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TEL Is a Sequence-specific Transcriptional Repressor*

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Rodolphe G. Lopez‡§, Clémence Carron‡§, Cécile Oury‡¶, Paola Gardellin‡‖, Olivier Bernard, and Jacques Ghysdael‡ ‡‡**

*From the ‡CNRS UMR146, Institut Curie, Centre Universitaire, 91405 Orsay and **INSERM U 434, CEPH, 75010 Paris, France*

***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*[1](*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 oncoprotein (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′ CCGCTCGAGCGCTCAGGGCGGAGGAAGACTCGATCCG 3′ (5′ amplimer) and 5′ CATGCCATGGGAGACACTGACAGAGG 3′ (3′ amplimer). The mutagenized insert was subcloned into *Eco*RI + *Hin*dIII-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 *Bam*HI and *Bgl*II restriction enzyme sites and that encoding the ETS domain by *Xba*I and *Sal*I restriction enzyme sites. Specifically, the *Eco*RI- and *Hin*dIII-bordered TEL cDNA

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‡‡ To whom correspondence should be addressed: CNRS UMR146-Institut Curie, Section de Recherche, Centre Universitaire, Bâtiment 110, 91405 Orsay, France. Tel.: 33 1 69 86 31 52; Fax: 33 1 69 07 45 25; E-mail: Jacques.Ghysdael@curie.u-psud.fr.

[1] The abbreviations used are: TEL, translocated ETS leukemia; EBS, ETS-binding site; Luc, firefly luciferase; tk, thymidine kinase; PCR, polymerase chain reaction; HA, hemagglutinin; DBD, DNA binding domain.

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was inserted into *Eco*RI- and *Hin*dIII-restricted M13mp18. Site-directed mutagenesis was carried out using the sculptor *in vitro* mutagenesis kit (Amersham Pharmacia Biotech). We used the mutagenic primer M2 to create a *Bgl*II restriction enzyme site centered at position 382 of TEL cDNA and the mutagenic primer M3 to create a *Bam*HI restriction enzyme site centered at position 179. The M2 and M3 primers have been described previously (14). These mutations resulted in a His to Gln 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 *Xba*I restriction enzyme site centered at position 336 and a *Sal*I restriction enzyme site centered at position 431 of TEL, respectively. The first mutation resulted in Ile to Leu and an Ala to Leu substitution at codons 335 and 336, respectively. The second mutation resulted in a Thr to Val substitution at codon 431. The sequences of the primers were 5′ GCAGTCTACAGTCTAGAAGCCTCCCAATGGG 3′ (M4) and 5′ GCTCCAGACGGTCGACTCGGCCACTCATG 3′ (M5). The mutagenized *Eco*RI- and *Hin*dIII-bordered fragment was inserted either into *Eco*RI + *Hin*dIII-restricted psp65 or ΔEB-HA to generate psp65-TELmod or ΔEB-TELmod.

To generate TEL-ΔDsw-(331–426)-ETS1, an *Xba*I- and *Sal*I-bordered fragment obtained by PCR amplification of the ETS domain of chicken ETS-1 cDNA was subcloned into *Xba*I + *Sal*I-restricted psp65-TELmod. The amplimers were 5′ GCTCTAGACGGCAGTGGACCCATCCAAC 3′ (5′ amplimer) and 5′ ACGCGTCGACTGGTGTGTAGCCCAGCAGG 3′ (3′ amplimer). The entire mutagenized TEL sequence was next retrieved by *Eco*RI and *Hin*dIII digestion and cloned into *Eco*RI- and *Hin*dIII-restricted ΔEB-HA. To generate TEL-ΔBsw-(66–130)-ETS1, the *Bam*HI to *Bgl*II fragment of psp65-TEL-ΔDsw-(331–426)-ETS1 was replaced by the corresponding region (amino acids residues 66–130) of the chicken ETS1 cDNA. This was done by PCR amplification of the ETS1 cDNA using 5′ CAGGATCCTCCCCAAAGATCCCCAGCAGTG 3′ as 5′ amplimer and 5′ GGAGATCTTCTCCAGGTGTTCCCAAAGGATATC 3′ as 3′ amplimer and subcloning of the amplified fragment into *Bam*HI- and *Bgl*II-restricted psp65-TEL-ΔDsw-(331–426). Similarly, to generate TEL-ΔBsw-(193–244)-EB1, the *Bam*HI to *Bgl*II fragment of psp65-TEL-ΔDsw-(331–426)-ETS1 was replaced by the region (amino acids residues 193–244) encoding the coiled-coil region of the Epstein-Barr virus EB1/Zebra cDNA. This was done by PCR amplification of the EB1 cDNA (a generous gift of Dr. M. Castellazi, ENS, Lyon, France) using 5′ GAAGATCTTGTTTAAGCAACTGCTGCAGCACTAC 3′ as 5′ amplimer and 5′ GAAGATCTGGAAATTTAAGAGATCCTCGTG 3′ as 3′ amplimer. The corresponding ΔEB derivatives were obtained by subcloning of the respective *Eco*RI to *Bgl*II fragments into *Eco*RI/*Bgl*II-restricted ΔEB-TELmod. Finally, the *Sac*I-bordered fragments of these B domain substitution mutants were exchanged with the corresponding *Sac*I-bordered fragment of ΔEB-HA-TEL. To generate TEL-ΔB, psp65-TELmodΔB was first generated by substituting the *Eco*RI to *Bgl*II fragment of psp65-TELmod with the *Eco*RI to *Bam*HI fragment of psp65-TELmod. The entire mutagenized TEL sequence was next subcloned into *Eco*RI- and *Hin*dIII-restricted ΔEB-HA. Finally, the *Sac*I-bordered fragment of ΔEB-TELmodΔB was exchanged with the corresponding fragment of ΔEB-HATEL. To generate TEL-ΔCsw-(131–331)-ETS1, a *Bgl*II- and *Xba*I-bordered insert was obtained by PCR amplification of the chicken ETS-1 cDNA and subcloned into *Bgl*II/*Xba*I-restricted psp65-TELmod. The amplimers used for PCR amplification were 5′ GAAGATCTTGCAGAAAGAAGAGGCAAAACC 3′ (5′ amplimer) and 5′ GCTCTAGACCTGTGTAGCCGGCGAG 3′ (3′ amplimer). The mutagenized TEL cDNA was next retrieved by digestion with *Eco*RI and *Hin*dIII and subcloned into *Eco*RI/*Hin*dIII-restricted ΔEB-HA. To generate TEL-ΔC, a *Bgl*II/*Xba*I adapter was first inserted into *Bgl*II/*Xba*I-restricted psp65-TELmod. The entire insert was PCR-amplified and bordered with *Hin*dIII and *Kpn*I restriction enzyme sites, using 5′ CCCAAGCTTGAGACATGTCTGAGACTCCTGCTCAG 3′ as 5′ amplimer and 5′ GGGGTACCTCAGCATTCATCTTCTTGG 3′ as 3′ amplimer. This fragment was next digested with *Hin*dIII + *Kpn*I and inserted into *Hin*dIII- and *Kpn*I-restricted pG4MpolyII (19) to generate ΔEB-TEL-ΔC. To obtain ΔEB-TEL-ΔE, the *Eco*RI to *Sal*I fragment of psp65-TELmod was subcloned into *Eco*RI- and *Xho*I-restricted pG4MpolyII (19).

pGal4-TEL-(120–452) was obtained by insertion of a *Xho*I- and *Kpn*I-bordered insert obtained by PCR amplification of the human TEL cDNA into *Xho*I- and *Kpn*I-restricted pG4MpolyII. The amplimers used were 5′ CCGCTCGAGTGATTCTGAAGCAGAGGAAACCTCGG 3′ (5′ amplimer) and 5′ GGGGTACCTCAGCATTCATCTTCTTGG 3′ (3′ amplimer). pGal4-TEL-(120–421) and pGal-TEL-(335–452) were obtained by insertion of the respective *Bgl*II-bordered inserts obtained by PCR amplification of the appropriate region of human TEL cDNA into *Bam*HI-restricted and dephosphorylated pG4MpolyII. The amplimers used were 5′ GGAGATCTGATTCTGAAGCAGAGG 3′ (5′ amplimer) and 5′ GGAAGATCTGTTTTCATAAACCTGAACAAAAGCC 3′ (3′ amplimer) for pGal4-TEL-(120–421), and 5′ GGAAGATCTGATAGCAGACTGTAGACTGC 3′ (5′ amplimer) and 5′ GGAAGATCTGCA TTCATCTTCTTGG 3′ (3′ amplimer) for pGal4-TEL-(335–452).

pGal4-TEL-(119–334) and pGal4-TEL-(422–452) were obtained by insertion of *Bgl*II/*Bam*HI-bordered insert obtained by PCR amplification of the appropriate regions of TEL cDNA into *Bam*HI-restricted and dephosphorylated pG4MpolyII. The amplimers used for the PCR amplifications were 5′ GGAGATCTGATTCTGAAGCAGAGG 3′ (5′ amplimer) and 5′ GGGGATCCTCTCCCAATGGGCATGG 3′ (3′ amplimer) for pGal4-TEL-(119–334), and 5′ GGAGATCTGACCCCAGATGAAATCATGAGTGGC 3′ (5′ amplimer) and 5′ GGGGAT CCGCATTCATCTTCTTGG 3′ (3′ amplimer) for pGal4-TEL-(422–452).

pGal4-TEL-(171–421), pGal4-TEL-(215–421), and pGal4-TEL-(284–421) were obtained by insertion of the respective *Bgl*II-bordered inserts obtained by PCR amplification of the appropriate region of human TEL cDNA into *Bam*HI-restricted and dephosphorylated pG4MpolyII. The 3′ amplimer used was 5′ GGAGATCTGATTCTGAAGCAGAGG 3′, and the 5′amplimers were 5′ GAAGATCTCCATAACCCTCCCACCATTGAAC 3′ for pGal4-TEL-(171–421), 5′ GAAGATCTGGCTGAGAGAGCTCAGGAACCC 3′ for pGal4-TEL-(215–421), and 5′ GAAGATCTCTCCGTGGATTTCAAACAGTCC3′ for pGal4-TEL-(284–421).

All mutated TEL cDNA and all the fragments generated by PCR amplification were completely sequenced to ensure for 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 cDNA fragment was obtained by PCR amplification using a 5′ amplimer bordered by a *Bam*HI restriction enzyme site and a 3′ amplimer bordered by a *Bgl*II restriction enzyme site. After *Bam*HI and *Bgl*II restriction enzyme site digestion, the amplified product was subcloned into *Bam*HI-restricted pGEX4T-1 (Amersham Pharmacia Biotech). The sequence of the 5′ and 3′ amplimers were 5′ CCGGATCCATGTCTGAGACTCCTGCTCAGT GTAGC 3′ and 5′ GGAGATCTCGCAGGCAGGCGGATCGAGTCTTCC 3′, respectively.

Immunoprecipitation Analyses—Transfected cells were processed for metabolic labeling and lysates subjected to immunoprecipitation as described previously (14), using an excess of either rabbit antiserum 68, raised against the carboxyl-terminal part of TEL (12), or serum 71. Immunoprecipitates were analyzed by SDS-PAGE followed by fluorography.

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 $E74_3$tk80Luc. This reporter plasmid contains the luciferase gene driven by an enhancer/promoter cassette composed of three tandem copies of the E74 EBS, inserted 5′ of the herpes simplex virus thymidine kinase (−80 to +52) promoter (20). As shown in Fig. 1*A*, this reporter plasmid 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 $E74_3$tk80Luc along with an expression plasmid encoding TEL resulted in a dose-dependent inhibition of luciferase expression (Fig. 1*A*). This trans-repressing activity was dependent upon the presence of

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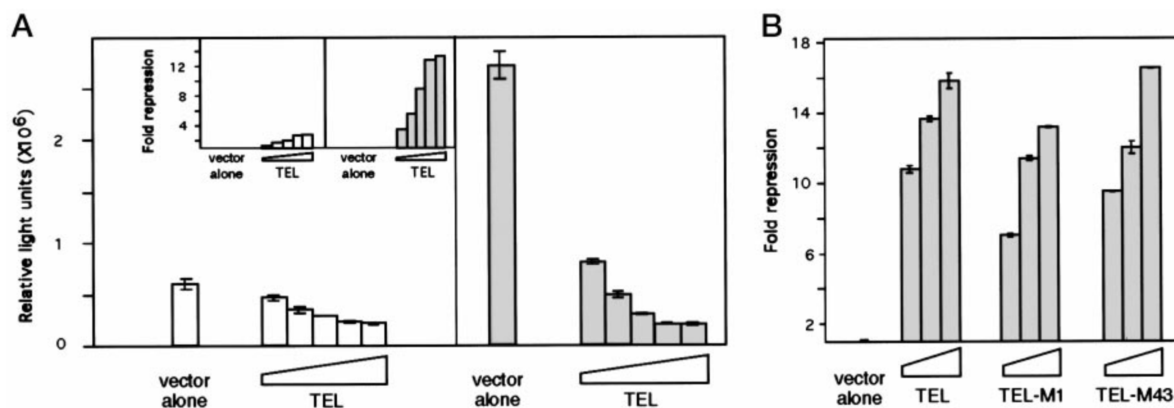

FIG. 1. **TEL is a repressor of EBS-driven transcription.** *A,* HeLa cells were transfected with 1 μg of the $E74_3tk_{80}Luc$ reporter plasmid (*right panel*) or with the control $tk_{80}Luc$ reporter (*left panel*) along with 25, 50, 100, 250, or 500 ng of expression vector for TEL or the empty vector. 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 $E74_3tk_{80}Luc$ 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 E74-binding sites since TEL only marginally affected the activity of the tk80Luc reporter (Fig. 1*A*). 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 $E74_3$tk80Luc in a dose-dependent manner (Fig. 1*B*). 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. 3*A*). To establish this point, we first generated an antiserum specific to the 52 amino-terminal residues of TEL (antiserum 71, see "Experimental Procedures"). To demonstrate the specificity of this antiserum, HeLa cells were transfected with expression plasmids encoding either TEL-M1 or TEL-M43. Cells were metabolically labeled with L-[^{35}S]methionine/L-[^{35}S]cysteine, and cell lysates were analyzed by immunoprecipitation. As expected, both proteins were immunoprecipitated by antiserum 68 specific to the carboxyl-terminal half of TEL (Fig. 3*A*, *lanes 3* and *5*), whereas only TEL-M1 but not TEL-M43 was immunoprecipitated by antiserum 71 (Fig. 3*A*, compare *lanes 4* and *6*). However, when

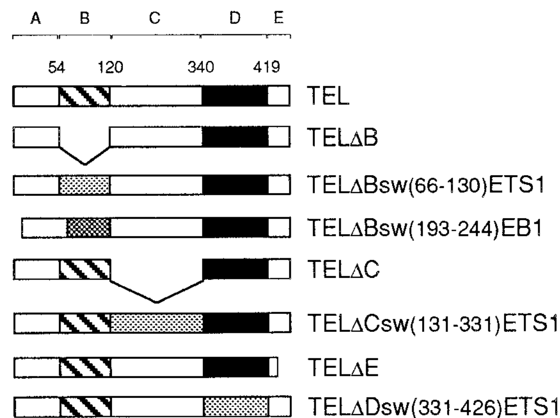

FIG. 2. **Schematic representation of TEL mutants.** The schematic structure of wild type TEL is depicted on the *top*. The ETS domain is indicated as a *black box,* and the oligomerization/B domain as a *hatched box*. Deletion mutants in specific region (*A-E*) are referred to as Δ mutants; for substitution mutants the amino acid borders of the swapped domains derived from ETS1 or EB1/Zebra are indicated. Swapped domains are depicted in *gray*.

TEL-M1 and TEL-M43 were co-expressed, either of these antibodies was found to precipitate both proteins, demonstrating their association as mixed M1/M43 oligomers (Fig. 3*A*, *lanes 7* and *8*). Similarly, the M1 and M43 forms normally expressed from the wild type TEL mRNA were found to associate as evidenced by their co-precipitation using antiserum 71 (Fig. 3*B*, *lane 4*). In contrast, the M43 forms of neither TEL-ΔB (Fig. 3*B*, *lane 6*) nor TEL-ΔBsw-(66–130)-ETS1 (Fig. 3*B*, *lane 2*) were found to associate with their respective M1 forms. This demonstrates that deletion of the B domain of TEL or its substitution by the corresponding domain of ETS1 impaired the ability of TEL to self-associate *in vivo*.

The results of Fig. 3*C* show that both TEL-ΔB and TEL-ΔBsw-(66–130)-ETS1 were severely impaired in their ability to repress the activity of $E74_3$tk80Luc, 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).

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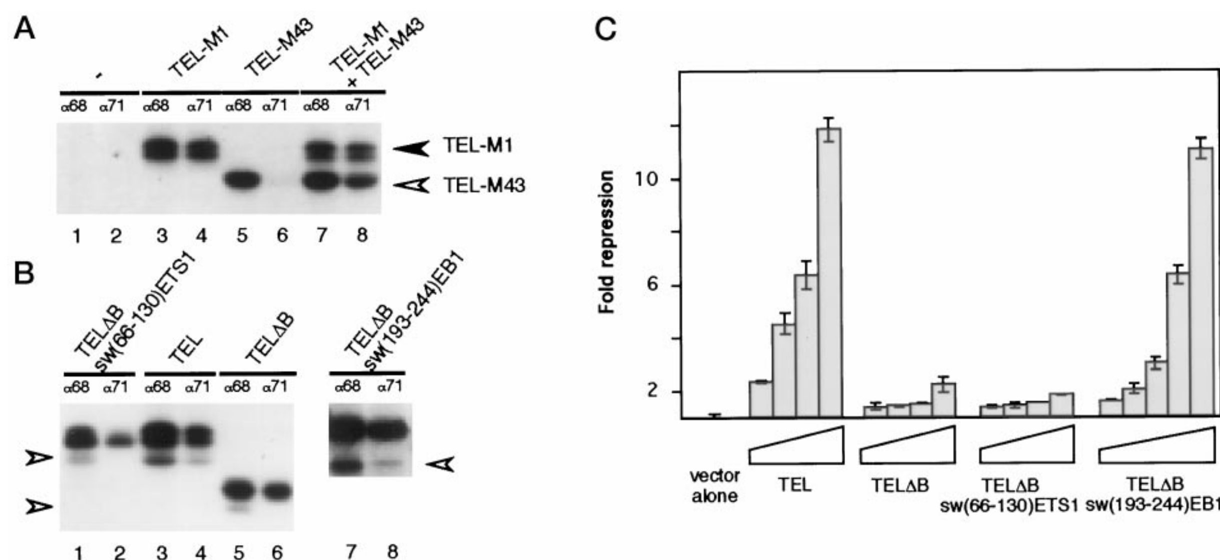

FIG. 3. **Oligomerization and transcriptional properties of TEL deletion and substitution mutants.** *A,* characterization of an antiserum specific to TEL-M1. HeLa cells were transfected with the control expression vector (*lanes 1* and *2*); with expression plasmids encoding TEL-M1 (*lanes 3* and *4*) 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 L-[^{35}S]methionine and L-[^{35}S]cysteine and lysed. Immunoprecipitation was carried out on 10^7 acid-insoluble counts of each lysates 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 L-[^{35}S]methionine and L-[^{35}S]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 $E74_3tk_{80}Luc$ 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.

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. 3*B, lane 8*) and to trans-repress $E74_3tk80Luc$ (Fig. 3*C*). These data show that the ability of TEL to self-oligomerize is essential to its repressing activity.

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* exon 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 $E74_3tk80Luc$ (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-ΔDsw-(331–426)-ETS1). This mutant is an efficient repressor of $E74_3tk80Luc$ (Fig. 4), indicating that the ETS domain of TEL is not specifically required for its ability to repress transcription.

We next analyzed whether TEL is also able to repress the activity of a cellular promoter that is known to be targeted by transcriptional activators of the ETS family. Recent studies have identified a novel promoter in the −270/−41 region of the *FLI-1* gene, which contains two EBS elements essential for its transactivation by Spi-1/PU.1 in spleen focus forming virus-transformed cells (24). Reporter constructs in which the luciferase gene is driven either by the wild type mouse −270/−41 *FLI-1* promoter or by the same promoter carrying mutated EBSs were co-transfected in HeLa cells along with expression plasmid for TEL and TEL-derived mutants. The results of Fig.

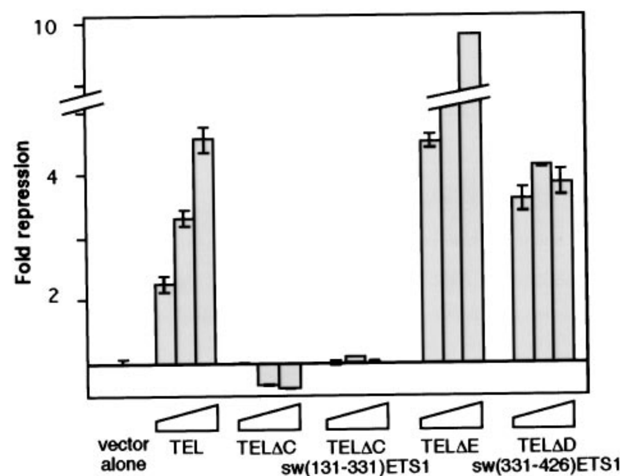

FIG. 4. **Study of the activity of TEL deletion and substitution mutants.** HeLa cells were transfected with 1 μg of $E74_3tk_{80}Luc$ along with 50, 100, or 250 ng of expression vector for the indicated proteins or the empty expression vector. Luciferase activity was measured as in Fig. 1*A*. The results 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.

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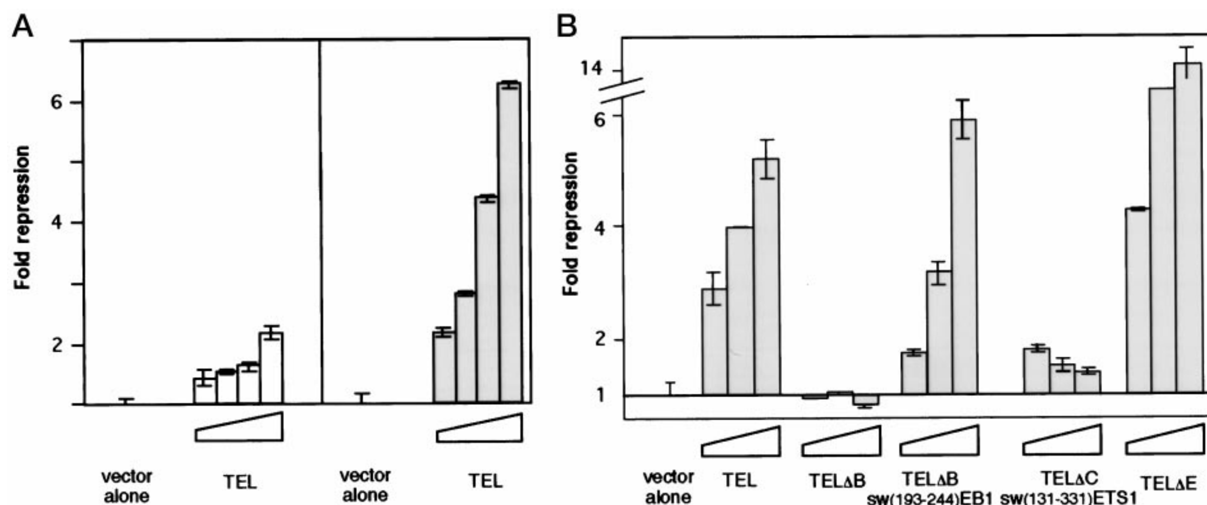

FIG. 5. **TEL represses the mouse (−270/−41) *FLI-1* promoter.** *A,* HeLa cells were transfected with 1 μg of (−270/−41)-*FLI-1*-Luc reporter plasmid (*right panel*) or with EBSmut-(−270/−41)-*FLI*-1-Luc (*left panel*) and 100, 250, 500, or 1000 ng of expression vector for TEL, or the empty vector. 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 as compared with the control expression vector. *B,* transcriptional regulatory properties of TEL mutants on (−270/−41)-*FLI*-1 promoter. HeLa cells were transfected with (−270/−41)-*FLI-1*-Luc along with 100, 500, or 1000 ng of expression vector for the indicated protein or with the empty vector. 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.

5*A* show that TEL repressed −270/−41 *FLI-1*-Luc in a dose-dependent manner. Binding of TEL to the *FLI-1* promoter is required for repression since mutation of the EBSs core sequence abolished its ability to repress. Self-oligomerization is also required for TEL to repress the *FLI-1* promoter since TEL-ΔB failed to repress, whereas TEL-ΔBsw-(193–244)-EB1 fully repressed (Fig. 5*B*). The exon 5-encoded domain of TEL was also required for repression of the *FLI-1* promoter since its substitution in TEL-ΔCsw-(131–331)-ETS1 generated an inactive protein. In contrast, deletion of the carboxyl-terminal domain (TEL-ΔE) enhanced repression (Fig. 5*B*).

These results show that the repressive activity of TEL requires the integrity of the exon 5-encoded central region and that the determinants that are essential to its repressive activity on model reporters are also required for TEL to repress a natural promoter.

TEL Contains an Autonomous Repression Domain—To determine whether TEL contains an autonomous repression domain, we tested if its repressive properties could be transferred to a heterologous, unrelated DNA binding domain. Different domains of TEL were fused to the DNA binding domain of *Saccharomyces cerevisiae* Gal4 protein (Gal4-DBD) (see Fig. 6*A*, for a schematic of the constructs). In addition to specific DNA binding, Gal4-DBD also directs oligomerization of Gal4 and Gal4-derived proteins. These fusion proteins were analyzed for their ability to regulate a luciferase reporter construct in which two copies of a consensus Gal4 DNA-binding site were inserted 5′ of the herpes simplex virus tk (−80 to +52) promoter (Gal_2tk80Luc (20)). In contrast to Gal4-DBD which only marginally affected the activity of Gal_2tk80Luc, a fusion protein containing TEL amino acid residues 120–452-(Gal4-TEL-(120–452)) repressed Gal_2tk80Luc in a dose-dependent manner to reach about 20-fold repression of transcription relative to the control expression plasmid (Fig. 6*B*). To delineate the domain(s) involved in the repressive activity of Gal4-TEL-(120–452), we analyzed the activity of a series of deletion mutants in its TEL-derived moiety (Fig. 6*A*). Gal4-TEL-(422–452), containing the 30 carboxyl-terminal residues of TEL, and Gal4-TEL-(335–452), which includes in addition the entire ETS domain, were inactive, showing that the carboxyl-terminal region 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. 6*B*). 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. 6*B*). 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. 6*B*). 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 (20% 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

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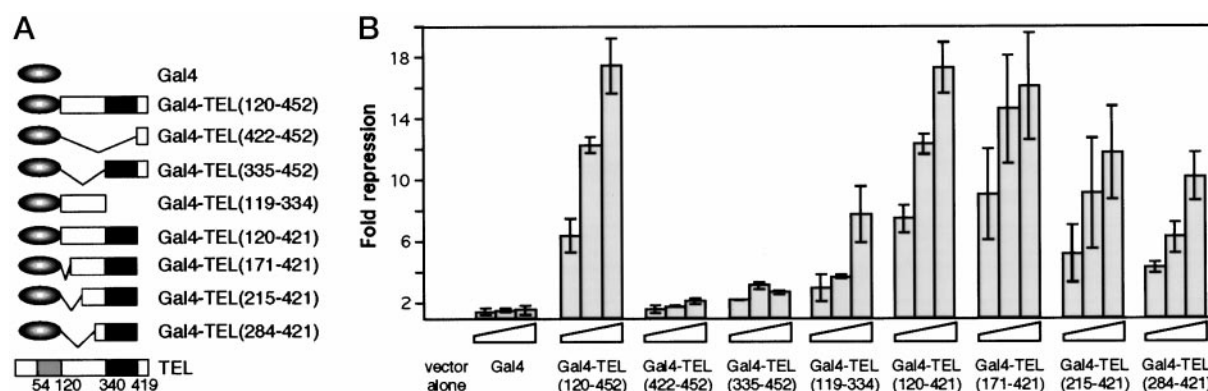

FIG. 6. **Transcriptional activity of Gal4 chimeras.** *A,* schematic structure of TEL fusions with the DNA binding domain (residues 1–147) of Gal4. *B,* HeLa cells were transfected with 1 μg of $Gal4_2tk_{80}Luc$ along with 400, 800, or 1600 ng of expression vector for Gal4-DBD, or the indicated fusion proteins, or the empty expression vector. The total amount of expression vector was kept constant to 1600 ng by addition of the control expression vector. 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 Gal4 fusion proteins as compared with the control expression vector.

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.[2] However, the fact that the unrelated oligomerization domain of Zebra and Gal4[2] 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.[2] 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.[2] Second, immunofluorescence analyses show that TEL does not co-localize with the large nuclear domains formed by *Polycomb* group proteins in mammalian cells.[3]

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.[2] 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 NH_2-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 (40, 41). 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

[2] R. G. Lopez, C. Carron, C. Oury, P. Gardellin, O. Bernard, and J. Ghysdael, unpublished observations.
[3] A. Otte and J. G., unpublished observations.

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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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