the normal function of TEL, its relation to other ETS proteins, and its role in leukemogenesis.

Genes of the ETS family encode transcriptional regulators that are essential for a variety of developmental processes and for the response of cells to extracellular stimuli (for review see Ref. 1).

Specific ETS genes are frequently rearranged in human solid tumors and leukemias as the result of chromosomal translocations. TEL*(ETV6) is an ETS family member that was originally identified by virtue of its fusion to the 3’-half of the gene encoding the platelet-derived growth factor β receptor in chronic myelomonocytic leukemia harboring a t(5;12)(q33;p13) chromosomal translocation (2). Other translocations in either leukemia or sarcoma also result in the fusion of TEL either to genes encoding other protein tyrosine kinases, including c-ABL (3, 4), JAK2 (5, 6), and TRKC (7) or to genes encoding known or alleged transcriptional regulators (8–11).

TEL is widely expressed throughout mouse embryonic development and in most human and mouse tissues and cell lines (12, 13). It is essential to mouse development as its inactivation by homologous recombination results in early lethality. Embryos show defects in yolk sac angiogenesis and in the survival of select mesenchymal and neural cells (13). TEL shares with other ETS proteins an evolutionarily conserved domain (ETS domain) that is responsible for its ability to bind consensus ETS-binding site (EBS) DNA elements (12). It also shares with a subset of other ETS proteins a conserved amino-terminal domain that is referred to as the B domain, the pointed domain, or the helix-loop-helix domain (2, 14). The recent elucidation of the structure of the B/pointed domain of ETS1 by NMR shows that this domain identifies a novel fold, unrelated to the helix-loop-helix motif (15). Although its precise function is unknown, the B/pointed domain of several ETS proteins appears to modulate their transcriptional activation properties, presumably via specific protein-protein interactions (for review, see Ref. 1).

The B domain of TEL has the unique property of inducing its stable homotypic oligomerization as well as that of TEL-derived fusion proteins (4, 14, 16). The ability of this domain to induce protein self-association results in the constitutive activation of the tyrosine kinase activity of TEL-ABL, TEL-platelet-derived growth factor β receptor, and TEL-JAK2, a property that is essential to their transforming and leukemogenic properties (4, 5, 14, 16).

Except for its ability to interfere with the activity of the FLI-1 oncprotein (17), the transcriptional regulatory properties of TEL are unknown. We show here that TEL is a potent sequence-specific transcriptional repressor of both model and natural EBS-containing promoters/enhancers. TEL repressive activity is shown to depend upon both its ability to oligomerize through the B domain and the presence of distinct autonomous repression domains.

**EXPERIMENTAL PROCEDURES**

Construction of TEL Mutants—The SV40-based expression plasmid encoding an HA epitope-tagged TEL (ΔEB-HATEL) and the ΔEB-TEL-M43 plasmid have been described elsewhere (12). Mutant TEL-M1 was generated as follows: the ATG encoding methionine 43 was changed for an alanine codon by PCR mutagenesis (18). The amplimers used were: 5’ CCGCTCGAGCGCTAGGCGAGGGAAAGACTTCGATCCG 3’ (5’ amplimer) and 5’ CATGCCATGGGAGAAGCTGAGAGG 3’ (3’ amplimer). The mutated insert was subcloned into EcoRI + HindIII-restricted ΔEB-HA (19).

TEL substitution and deletion mutants were generated from a modified TEL cDNA (TELmod) in which the nucleotide sequence encoding the B domain was bordered by BamHI and Bg/II restriction enzyme sites and that encoding the ETS domain by XhoI and SalI restriction enzyme sites. Specifically, the EcoRI- and HindIII-bordered TEL cDNA...
directed mutagenesis was carried out using the sculptor kit (Amersham Pharmacia Biotech). We used the mutagenic primers M2 to create a BgII restriction enzyme site centered at position 382 of TEL cDNA and the mutagenic primer M3 to create a BamHI restriction enzyme site centered at position 313. The position of M2 and M3 primers has been described previously (14). These mutations resulted in a His to Gin substitution at codon 119 and in Ala to Gly and His to Ile substitutions at codons 52 and 53, respectively. The mutagenic primers M4 and M5 were used similarly to create an XbaI restriction enzyme site centered at position 336 and a SpeI restriction enzyme site centered at position 431 of TEL, respectively. The first two mutations resulted in a Thr to Val substitution at codon 431. The mutagenic primers M4 and M5 were then subcloned into pG4MpolyII. The amplicers used for the PCR amplifications were 5′ GGAATCTGGTGCTAGAGCAGAGAGG (3′ primer) and 5′ GGAATCTGGTGCTAGAGCAGAGAGG (5′ primer) for pGal4-TEL (120–421), and 5′ GGAATCTGGTGCTAGAGCAGAGAGG (3′ primer) and 5′ GGAATCTGGTGCTAGAGCAGAGAGG (5′ primer) for pGal4-TEL (335–452). They were then digested with EcoRI and HindIII and subcloned into EcoRI/HindIII-restricted pGEX4T-1 (Amersham Pharmacia Biotech). The sequence in the −5′ upstream region of human TEL DNA was used to generate TEL-C. A BglII/XbaI adapter was first inserted into BglII/XbaI-restricted p65-TELmod. The PCR amplifiers used for PCR amplification were completely sequenced to ensure the presence of the expected modifications and the absence of unwanted mutations.

**Transient Transfection Assays**—HeLa cells were transfected by the calcium phosphate co-precipitation method as described previously (19). The transfection mixture included 1 µg of the indicated reporter gene constructs, the indicated amounts of expression plasmid, and 50 ng of pEF-BosLacZ to normalize for transfection efficiency. The total amount of expression plasmid was kept constant to 1 µg by addition of empty ΔEB vector, and the total amount of DNA was kept constant (10 µg) by addition of carrier plasmid DNA. Cell lysates were prepared 48 h after transfection and assayed for luciferase activity using the luciferase assay system kit (Promega). The results shown represent the average luciferase activity and standard deviation from at least three independent experiments. β-Galactosidase activity was assayed using the Galacto-Star kit (Tropix).

**Preparation of a TEL-specific Antiserum**—A rabbit antiserum specific to the amino terminus of human TEL (serum 71) was obtained by injection of a glutathione S-transferase protein fused to amino acid residues 1–52 of TEL. The corresponding DNA fragment was obtained by PCR amplification using a 5′ primer and a 3′ primer, a BamHI restriction enzyme site centered at position 313 of TEL, and an XbaI restriction enzyme site centered at position 431 of TEL, respectively.

**Immunoprecipitation Analyses**—Transfected cells were processed for immunoprecipitation with a TEL specific antibody. The transfection mixture included 1 µg of the indicated reporter gene constructs, the indicated amounts of expression plasmid, and 50 ng of pEF-BosLacZ to normalize for transfection efficiency. The total amount of expression plasmid was kept constant to 1 µg by addition of empty ΔEB vector, and the total amount of DNA was kept constant (10 µg) by addition of carrier plasmid DNA. Cell lysates were prepared 48 h after transfection and assayed for luciferase activity using the luciferase assay system kit (Promega). The results shown represent the average luciferase activity and standard deviation from at least three independent experiments. β-Galactosidase activity was assayed using the Galacto-Star kit (Tropix).

**RESULTS**

**TEL Represses ETS-Binding Site (EBS)-directed Transcription**—In a previous study, we showed TEL to be a sequence-specific DNA-binding protein that recognizes conventional EBS such as the E74 oligonucleotide (12). To investigate the transcriptional regulatory properties of TEL, we therefore analyzed its activity on E74, tk80Luc. This reporter plasmid contains the luciferase gene driven by an enhancer/promoter cassette composed of three tandem copies of the E74 EBS, immediately 5′ of the herpes simplex virus thymidine kinase (−80 to +52) promoter (20). As shown in Fig. 1A, this pGal4-TEL-(335–452) reporter drives high levels of luciferase activity in HeLa as compared with the control tk80Luc reporter, reflecting the enhancer activity of the E74 EBS in these cells. Co-transfection of E74, tk80Luc along with an expression plasmid encoding TEL resulted in a dose-dependent inhibition of luciferase expression (Fig. 1A). This trans-repressing activity was dependent upon the presence of
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Luciferase activity (relative light units) was evaluated in cell extracts and normalized relative to the vector. To investigate the importance of TEL self-association to its repressive properties, we generated mutant properties (14, 15). To assess the in vivo repressive properties of these mutants, we used the E74-binding sites since TEL only marginally affected the activity of the tk60Luc reporter (Fig. 1A). A reporter in which a palindromic EBS is inserted upstream of a minimal (−56 to +119) c-fos promoter/chloramphenicol acetyltransferase cassette (21) was also repressed by TEL. In contrast, TEL did not affect the activity of the same reporter carrying mutated EBS (data not shown).

TEL is expressed in a variety of cell types as two protein isoforms corresponding to translation initiation of TEL mRNA at two successive ATG codons at positions 1 (TEL-M1) and 43 (TEL-M43), respectively (12). Expression plasmids encoding either TEL-M1 or TEL-M43 were constructed and compared for their transcriptional regulatory properties. Both proteins were expressed at similar levels (data not shown) and inhibited the activity of E74tk80Luc in a dose-dependent manner (Fig. 1B). We conclude from these results that both TEL isoforms are sequence-specific transcriptional repressors of EBS-directed transcription.

Repression of EBS-directed Transcription Depends upon TEL Self-association—TEL is unusual among other vertebrate ETS proteins in that it forms stable homotypic oligomers in vivo (14). This self-oligomerization property maps to a 65-amino acid domain (B domain) that is evolutionarily conserved in a subset of ETS proteins. Despite this conservation, the B domain of other ETS proteins is not endowed with self-association properties (14, 15). To investigate the importance of TEL self-association to its repressive properties, we generated mutant TEL proteins in which the B domain is either deleted (TEL-ΔB) or swapped for the homologous domain of ETS1 (TEL-ΔBsw-(66–130)-ETS1; see Fig. 2 for a schematic of the constructs). To assess the in vivo self-associating properties of these mutants, we made use of the fact that TEL-M1 and TEL-M43 are able to form M1/M43 oligomers (Fig. 3A). To establish this point, we first generated an antisera specific to the 52 amino-terminal residues of TEL (antisera 71, see "Experimental Procedures"). To demonstrate the specificity of this antisera, HeLa cells were transfected with expression plasmids encoding either TEL-M1 or TEL-M43. Cells were metabolically labeled with [35S]methionine/[35S]cysteine, and cell lysates were analyzed by immunoprecipitation. As expected, both proteins were immunoprecipitated by antisera 68 specific to the carboxyl-terminal half of TEL (Fig. 3A, lanes 3 and 5), whereas only TEL-M1 but not TEL-M43 was immunoprecipitated by antisera 71 (Fig. 3A, compare lanes 4 and 6). However, when

FIG. 1. TEL is a repressor of EBS-driven transcription. A, HeLa cells were transfected with 1 μg of the E74tk80Luc reporter plasmid (right panel) or with the control tk80Luc reporter (left panel) along with 25, 50, 100, 250, or 500 ng of expression vector for TEL or the empty vector. Luciferase activity (relative light units) was evaluated in cell extracts and normalized relative to the β-galactosidase activity encoded by a co-transfected LacZ expression plasmid. The inset represents the same data expressed as fold repression relative to the empty expression vector. In this representation, a 10-fold repression corresponds to 90% inhibition of promoter activity by TEL as compared with the control expression vector. B, HeLa cells were transfected as above with E74tk80Luc along with 100, 250, or 500 ng of expression vector for TEL, TEL-M1, or TEL-M43. The results are presented as the fold repression relative to the empty expression plasmid. In this representation, a 10-fold repression corresponds to 90% inhibition of promoter activity by TEL as compared with the control expression vector.

The results of Fig. 3C show that both TEL-ΔB and TEL-ΔBsw-(66–130)-ETS1 were severely impaired in their ability to repress the activity of E74tk80Luc, indicating that the repressive activity of TEL requires the integrity of its oligomerization domain. This difference in activity is not due to differences in the level of protein expression or nuclear localization of the mutant proteins as compared with wild type. It is also not explained by a defect in DNA binding as TEL-ΔB was found to bind efficiently an E74 oligonucleotide probe in electrophoretic mobility shift assays (data not shown).
Transcriptional Repression by TEL Requires Specific Domains—Transcriptional repression may result either from passive competition with endogenous activators for DNA binding or from active mechanisms involving protein-protein interactions (for review see Ref. 23). To distinguish between these alternatives, we studied the properties of additional deletion and substitution TEL mutants (see Fig. 2). All mutants were found to be expressed at similar levels as wild type TEL and to accumulate in the nucleus of transfected cells (data not shown).

Deletion of the 181 amino acid domain encoded by TEL exons 5 (TEL-ΔC) or its substitution by the topologically equivalent domain of ETS1 (TEL-ΔCsw-(131–331)-ETS1) abolished the ability of TEL to trans-repress E74tk80Luc (Fig. 4). In contrast, deletion of the 22 carboxyl-terminal residues of TEL (TEL-ΔE) enhanced repression (Fig. 4). To analyze whether the ETS domain of TEL is specifically required for TEL to repress EBS-driven transcription, we replaced the ETS domain of TEL by that of ETS1 (TEL-ΔEsw-(331–426)-ETS1). This mutant is an efficient repressor of E74tk80Luc (Fig. 4), indicating that the ETS domain of TEL is not specifically required for its ability to repress transcription.

To establish further the importance of self-oligomerization in the ability of TEL to repress transcription, we substituted the B domain of TEL by the unrelated coiled-coil oligomerization domain of Epstein-Barr virus encoded EB1/Zebra (22) to generate TEL-ΔBsw-(193–244)-EB1 (Fig. 2). The resulting chimera was found to self-associate as assessed by co-precipitation analysis (Fig. 3B, lane 8) and to trans-repress E74tk80Luc (Fig. 3C). These data show that the ability of TEL to self-oligomerize is essential to its repressing activity.

FIG. 3. Oligomerization and transcriptional properties of TEL deletion and substitution mutants. A, characterization of an antiserum specific to TEL-M1. HeLa cells were transfected with the control expression vector (lanes 1 and 2); with expression plasmids encoding TEL-M1 (lanes 3 and 4) or TEL-M43 (lanes 5 and 6); with both the TEL-M1 and TEL-M43 expression vectors (lanes 7 and 8). Cells were labeled with [35S]methionine and [35S]cysteine and lysed. Immunoprecipitation was carried out on 107 acid-insoluble counts of each lysate with either antibody 68 which is directed to the carboxyl-terminal half of TEL, a region shared by these proteins (odd-numbered lanes), or with antibody 71, specific for the amino terminus of TEL-M1 (even-numbered lanes), and analyzed by polyacrylamide gel electrophoresis. TEL-M1 is indicated by a filled arrowhead and TEL-M43 by an open arrowhead. Note that TEL-M43 is only found in the antiserum 71 immunoprecipitates only when it is co-expressed with TEL-M1. B, self-association of TEL mutants. HeLa cells were transfected with expression plasmids for TEL, TEL-ΔB, TEL-ΔBsw-(66–130)-ETS1, or TEL-ΔBsw-(193–244)-EB1, metabolically labeled with [35S]methionine and [35S]cysteine, lysed, and subjected to immunoprecipitation analysis. Self-association was assessed as described in A by the ability of the M43 isoform of each mutant (indicated by open arrowheads) to co-precipitate with its respective M1 isoform, using antibody 71 (even numbered lanes). Immune precipitation with antibody 68 is used as expression control (odd-numbered lanes). C, HeLa cells were transfected with E74tk80Luc along with 25, 50, 100, 250, or 500 ng of expression vector for the indicated proteins and luciferase activity evaluated in cell extracts. The results are presented as fold repression relative to the empty expression plasmid. In this representation, a 10-fold repression corresponds to 90% inhibition of promoter activity by TEL and TEL mutants as compared with the control expression vector.

FIG. 4. Study of the activity of TEL deletion and substitution mutants. HeLa cells were transfected with 1 μg of E74tk80Luc along with 50, 100, or 250 ng of expression vector for the indicated proteins or the empty expression vector. Luciferase activity was measured as in Fig. 1A. The results are represented as the fold repression relative to the empty expression vector. In this representation, a 10-fold repression corresponds to 90% inhibition of promoter activity by TEL and TEL mutants as compared with the control expression vector.
of TEL is not sufficient for repression. The ETS domain was, however, found to be necessary for full repression since Gal4-TEL-(119–334) was significantly impaired in its repressive activity as compared with wild type (Fig. 6B). In contrast, deletion of the 30 carboxyl-terminal residues had no effect on repression (Gal4-TEL-(120–421)). Analysis of progressive amino-terminal deletions showed that deletion of TEL residues 119–170 in Gal4-TEL-(171–421) did not affect repression, whereas further deletion of 43 residues impaired repression (Gal4-TEL-(215–421)) (Fig. 6B). Deletion of an additional 69 residues had no major effect since Gal4-TEL-(284–421) showed an activity similar to that of Gal4-TEL-(215–421) (Fig. 6B). We conclude from these experiments that the intrinsic repressive properties of TEL depend upon two domains as follows: the first includes residues 171–215 of the central exon-5 encoded region, whereas the second encompasses the ETS domain and the last 55 residues of the central region.

DISCUSSION

This study shows that TEL encodes a sequence-specific transcriptional repressor. TEL-repressive activity depends upon two autonomous transcriptional repression domains. The first maps to a region rich in proline residues (22% proline between amino acid residues 171 and 285). High content in proline is a feature found in other transcriptional repression domains (23). The second encompasses the 55 carboxyl-terminal residues of the exon 5-derived region and the adjacent ETS domain. Recent studies have shown that, in addition to its role in nuclear localization and specific DNA binding, the ETS domain also mediates protein-protein interactions with unrelated factors either on its own or in combination with an adjacent domain (25–27). Some of these interactions are rather promiscuous with several ETS domains being able to interact with the same partner, whereas others are highly specific. The ETS domain of ETS1 can replace that of TEL, suggesting that the exon 5-encoded moiety of this repression domain is essential to its specificity. The analysis of deletion and substitution mutants in the oligomerization/B domain show that in order to repress EBS-driven transcription, TEL needs to assemble into oligomers. This suggests that self-association is likely to release TEL...
repression domains from inhibitory constraints to activate their interaction with either transcriptional co-repressors or with components of the RNA polymerase II initiation complex. Whether the oligomerization/B domain of TEL is also associated with intrinsic repressive properties could not be addressed directly since its fusion to the DNA binding domains of either Gal4 or LexA resulted in insoluble proteins. However, the fact that the unrelated oligomerization domain of Zebra and Gal4 can replace the oligomerization domain of TEL to generate an active repressor does not support this notion. Like Gal4-DBD, the Zebra coiled-coil domain is not a repression domain as evidenced by its inability to regulate LexA operator-driven transcription when fused to the LexA DNA binding domain. We therefore favor a model in which the main contribution of the B domain of TEL to EBS-mediated repression is to induce protein self-association.

The oligomerization domain of TEL shares significant homology to the SPM domain found in a subset of the Polycomb group of transcriptional repressors and their vertebrate homologs (28). The SPM domain is important to both homotypic and heterotypic interactions between these proteins and the assembly of multiprotein complexes (29). Two lines of evidence suggest that TEL is unlikely to be a component of Polycomb group complexes. First, we failed to detect heteromer formation between TEL and Rae28, the mouse homolog of Drosophila Polyhomeotic. Second, immunofluorescence analyses show that TEL does not co-localize with the large nuclear domains formed by Polycomb group proteins in mammalian cells.

In the ETS family, TEL is most closely related to Drosophila YAN in both the ETS and B domains. YAN was genetically identified as an inhibitor of the Sevenless signaling pathway in R7 photoreceptor cell development (30) and more generally in establishing proper regulation of several developmental decisions (31–33). YAN is also a repressor of EBS-driven transcription that can compete for DNA binding with transcriptional activators of the ETS family like PntP2 or interfere with the activity of unrelated factors like D-Jun (34, 35). Whether the repressive activity of YAN also requires its B domain and whether it depends upon an intrinsic repression domain is unknown. The B domain of YAN is also endowed with self-oligomerization properties, although the strength of the interaction is weaker than that of TEL. This suggests that YAN-repressive function may also require self-association. YAN function is negatively controlled by extracellular signal-regulated kinase in cell fate specification in the eye and by c-Jun NH2-terminal kinase in dorsal closure, a property that appears to result from its direct phosphorylation by these kinases on several serine and threonine residues (31, 32). TEL phosphorylation is also induced following activation of the extracellular signal-regulated kinase pathway in mammalian cells. TEL therefore appears to belong to the small class of ETS transcriptional repressors including YAN, ERF (36), and NET (37) whose activity is controlled by mitogenic and/or cell cycle-dependent signals.

Although frequently altered in human leukemia, TEL is not essential for the differentiation of mouse hematopoietic progenitors in vitro and fetal liver hematopoiesis in vivo (13). However, TEL appears to be required for hematopoietic stem cells and/or committed progenitors of all lineages to stably colonize bone marrow (38). This suggests that TEL could act in concert either with specific activators of the ETS family or with unrelated activators to control the response of hematopoietic stem and progenitor cells to stroma-derived signals. Such a dual control could ensure that transient stroma-controlled intracellular signals result in important changes in the expression of genes involved in either migration, homing, proliferation, and/or differentiation of these cells.

The most frequent chromosomal translocation involving TEL in leukemia is the t(12;21)(p13;q22) which is found in about 25% of the cases of childhood pre-B acute lymphoblastic leukemia. The molecular consequence of this translocation is the expression of a TEL-AML1 chimeric protein in which the 336 amino-terminal residues of TEL are fused to most of AML1B, a Runt family protein (8, 9). Depending on the promoter context, AML1B is either an activator or a repressor of transcription (for review see Ref. 39). Previous studies have shown that TEL-AML1 is a repressor of model promoters normally activated by AML1B in transient transfection assays, suggesting that its leukemogenic properties may result from repression of genes normally activated by AML1B. One of the repression domains of TEL identified in our study is retained in TEL-AML1. If this domain turns out to be active in TEL-AML1 to repress physiologically important genes, leukemogenesis by TEL-AML1 could also involve the abnormal regulation of genes normally repressed by AML1 through the use of TEL-specific repressive mechanisms.

A frequent feature of TEL-AML1-associated leukemia is the loss of the non-rearranged TEL allele, a property that appears to be associated with disease progression (8, 9, 42). As TEL and TEL-AML1 are able to form hetero-oligomers in vitro through
their B domain (43), it is possible that expression of TEL in t(12;21) leukemic cells interferes with the activity of TEL-AML1. However, TEL appears unable to override the repressive activity of TEL-AML1 in transient assays (40). Alternatively, loss of TEL function could activate a pathway that cooperates with TEL-AML1 in leukemogenesis. Our study shows that TEL is a repressor of the FLI-1 promoter, suggesting that loss of TEL could lead to the deregulated expression of FLI-1 in t(12;21) leukemic cells. Activation of FLI-1 expression is observed in >75% of Friend murine leukemia virus-induced mouse erythroleukemia, and enforced expression of FLI-1 is sufficient to inhibit Epo-induced differentiation and to induce proliferation of primary erythroblasts (44). In addition, gain of function mutations of FLI-1 or of the closely related ERG protein as the result of specific chromosomal translocations is a frequent event in human cancer (for review see Ref. 1). If TEL indeed controls the expression of FLI-1 in TEL-AML1 leukemic cells, disruption of a FLI-1 pathway could have a more general role in leukemia than previously anticipated.

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