**The EMBO Journal** Vol.16 No.1 pp.69–82, 1997

# A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL–PDGFRβ oncoprotein

**Christine Jousset, Clémence Carron, Anthony Boureux, Christine Tran Quang, Cécile Oury, Isabelle Dusanter-Fourt[1], Martine Charon[1], Jonathan Levin[2], Olivier Bernard[3] and Jacques Ghysdael**

CNRS UMR 146, Institut Curie-Section de Recherche, Centre Universitaire, 91405 Orsay, [1]INSERM U 363, Institut Cochin de Génétique Moléculaire, Hôpital Cochin, 75014 Paris, [2]Unité d'Ingéniérie des Protéines, Institut National de la Recherche Agronomique, Domaine de Vilvert, 78352 Jouy-en-Josas and [3]INSERM U 301, Institut de Génétique Moléculaire, Hôpital Saint-Louis, 75010 Paris, France

C.Jousset and C.Carron contributed equally to this work

**TEL is a novel member of the ETS family of transcriptional regulators which is frequently involved in human leukemias as the result of specific chromosomal translocations. We show here by co-immunoprecipitation and GST chromatography analyses that TEL and TEL-derived fusion proteins form homotypic oligomers *in vitro* and *in vivo*. Deletion mutagenesis identifies the TEL oligomerization domain as a 65 amino acid region which is conserved in a subset of the ETS proteins including ETS-1, ETS-2, FLI-1, ERG-2 and GABPα in vertebrates and PNTP2, YAN and ELG in *Drosophila*. TEL-induced oligomerization is shown to be essential for the constitutive activation of the protein kinase activity and mitogenic properties of TEL–platelet derived growth factor receptor β (PDGFRβ), a fusion oncoprotein characteristic of the leukemic cells of chronic myelomonocytic leukemia harboring a t(5;12) chromosomal translocation. Swapping experiments in which the TEL oligomerization domain was exchanged by the homologous domains of representative vertebrate ETS proteins including ETS-1, ERG-2 and GABPα show that oligomerization is a specific property of the TEL amino-terminal conserved domain. These results indicate that the amino-terminal domain conserved in a subset of the ETS proteins has evolved to generate a specialized protein–protein interaction interface which is likely to be an important determinant of their specificity as transcriptional regulators.**

*Keywords*: ETS family/leukemia/oligomerization/TEL/TEL–PDGFRβ

## Introduction

ETS proteins form a class of >20 different sequence-specific transcriptional regulators involved in the response of cells to a variety of developmental and environmental cues (for review, see Scott *et al*., 1994; Bories *et al.*, 1995; Hill and Treisman, 1995; Janknecht *et al.*, 1995; Muthusamy *et al*., 1995; Wassarman *et al.*, 1995). Six members of this family are implicated to date in a variety of oncogenic processes in both animal species and man. c-*ets1* is the cellular homolog of the v-*ets* oncogene of avian leukemia virus E26 which, together with a co-transduced v-*myb* oncogene and a partial *gag* gene, encode the Gag-Myb-Ets transforming protein (Leprince *et al.*, 1983; Nunn *et al.*, 1983). Both v-*myb* and v-*ets* contribute in an interdependent and synergistic way to the transforming and leukemogenic properties of E26 (Metz and Graf, 1991a,b). Spi-1/PU1 and FLI-1 are overexpressed in the leukemic cells of the erythroleukemias induced in mice by the Friend virus complex and the Friend helper virus, respectively (Moreau-Gachelin *et al.*, 1988; Ben-David *et al.*, 1991). Overexpression of Spi-1 has been shown to affect various aspects of the differentiation and proliferation control of primary erythroid progenitors and of Friend leukemic cell lines (Schuetze *et al.*, 1993; Delgado *et al.*, 1994; Tran Quang *et al.*, 1995). In man, the carboxy-terminal domains of FLI-1, ERG-2 or ETV-1 including their ETS domain are fused to the amino-terminal half of EWS, an RNA binding protein encoded by the *EWS* gene on chromosome 22, in Ewing sarcomas and related tumors of childhood as the result of specific chromosomal translocations (Delattre *et al.*, 1992; Zucman *et al.*, 1993; Jeon *et al.*, 1995). Similarly, the ERG-2 DNA binding domain is fused to TLS, a protein related to EWS, in several forms of acute myelogenous leukemias harboring a t(16;21) translocation (Shimizu *et al.*, 1993; Ichikawa *et al.*, 1994). In these cases, substitution of the amino-terminal moiety of FLI-1, ERG-2 or ETV-1 by either EWS or TLS sequences confers aberrant transcriptional activation properties and activates the transforming properties of these proteins (May *et al.*, 1993a,b; Bailly *et al.*, 1994; Prasad *et al.*, 1994). Finally, a series of leukemias are associated with specific translocations which result in the fusion of the amino-terminal domain of TEL to a variety of partners. These include the carboxy-terminal domain of the platelet-derived growth factor receptor β (PDGFRβ) and c-ABL tyrosine-specific protein kinases in chronic myelomonocytic leukemia (CMML) with t(5;12) and acute lymphoid leukemia with t(9;12), respectively (Golub *et al.*, 1994; Papadopoulos *et al.*, 1995); and the AML1/CBFα subunit of core binding factor (CBF), a tissue-specific transcriptional regulator, in childhood B-cell acute lymphoid leukemia with t(12;21) (Golub *et al.*, 1995; Romana *et al.*, 1995). TEL is also involved in reciprocal t(12;22) chromosomal translocations involving *MN1*, a gene of unknown function (Buijs *et al.*, 1995).

The common feature of ETS proteins is a domain of 85 amino acid residues (ETS domain) which is structured as a winged helix–turn–helix motif responsible for their binding to specific sequences centered over a conserved GGAA/T core motif (Donaldson *et al.*, 1994, 1996; Liang *et al.*, 1994; Kodandapani *et al.*, 1996). Moreover, a subset

of ETS proteins, including ETS-1, ETS-2, ERG-2, FLI-1, GABPα and TEL in vertebrates and PNTP2, ELG and YAN in *Drosophila* also share a region of homology of ~65 amino acid residues which has been referred to diversely as the B domain (Boulukos *et al.*, 1989), the *pointed* domain (Klambt, 1993) or the HLH domain because of a loose resemblance to the helix–loop–helix motif of other transcriptional regulators (Seth and Papas, 1990). Although deletion analyses indicate that the amino-terminal conserved region of ETS-1, ETS-2 and FLI-1 modulates the transcriptional properties of these proteins (Gegonne *et al.*, 1992; Schneikert *et al.*, 1992; Rao *et al.*, 1993), its precise mode of action remains unclear.

The TEL–PDGFRβ and TEL–ABL fusion proteins are formed by the fusion of the 154 amino-terminal residues of TEL, including the conserved amino-terminal domain (B domain), to either the transmembrane and catalytic domain of PDGFRβ or the SH2, SH3 and catalytic domains of c-ABL, respectively (Golub *et al.*, 1994; Papadopoulos *et al.*, 1995). Since normal ligand-induced activation of PDGFRβ depends upon ligand-induced dimerization (for review, see Heldin, 1995), it has been suggested that the TEL-derived sequence might contribute to the oncogenic properties of TEL–PDGFRβ by favoring constitutive dimerization and activation of the protein kinase activity of the PDGFRβ moiety of the fusion protein (Golub *et al.*, 1994). Likewise, TEL-induced oligomerization might activate the protein kinase activity (Papadopoulos *et al.*, 1995) and transforming properties of TEL–ABL in a way reminiscent of the BCR-induced oligomerization of the BCR–ABL fusion protein of Philadelphia-positive human leukemias (McWhirter *et al.*, 1993).

Here, we show that the amino-terminal conserved domain of TEL forms a homotypic interaction interface which mediates oligomerization of TEL and TEL-derived fusion proteins. This property appears to be specific to TEL, since the amino-terminal conserved domain of other vertebrate proteins fails to mediate oligomerization. TEL-induced oligomerization is shown to play an essential role in the activation of the protein kinase activity and mitogenic property of TEL–PDGFRβ.

## Results

To determine whether the 154 amino-terminal residues of TEL encode an oligomerization interface, SV40 early promoter-based expression plasmids encoding intact TEL–PDGFRβ (TP-0) or a hemagglutinin (HA) epitope-tagged version of a mutant derived thereof by deletion of the 448 carboxy-terminal residues, HA-TP-0 (tt), were transfected either alone or co-transfected in HeLa cells (see Figure 1 for a schematic of the proteins used in this study). Transfected cells were metabolically labeled with L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine, and the respective proteins were immunoprecipitated from cell lysates using specific antibodies. The results of Figure 2 show that a monoclonal antibody to the HA epitope specifically precipitated HA-TP-0 (tt) (Figure 2A, compare lanes 5 and 6) whereas a rabbit antiserum directed to the carboxy-terminal end of PDGFRβ only precipitated TP-0 (Figure 2A, compare lanes 3 and 4). In contrast, immunoprecipitation of lysates of cells co-expressing both proteins with either of these antibodies resulted in the co-precipitation of TP-0 and HA-TP-0 (tt) (Figure 2A, lanes 7 and 8). Similar results were obtained when these proteins were co-expressed following *in vitro* translation of their respective mRNAs in rabbit reticulocyte lysates and immunoprecipitated with the same set of antibodies (Figure 2B, lanes 4–9). We conclude from these experiments that TP-0 and truncated forms derived thereof form a complex *in vivo* and *in vitro*.

To analyze whether the TEL moiety is required for this association, a series of truncated polypeptides that contained TEL amino- and carboxy-terminal deletions was generated (see Figure 1A). The ability of these polypeptides to interact with intact TP-0 was analyzed by co-immunoprecipitation assays using antibodies specific of each of them (Figure 3A and B, lanes 1–6). TEL amino acids 1–53 were found to be dispensable for complex formation since their deletion in HA-TP-3 (t) did not affect the ability of this protein to associate with TP-0 (Figure 3A, lanes 7 and 8). In contrast, further deletion of 65 amino acid residues in HA-TP-4 (t) or complete deletion of TEL-derived sequences in HA-TP-5 (t) abolished the ability of these proteins to co-precipitate with TP-0 (Figure 3A, lanes 9–12), indicating that complex formation is dependent upon specific sequences in the TEL domain. Analysis of TEL–PDGFRβ mutants carrying carboxy-terminal deletions in the TEL moiety showed that removal of TEL amino acid residues 119–154 (TP-11) did not affect complex formation with HA-TP-0 (t) (Figure 3B, lanes 7 and 8), whereas further deletion into the central, conserved domain of TEL in TP-13 abolished it (Figure 3B, lanes 9 and 10). The essential role of the conserved domain of TEL in complex formation is borne out further by the inability of a mutant TEL–PDGFRβ deleted in this domain (TP-10) to associate with HA-TP-0 (t) (Figure 3B, lanes 11 and 12). Moreover, fusion of the TEL conserved domain (amino acid residues 54–119 of TEL) to 132 residues of PDGFRβ sequences, HA-TP-12 (t), is sufficient to engage these proteins in complex formation with TP-0 *in vitro* (Figure 3C, lanes 3 and 4; compare also with Figure 3A, lanes 9 and 10). Complex formation between TP-0 and HA-TP-12 (tt) was also observed *in vivo* in HeLa cells transfected with the respective expression plasmids (data not shown).

The studies reported so far were carried out using TEL deletion mutants made in the context of the TEL–PDGFRβ fusion protein. To determine whether the amino-terminal conserved domain of TEL also mediates complex formation of TEL and TEL-derived nuclear proteins, we analyzed the ability of intact TEL and TEL derivatives to associate *in vivo* and *in vitro*. Expression plasmids encoding an HA-tagged version of TEL or an ETS-1 chimeric protein in which the 129 amino-terminal residues of ETS-1 are replaced by the corresponding residues of TEL, TEL(1–119)/ETS-1(130–441) (see Figure 1C for a schematic of these constructs), were transfected either alone or co-transfected in HeLa cells. Cells were metabolically labeled with L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine and the respective proteins were immunoprecipitated using antibodies specific for each of them (Figure 4A, lanes 3–6). The results of Figure 4A show that an antibody specific for ETS-1 was able to precipitate TEL only when it was co-expressed with the chimeric TEL(1–119)/ETS-1(130–441)

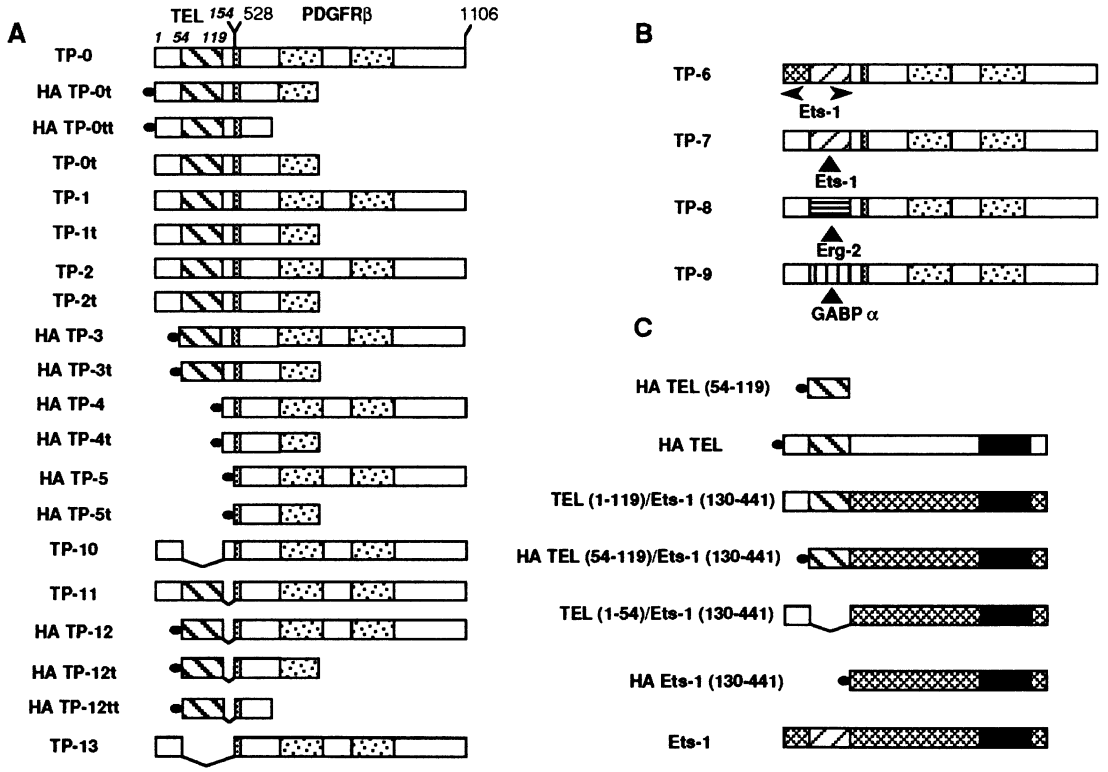


**Fig. 1.** Schematic structure of TEL derivatives used in this study. (**A**) Schematic of intact TEL–PDGFRβ (TP-0) and of deletion mutants derived thereof. Mutants in the TEL-derived portion of TP-0 are depicted as TP-1–TP-13. TP-1 and TP-2 were obtained following site-directed mutagenesis to generate in-frame *Bam*HI and *Bgl*II restriction sites flanking the amino-terminal conserved domain of TEL (depicted as a hatched box). The amino acid substitutions created by these mutations were found not to affect the ability of TP-1 and TP-2 to associate with intact TEL–PDGFRβ (data not shown). TEL–PDGFRβ 5′ deletion mutants (HA-TP-3, HA-TP-4 and HA-TP-5), missense mutants (TP-1 and TP-2) and internal deletion mutants (TP-10, TP-11, HA-TP-12 and TP-13) were generated either as fusions containing the entire PDGFRβ-derived sequences or as truncated versions of this domain. Truncations were obtained by digestion of PDGFRβ DNA at either *Apa*I (in which case the mutants are referred to as t mutants containing only 132 amino acids of PDGFRβ sequences, spanning the transmembrane and part of the cytoplasmic domains) or *Sac*I (referred to as tt mutants containing 73 amino acids of PDGFRβ spanning the transmembrane and part of the cytoplasmic domains). Some of the mutants were generated as HA-tagged versions (the HA tag is shown as a black oval). The split catalytic domain and the transmembrane region of PDGFRβ are shown as light stippled and stippled boxes, respectively. (**B**) Schematic of TEL–PDGFRβ substitution mutants. Mutant TP-6 was obtained by substitution of residues 1–119 of TEL by the 1–130 amino-terminal residues of c-Ets-1. Mutants TP-7, TP-8 and TP-9 were obtained by substitution of the conserved amino-terminal domain of TEL (amino acids residues 54–119) by the homologous domain of ETS-1, ERG-2 and GABPα, respectively. The amino-terminal conserved domains of TEL, ETS-1, ERG-2 and GABPα are shown as distinct hatched boxes. The catalytic and the transmembrane regions of PDGFRβ are shown as light stippled and stippled boxes, respectively. (**C**) Schematic of TEL deletion mutants and ETS-1 chimeric proteins. Deletion mutant ETS-1(130–441) has been described previously (Gegonne *et al.*, 1992). The ETS (DNA binding) domain is shown as a black box. When present, the HA epitope is shown as a black oval.

protein (Figure 4A, compare lanes 4, 6 and 8). Conversely, in anti-HA immunoprecipitates, the TEL(1–119)/ETS-1(130–441) protein was detected only when it was co-expressed with HA-TEL (Figure 4A, compare lanes 3, 5 and 7). Complex formation between TEL(1–119)/ETS-1(130–441) and TEL is dependent upon the presence of TEL sequences since HA-ETS-1(130–441) was found to be unable to co-precipitate with HA-TEL (Figure 4B, compare lanes 2, 4 and 6). To analyze whether the conserved domain of TEL is sufficient to mediate complex formation, we co-expressed HA-TEL with a TEL–ETS-1 chimera limited to this domain of TEL, HA-TEL(54–119)/ETS-1(130–441), and analyzed the association of these proteins using antibodies specific for their respective carboxy-terminal domains (ETS domains). The results in Figure 4B show that immunoprecipitation of cell lysates co-expressing both proteins with either the ETS-1-specific antibody or the TEL-specific antibody resulted in the co-precipitation of HA-TEL and HA-TEL(54–119)/ETS-1(130–441) (Figure 4B, compare lanes 1 and 7–10). The same results were obtained using *in vitro* translated proteins in reticulocyte lysates (data not shown). We conclude from these experiments that the TEL amino-terminal conserved domain is essential to engage TEL in complex formation *in vivo* and *in vitro* independently of its protein context.

To analyze whether this domain alone is by itself sufficient for complex formation, we generated an HA-TEL miniprotein restricted to this domain, HA-TEL(54–119), and analyzed its ability to co-precipitate with either TP-0 (t) or TEL(1–119)/ETS-1(130–441) *in vitro*. As shown in Figure 4C, immunoprecipitation of TP-0 (t) or TEL(1–119)/ETS-1(130–441) with the anti-HA specific antibody was only observed when co-expressed with HA-TEL(54–119) (Figure 4C, compare lanes 1–5). To determine whether the TEL amino-terminal conserved domain is able to interact with itself, a GST fusion protein containing amino acids 54–119 of TEL was immobilized on glutathione–agarose beads and incubated with *in vitro* translated L-[$^{35}$S]methionine-labeled TP-0 or

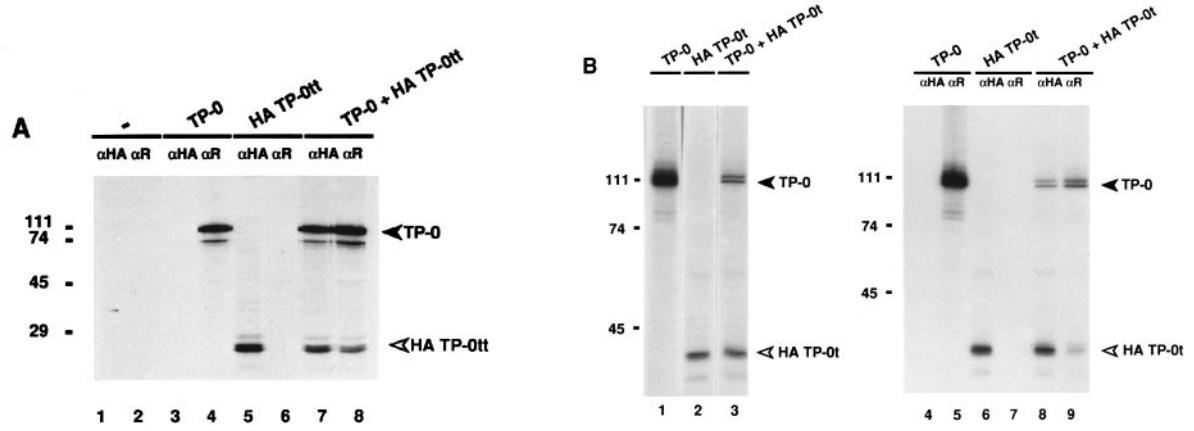


**Fig. 2.** *In vivo* and *in vitro* complex formation of TEL–PDGFRβ and mutants derived thereof. (**A**) HeLa cells were transfected with expression plasmids for either intact TEL–PDGFRβ (TP-0) (lanes 3 and 4) or the HA-TP-0 (tt) deletion mutant (lanes 5 and 6), or were co-transfected with both TP-0 and HA-TP-0 (tt) (lanes 7 and 8). HeLa cells transfected with the empty expression vector were used as negative control (lanes 1 and 2). Transfected cells were metabolically labeled with L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine and lysed. Immunoprecipitations were carried out on $10^7$ acid-insoluble radioactive counts of each lysate using either the HA-specific monoclonal antibody (odd-numbered lanes) or a rabbit antiserum specific to the carboxy-terminus of the PDGFRβ (even-numbered lanes). Immunoprecipitates were analyzed by electrophoresis on polyacrylamide gels (10% acrylamide) in the presence of SDS followed by fluorography. Note that these proteins migrate as doublets as the result of translation initiation at both the first initiation codon of the TEL open reading frame or at the codon encoding Met43 (data not shown). (**B**) pBS plasmids encoding either TP-0 (lanes 1, 4 and 5) or HA-TP-0 (t) (lanes 2, 6 and 7) were translated alone or co-translated (lanes 3, 8 and 9) following coupled transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine. Translated proteins were analyzed directly (lanes 1–3) or immunoprecipitated using the HA-specific monoclonal antibody (lanes 4, 6 and 8) or the rabbit antiserum to the carboxy-terminus of PDGFRβ (lanes 5, 7 and 9). Immunoprecipitates were analyzed by electrophoresis on polyacrylamide gels (10% acrylamide) in the presence of SDS followed by fluorography.

HA-TEL (54–119). As shown in Figure 5, both TP-0 (Figure 5A, lanes 6–10) and HA-TEL(54–119) (Figure 5B, lanes 6–10) bound to the GST–TEL(54–119) affinity matrix. In contrast, neither GST alone nor a GST fusion protein containing the amino-terminal conserved domain of ETS-1 (data not shown) was found to be able to bind TP-0 (Figure 5A, lanes 1–5) and HA-TEL(54–119) (Figure 5B, lanes 1–5). We conclude from these experiments that the conserved amino-terminal of TEL is sufficient for oligomerization.

The domain of TEL responsible for oligomerization is phylogenetically conserved in a subset of the ETS family members which include, in vertebrates, the highly related ETS-1 and ETS-2, the related FLI-1 and ERG-2 and the α subunit of GABP and, in *Drosophila*, PNTP2, ELG and YAN. To analyze whether the corresponding domains of other vertebrate ETS proteins are also involved in complex formation, TEL–PDGFRβ fusion proteins were created in which the conserved domain of TEL was substituted for those of ETS-1, ERG-2 or GABPα to generate TP-7, TP-8 and TP-9 (see Figure 1B for a schematic of these mutants). The properties of a TEL–PDGFRβ fusion protein (TP-6) in which the 119 amino-terminal residues of TEL were replaced by the first 130 amino acid residues of ETS-1 were also analyzed. These fusion proteins were co-translated *in vitro* with ETS-1, ERG-2 and GABPα, respectively, and complex formation analyzed by co-immunoprecipitation analyses using specific antisera (Figure 6A, lanes 1–4 and 7–8; B, lanes 1–4 and 7–10). The results of Figure 6 show that in conditions in which intact TEL–PDGFRβ co-immunoprecipitated with TEL (Figure 6C), none of the fusion proteins in which the TEL amino-terminal conserved region was exchanged for that of ETS-1 (Figure 6A, lanes 5–6 and 9–10), ERG-2 (Figure 6B, lanes 5–6) or GABPα (Figure 6B, lanes 11–12) were able to form homotypic complexes as evidenced by their failure to co-precipitate with ETS-1, ERG-2 or GABPα, respectively. Also, unlike intact TP-0, neither TP-6, TP-7, TP-8 nor TP-9 was found to be able to associate with TEL (data not shown). We conclude from these experiments that the ability to oligomerize is a specific property of the amino-terminal conserved domain of TEL and that TEL-induced oligomerization is homotypic. Intact TEL–PDGFRβ (TP-0) was found to localize to the cytoplasm of transfected cells (Figure 7C, compare panels 1 and 2); this property is independent of the presence of the oligomerization domain since no difference in subcellular localization was observed between TP-0 and TP-10 (Figure 7C, compare panels 2 and 3).

The mitogenic and chemotactic properties of PDGFRβ are dependent upon PDGF-induced dimerization and subsequent activation of its tyrosine-specific protein kinase activity (for review, see Heldin, 1995). To analyze whether TEL-mediated oligomerization is involved in the activation of the tyrosine-specific protein kinase activity of TEL–PDGFRβ, we compared the autophosphorylation properties of TP-0 and of a series of deletion or substitution mutants derived thereof which either maintain or abolish the ability to oligomerize. Proteins were translated in reticulocyte lysates and aliquots of each lysate were electrophoresed on polyacrylamide gels and, after transfer to nitrocellulose, the blots were revealed with either an antibody to the carboxy-terminal end of PDGFRβ to compare for expression levels or with a phosphotyrosine-specific antibody to analyze for autophosphorylation of the PDGFRβ-derived moiety. The results of Figure 7B show that all fusion proteins tested were expressed at a similar level. Analysis of the same blot with an anti-phosphotyrosine antibody revealed a high level of tyrosine phosphorylation associated with TP-0 (Figure 7A, lane 1). This reflects TEL–PDGFRβ autophosphorylation since a TEL–PDGFRβ mutant carrying a K634A substitution in

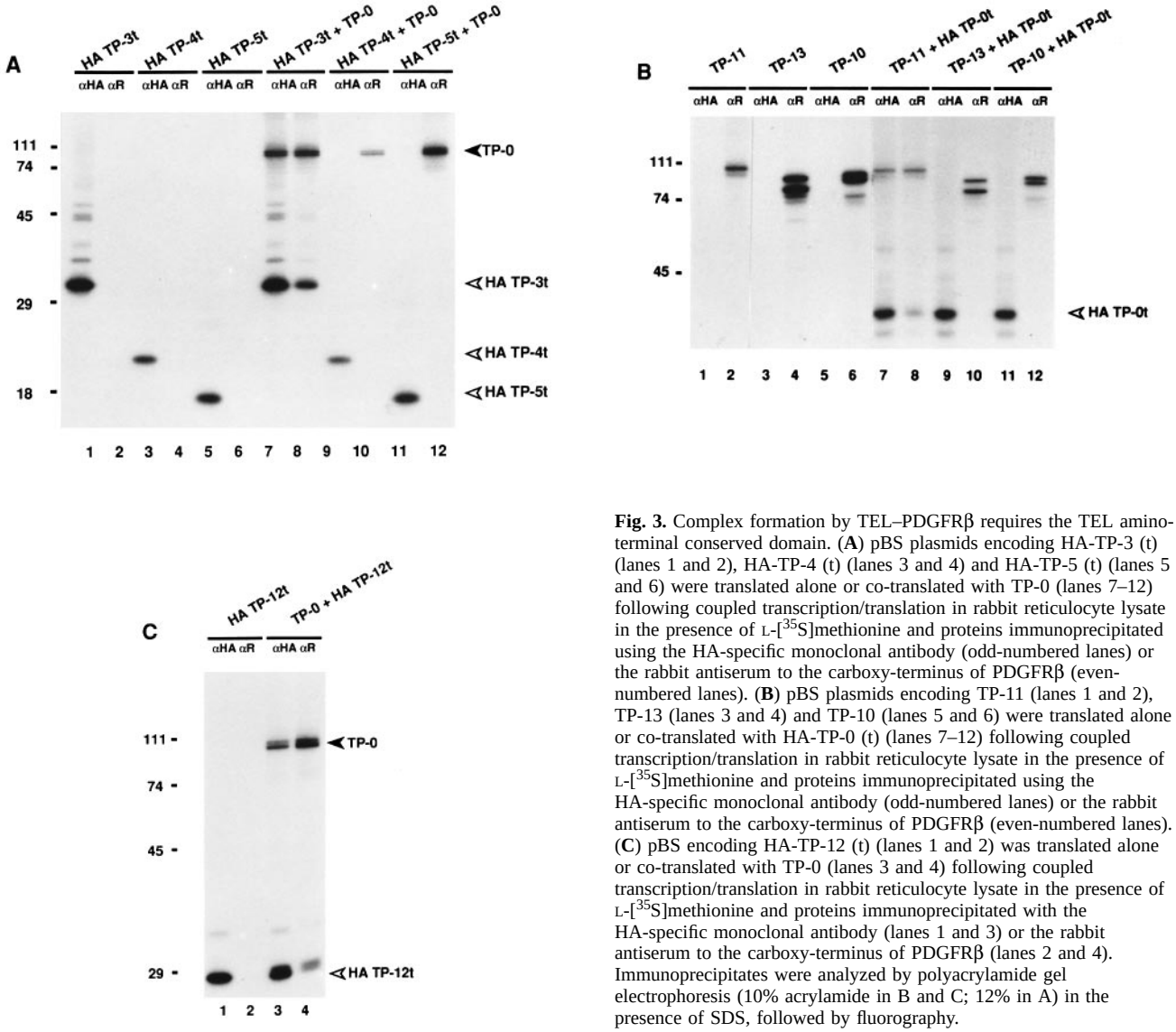


**Fig. 3.** Complex formation by TEL–PDGFRβ requires the TEL amino-terminal conserved domain. (**A**) pBS plasmids encoding HA-TP-3 (t) (lanes 1 and 2), HA-TP-4 (t) (lanes 3 and 4) and HA-TP-5 (t) (lanes 5 and 6) were translated alone or co-translated with TP-0 (lanes 7–12) following coupled transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine and proteins immunoprecipitated using the HA-specific monoclonal antibody (odd-numbered lanes) or the rabbit antiserum to the carboxy-terminus of PDGFRβ (even-numbered lanes). (**B**) pBS plasmids encoding TP-11 (lanes 1 and 2), TP-13 (lanes 3 and 4) and TP-10 (lanes 5 and 6) were translated alone or co-translated with HA-TP-0 (t) (lanes 7–12) following coupled transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine and proteins immunoprecipitated using the HA-specific monoclonal antibody (odd-numbered lanes) or the rabbit antiserum to the carboxy-terminus of PDGFRβ (even-numbered lanes). (**C**) pBS encoding HA-TP-12 (t) (lanes 1 and 2) was translated alone or co-translated with TP-0 (lanes 3 and 4) following coupled transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine and proteins immunoprecipitated with the HA-specific monoclonal antibody (lanes 1 and 3) or the rabbit antiserum to the carboxy-terminus of PDGFRβ (lanes 2 and 4). Immunoprecipitates were analyzed by polyacrylamide gel electrophoresis (10% acrylamide in B and C; 12% in A) in the presence of SDS, followed by fluorography.

the ATP binding site, a mutation known to inactivate the intrinsic protein kinase activity of PDGFRβ (Williams, 1989), failed to react with the anti-phosphotyrosine antibody when tested in the same conditions (data not shown). All mutants of TEL–PDGFRβ which scored as oligomerization-defective in our co-precipitation assays, including the deletion mutants HA-TP-4, HA-TP-5, TP-10 and TP-13 (Figure 7A, lanes 2 and 4–6) and the substitution mutants TP-6 (Figure 7A, lane 3), TP-7, TP-8 and TP-9 (data not shown), were also found to lack protein kinase activity. In contrast, all TEL–PDGFRβ mutants which maintain their ability to oligomerize, including TP-11, HA-TP-3 and HA-TP-12, also maintain a high level of protein kinase activity (Figure 7A, lanes 7–9). We conclude from these experiments that oligomerization mediated by the TEL conserved amino-terminal domain is instrumental in the constitutive activation of the TEL–PDGFRβ protein tyrosine kinase activity.

To analyze whether the TEL-dependent oligomerization of TEL–PDGFRβ is important to its mitogenic properties, we made use of the ability of the interleukin 3 (IL-3)-dependent Ba/F3 murine hematopoietic cell line (Palacios and Steinmetz, 1985) to become independent of IL-3 for survival and proliferation upon expression of several receptor and non-receptor protein tyrosine kinases or cytokine receptors (Mathey-Prevot *et al.*, 1986; Daley and Baltimore, 1988; Daley *et al.*, 1992; McWhirter and Wang, 1993; Damen, 1995; Gobert *et al.*, 1995). The cDNAs encoding TP-0 as well as oligomerization-defective mutants derived thereof by either deletion of TEL sequences, including HA-TP-5 and TP-10, or by substitution of TEL sequences by the corresponding sequence of ETS-1 (TP-6) were subcloned into the pBabeNeo retroviral expression vector (Morgenstern and Land, 1990). These constructs as well as the pBabeNeo control were electroporated into Ba/F3 cells and transfected cells were selected in IL-3-containing growth medium in the presence of G418. Immunoprecipitation analyses of L-[$^{35}$S]methionine- and L-[$^{35}$S]cysteine-labeled cells showed that all transfectants expressed the expected proteins (Figure 8A). As expected from our previous analyses using *in vitro* translated proteins, only the intact TEL–PDGFRβ (TP-0) was active as a protein tyrosine kinase (Figure 8B, lane 2) whereas oligomerization-defective forms were inactive (Figure 8B, lanes 3–5). To assay for IL-3 independence, transfectants were switched to growth medium without

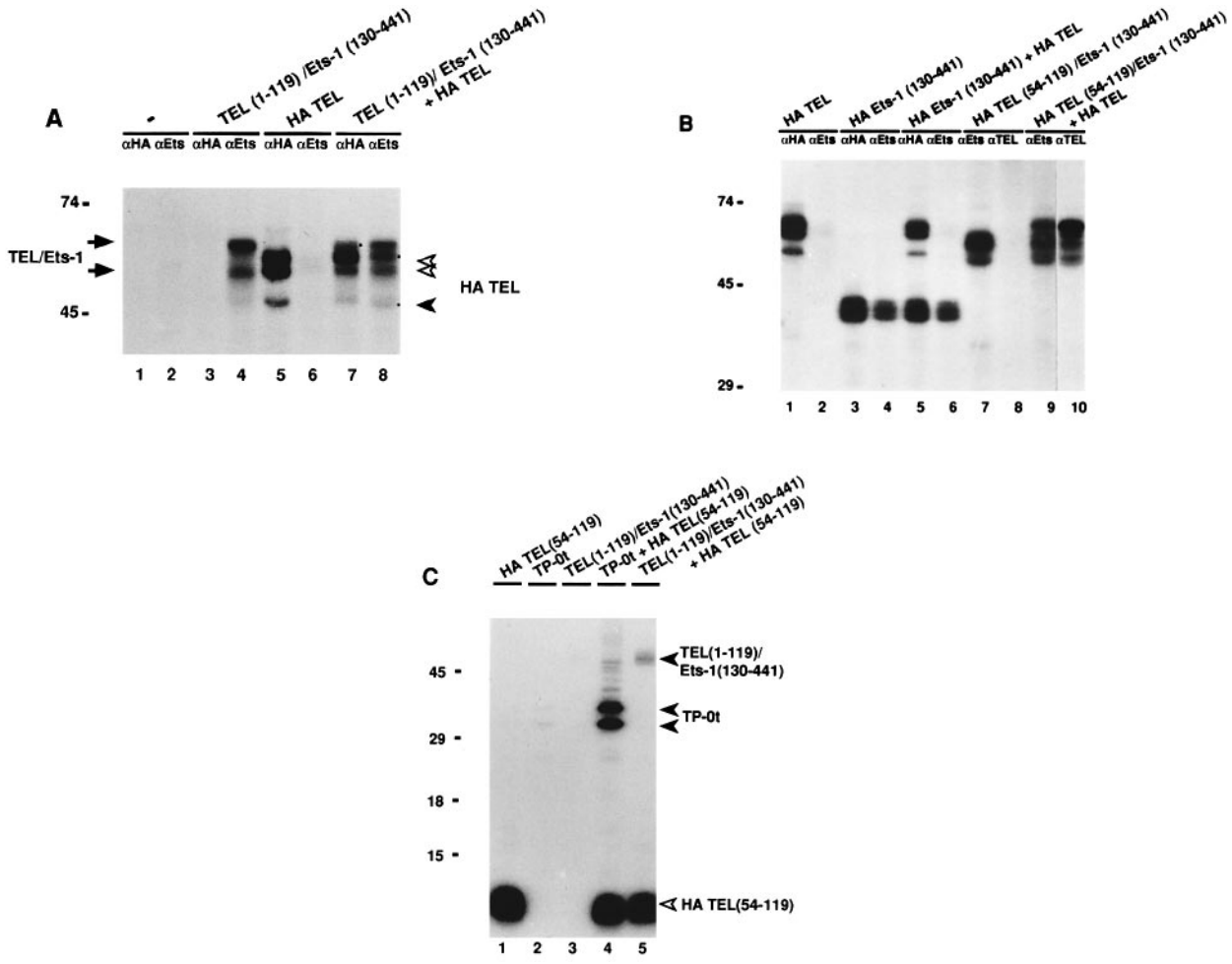


**Fig. 4.** Complex formation by TEL, TEL–ETS-1 chimeras and the TEL(54–119) miniprotein. (**A**) HeLa cells were transfected with expression plasmids for either TEL(1–119)/ETS-1(130–441) (lanes 3 and 4) or the HA-epitope tagged TEL (lanes 5 and 6), or were co-transfected with both expression plasmids (lanes 7 and 8). Cells transfected with the empty expression vector were used as negative control (lanes 1 and 2). Transfected cells were metabolically labeled in the presence of L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine and lysed. Immunoprecipitations were carried out on $10^7$ acid-insoluble counts of each lysate using either the HA-specific monoclonal antibody (odd-numbered lanes) or the rabbit antiserum to the DNA binding domain of ETS-1 (even-numbered lanes). In cells, TEL is modified by phosphorylation events to generate several isoforms (Poirel *et al.*, 1997) indicated by open arrowheads. The filled arrowhead points to the translation product initiated at Met43 (Poirel *et al.*, 1997). The TEL–ETS-1 isoforms are indicated by filled arrows. Dots in lanes 7 and 8 point to discriminatory bands in the respective immunoprecipitates. (**B**) HeLa cells were transfected with expression plasmids for either HA-TEL (lanes 1 and 2) or HA-ETS-1(130–441) (lanes 3 and 4) or HA-TEL(54–119)/ETS-1(130–441) (lanes 7 and 8), or co-transfected with HA-TEL and ETS-1(130–441) (lanes 5 and 6) or with HA-TEL and HA-TEL(54–119)/ETS-1(130–441) (lanes 9 and 10). Cells were metabolically labeled with L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine and lysed. Immunoprecipitations were carried out on $10^7$ acid-insoluble radioactive counts of each lysate using either the HA-specific monoclonal antibody (lanes 1, 3 and 5), the rabbit antiserum to the DNA binding domain of ETS-1 (lanes 2, 4, 6, 7 and 9) or the rabbit antiserum to the DNA binding domain of TEL (lanes 8 and 10). (**C**) pBS plasmids encoding either the HA-TEL(54–119) miniprotein (lane 1), TP-0 (t) (lane 2) or TEL(1–119)/ETS-1(130–441) (lane 3) were translated alone following coupled transcription/translation in rabbit reticulocyte lysates in the presence of L-[$^{35}$S]methionine. Co-translations of either HA-TEL(54–119) and TP-0 (t) or HA-TEL(54–119) and TEL(1–119)/ETS-1(130–441) were obtained in parallel (lanes 4 and 5). Translated proteins were immunoprecipitated using the HA-specific monoclonal antibody. Immunoprecipitated proteins were analyzed by polyacrylamide gel electrophoresis (8% acrylamide in A and B; 15% in C) in the presence of SDS followed by fluorography.

IL-3. Whereas the control transfectants and those expressing the oligomerization-defective TEL–PDGFRβ mutants were found to be unable to proliferate in these conditions, transfectants expressing TP-0 could proliferate in an IL-3-independent fashion (Figure 8C). Ba/F3 cells expressing TP-0 were found to proliferate in the absence of IL-3 for a period of >1 month at a rate which is, however, reduced as compared with that observed in the presence of IL-3 (Figure 8C). The control Ba/F3 cell line as well as all transfectants were found to proliferate at the same rate in the presence of IL-3 (data not shown). Supernatants from TP-0 cells failed to support the growth factor-independent proliferation of Ba/F3 cells, indicating that TP-0-induced proliferation does not result from induction of growth stimulatory cytokines but is a direct consequence of TEL–PDGFRβ signaling. We conclude from these experiments that TEL-induced oligomerization of TEL–PDGFRβ is essential for its activity as a protein tyrosine kinase and its mitogenic properties *in vivo*.

## Discussion

The results presented in this study show that the amino-terminal conserved domain of TEL (amino acid residues 54–119) is a homotypic oligomerization domain as assessed by co-immunoprecipitation analyses and by

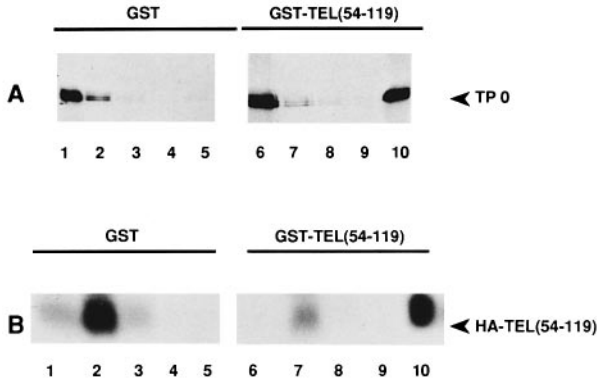


**Fig. 5.** *In vitro* association assay betweeen GST–TEL(54–119) immobilized on agarose beads and *in vitro* translated TP-0 and TEL(54–119). pBS plasmids encoding either TP-0 (**A**) or HA-TEL(54–119) (**B**) were translated following transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine. Translated proteins were incubated with either GST–agarose (lanes 1–5) or GST–TEL(54–119) (lanes 6–10) and the flow through (lanes 1 and 6) and wash fractions (lanes 2–4 and 7–9) were collected. Bound proteins (lanes 5 and 10) were eluted by boiling in SDS–polyacrylamide sample buffer. Equal amounts of each fraction were analyzed by electrophoresis on polyacrylamide gels and visualized by fluorography.

affinity chromatography using a specific GST matrix. TEL-induced oligomerization is an autonomous property of this domain since a miniprotein corresponding to the TEL oligomerization domain was found to engage in complex formation with itself, with TEL and with TEL fusion proteins maintaining an intact amino-terminal conserved domain.

The primary amino acid sequence of the oligomerization domain of TEL is highly conserved in a subset of the ETS family members, implying a conserved structural fold (Figure 9). Previous alignment studies have proposed this region to display similarity to the HLH motif of the basic-HLH (bHLH) proteins (Seth and Papas, 1990). To gain further insight into the possible secondary structure of this domain, the primary sequence of the amino-terminal conserved domains of ETS-1, ETS-2, ERG-2, FLI-1, GABPα and TEL, together with those of *Drosophila melanogaster* PNTP2, YAN and ELG and a sea urchin ETS protein, were aligned using the CLUSTAL W multiple alignment program (Thompson *et al.*, 1994). Secondary structure predictions were performed using the SIMPA 95 program (Levin and Garnier, 1988; J.Levin, in preparation). As shown in Figure 9, two helices are strongly predicted at each end of the domain, separated by a region with a low β-strand folding probability. The helix predicted at the carboxy-terminal end of the domain co-localizes with helix 2 of the presumed HLH. In contrast, the proposed region of similarity with helix 1 of *bona fide* bHLH proteins is not predicted to fold as an α-helix in the multiple alignment analysis. Furthermore, this region shows additional features which make it unlikely to resemble helix 1 of bHLH proteins. First, a phenylalanine residue (F129 in MyoD1; Ma *et al.*, 1994) which is conserved in the middle of helix 1 of *bona fide* bHLH proteins is substituted by glycine in all ETS proteins (Seth and Papas, 1990; see Figure 9). Finally, FLI-1, ERG-2 and PNTP2 show an extra amino acid residue in the middle of this region when compared with other ETS proteins or bHLH proteins (Seth and Papas, 1990; see Figure 9).

The non-conservation in ETS proteins of the hydrophobic residues necessary for stabilization of the putative helix 1 as well as the insertion observed in that region for some ETS proteins, together with our secondary structure prediction, suggest that this region is unlikely to fold as an α-helix. In the absence of experimental data about the structure adopted by this domain in either a monomeric or oligomeric state, its description as an HLH domain clearly appears to be premature.

The conserved fold of the amino-terminal domain of ETS proteins is likely to underlie a conserved function. Our results show, however, that the conserved amino-terminal domains of ETS-1, ERG-2 and GABPα are not homotypic oligomerization domains since they failed to replace the conserved amino-terminal domain of TEL in inducing oligomerization when analyzed in an identical setting (Figure 6). These data are in accordance with other experimental approaches which have led to the conclusion that ETS-1 and ETS-2 are monomeric in solution (for review, see Wasylyk *et al.*, 1993; our unpublished observations). We interpret these observations as suggesting that the amino-terminal conserved domain has evolved in different ETS proteins to generate a specialized protein–protein interaction interface which is likely to make an important contribution to their specificity. In line with this notion, the amino-terminal conserved domains of the closely related FLI-1 and ERG-2 proteins and of the ETS protein subclass formed by ETS-1, ETS-2 and PNTP2 are included in a more extended, subclass-specific, region of homology of ~200 and 100 amino acid residues, respectively. In the case of PNTP2, ETS-1 and ETS-2, this extended region of homology includes a conserved threonine residue which is the target for phosphorylation by members of the MAP kinase family, a modification which recently has been shown to play an essential role in the physiological and transcriptional response of these proteins to specific environmental signals involving MAP kinases as intracellular signaling components (Brunner *et al.*, 1994; O'Neil *et al.*, 1994; Rabault *et al.*, 1996; Yang *et al.*, 1996). Specificity and diversity in transcriptional regulation by ETS proteins is highly dependent upon their assembly into multiprotein complexes and in their synergy with other *cis*-linked transcriptional regulators (Wasylyk *et al.*, 1990; Hipskind *et al.*, 1991; Dalton and Treisman, 1992; Pongubala *et al.*, 1992; Gegonne *et al.*, 1993; Wotton *et al.*, 1993; Giese *et al.*, 1995; John *et al.*, 1995; Sieweke *et al.*, 1996). In many of these situations, the domains involved in the multiple contacts made by ETS proteins with other transcriptional regulators have not been identified. As ETS-1, ETS-2, FLI-1, ERG-2 and GABPα display a similar DNA binding specificity (Brown and McKnight, 1992; Nye *et al.*, 1992; Woods *et al.*, 1992), the specialized protein–protein interaction interface provided by their amino-terminal conserved domain could play an important role in governing either the specificity of the assembly or the operation of ETS-containing multiprotein complexes.

Like other ETS family members, TEL displays sequence-specific DNA binding properties towards consensus ETS binding sites *in vitro* (Poirel *et al.*, 1997). Unlike other ETS proteins, however, TEL is a homodimer

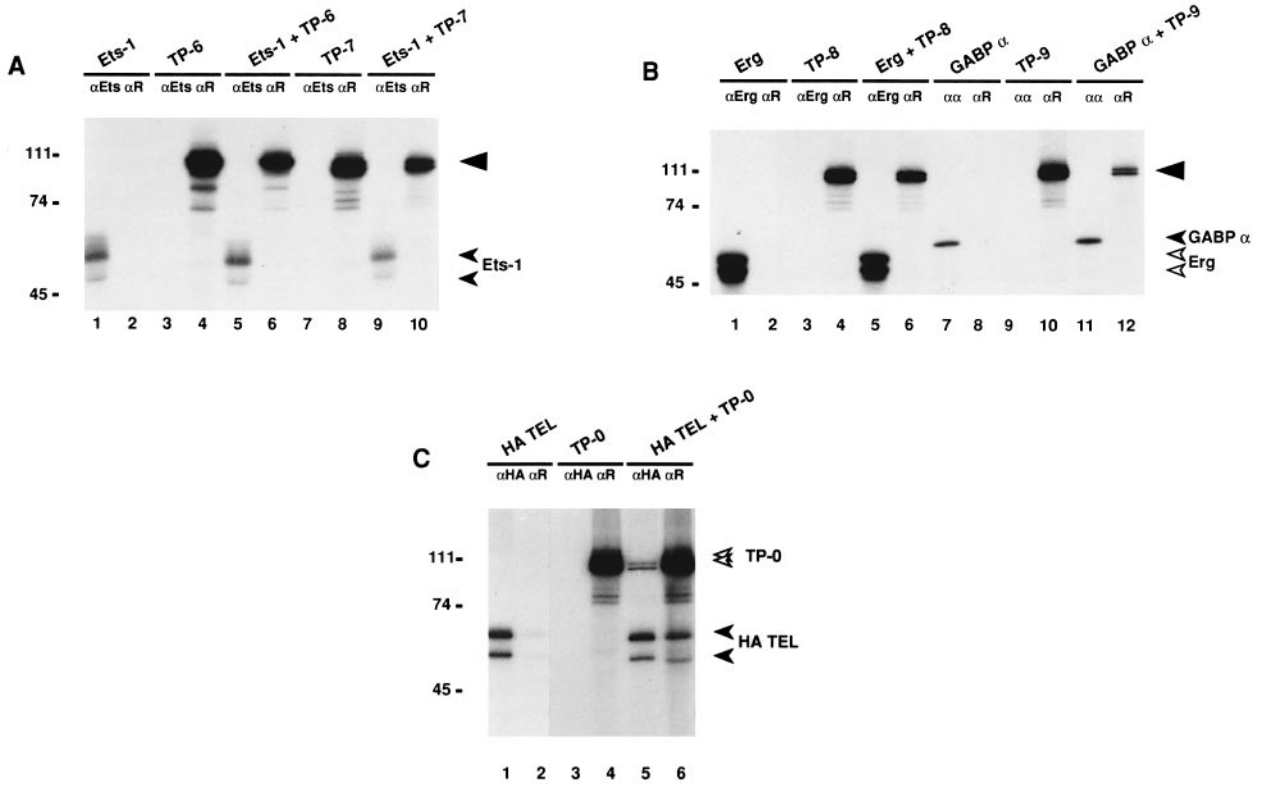


**Fig. 6.** The conserved amino-terminal domains of ETS-1, ERG-2 and GABPα fail to induce complex formation. (**A**) pBS plasmids encoding ETS-1 (lanes 1 and 2), TP-6 (lanes 3 and 4) and TP-7 (lanes 7 and 8) were translated alone following coupled transcription/translation in rabbit reticulocyte lysates in the presence of L-[$^{35}$S]methionine. Co-translations of either ETS-1 and TP-6 (lanes 5 and 6) or ETS-1 and TP-7 (lanes 9 and 10) were obtained in parallel. Translated proteins were immunoprecipitated using either the rabbit antiserum to the DNA binding domain of ETS-1 (odd-numbered lanes) or the rabbit antiserum to the carboxy-terminus of PDGFRβ (even-numbered lanes). (**B**) pBS plasmids encoding ERG-2 (lanes 1 and 2), TP-8 (lanes 3 and 4), GABPα (lanes 7 and 8) and TP-9 (lanes 9 and 10) were translated alone following coupled transcription/translation in rabbit reticulocyte lysate in the presence of L-[$^{35}$S]methionine. Co-translations of either ERG-2 and TP-8 (lanes 5 and 6) or GABPα and TP-9 (lanes 11 and 12) were obtained in parallel. Translated proteins were immunoprecipitated using the rabbit antiserum to the carboxy-terminus of ERG-2 (lanes 1, 3 and 5), the rabbit antiserum to GABPα (lanes 7, 9 and 11) and the rabbit antiserum to the carboxy-terminus of PDGFRβ (lanes 2, 4, 6, 8, 10 and 12). The large arrowheads point to the position of TEL–PDGFRβ substitution mutants. (**C**) pBS plasmids encoding either HA-TEL (lanes 1 and 2) or TP-0 (lanes 3 and 4) were translated alone following coupled transcription/translation in rabbit reticulocyte lysates in the presence of L-[$^{35}$S]methionine. Co-translation of HA-TEL and TP-0 was obtained in parallel. Translated proteins were immunoprecipitated using the HA-specific monoclonal antibody (odd-numbered lanes) or the rabbit antibody to the carboxy-terminus of PDGFRβ (even-numbered lanes).

or higher order oligomer, a property which might favor its binding to promoters/enhancers displaying tandemly arranged ETS binding sites and contribute, therefore, to its specificity with respect to other members of the family. This situation is reminiscent of the DNA binding properties of GABP and its human E4TF1/NRF-2 homolog (LaMarco *et al.*, 1991; Watanabe *et al.*, 1993) in which an ETS family α subunit is complexed to either of two isoforms of a β subunit. One of these isoforms include a carboxy-terminal dimerization domain which allows the formation of $\alpha_2\beta_2$ tetramers which display preferred DNA binding activity to tandem ETS binding sites (Thompson *et al.*, 1991; Brown and McKnight, 1992; Virbasius *et al.*, 1993; Gugneja *et al.*, 1995). Experiments are in progress to determine whether oligomerization of TEL is involved in the regulation of its DNA binding activity and DNA binding specificity.

Leukemic cells of childhood B cell acute lymphoblastic leukemias with t(12;21) express a specific TEL–AML1 fusion protein in which the 333 amino-terminal residues of TEL are fused to the N-terminus of AML1 (Golub *et al.*, 1995; Romana *et al.*, 1995). TEL–AML1 has been shown recently to interfere with the AML1B-dependent activation of the T-cell receptor β enhancer in transient transfection assays. Furthermore, a region including the TEL oligomerization domain identified here was found to be essential for TEL–AML1-mediated repression (Hiebert *et al.*, 1996). We found that substitution of the amino-terminal conserved domain of ETS-1 by that of TEL in TEL(1–119)/ETS-1(130–441) and HA-TEL(54–119)/ETS-1(130–441) resulted in the conversion of ETS-1 from an activator into a repressor of ETS-1-responsive model reporter genes (data not shown). These observations suggest that the TEL conserved amino-terminal domain either encodes a *bona fide* repression domain or acts by masking the AML1-B and ETS-1 activation domains through oligomerization. TEL is more closely related to *D.melanogaster* YAN/POK than to any other member of the ETS family in both its DNA binding and amino-terminal conserved domains. Genetically, YAN has been shown to be a negative regulator of R7 photoreceptor cell development, acting antagonistically to the Sevenless/Ras/

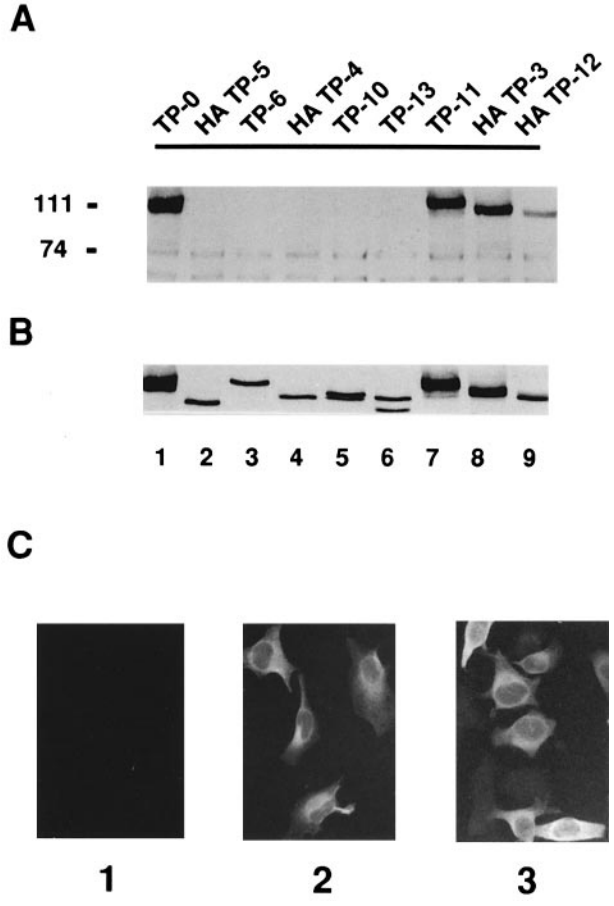


**Fig. 7.** Complex formation by TEL–PDGFRβ is essential to activation of its tyrosine kinase activity and does not affect its subcellular localization. pBS encoding TP-0, HA-TP-5, TP-6, HA-TP-4, TP-10, TP-13, TP-11, HA-TP-3 and HA-TP-12 were translated following coupled transcription/translation in rabbit reticulocyte lysates (lanes 1–9). Proteins were separated by polyacrylamide gel electrophoresis and blotted on nitrocellulose. (**A**) The blot was revealed with a phosphotyrosine-specific monoclonal antibody (4G10) to analyze protein kinase activity. (**B**) The blot was revealed with the rabbit antiserum to the carboxy-terminus of PDGFRβ to analyze expression levels. (**C**) Subcellular localization of TP-0 and TP-10. HeLa cells were transfected with the control ΔEB expression plasmid (1) or the expression plasmids for either TP-0 (2) or TP-10 (3) and seeded on a Lab-Tek slide. After 24 h, cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100 and incubated with the polyclonal anti-PDGFRβ rabbit antiserum followed by a fluorescein isothiocyanate-conjugated goat serum to rabbit immunoglobulin G. Epifluorescence analysis was performed at a ×200 magnification.

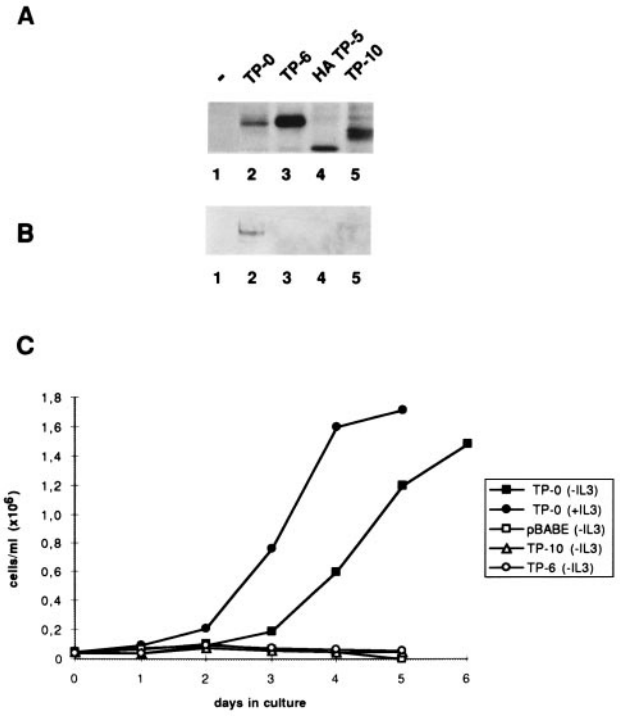


**Fig. 8.** Expression of TEL–PDGFRβ relieves the requirement of Ba/F3 cells for IL-3 for long-term proliferation. (**A**) Ba/F3 cells transfected with the pBabeNeo control plasmid (lane 1) or pBabeNeo derivatives encoding TP-0 (lane 2), TP-6 (lane 3), HA-TP-5 (lane 4) and TP-10 (lane 5) were metabolically labeled with L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine for 60 min and lysed. Immunoprecipitations were carried out on $6\times10^7$ acid-insoluble radioactive counts of each lysates using the polyclonal anti-PDGFRβ rabbit antiserum and analyzed by electrophoresis on polyacrylamide gels, followed by autoradiography. (**B**) Unlabeled Ba/F3 cells transfected with the pBabeNeo control plasmid (lane 1) or pBabeNeo derivatives encoding TP-0 (lane 2), TP-6 (lane 3), HA-TP-5 (lane 4) and TP-10 (lane 5) were lysed and immunoprecipitated as in (A). Immunoprecipitated proteins were separated by polyacrylamide electrophoresis, blotted on nitrocellulose and revealed with the phosphotyrosine-specific monoclonal antibody 4G10. (**C**) For proliferation assay, TP-0-expressing Ba/F3 cells were plated at $5\times10^4$ cells/ml in the presence or absence of IL-3. Control Ba/F3 cells and cells expressing TP-6 and TP-10 were seeded in parallel in the absence of IL-3. The number of viable cells was counted at various times using an electronic cell counter (CASY-1, Schärfe System, FRG).

MAP kinase proneural differentiation pathway (O'Neil *et al.*, 1994). Biochemical evidence indicates that YAN can function as a repressor of ETS-responsive reporter genes and that phosphorylation of YAN by Rolled/MAP kinase on several residues has been proposed to down-regulate its activity by decreasing its stability and by altering its subcellular localization (O'Neil *et al.*, 1994; Rebay and Rubin, 1995). In line with its close structural relatedness to the TEL oligomerization domain, we found that the amino-terminal conserved domain of YAN also appears to be able to induce homotypic oligomerization, although the stability of YAN oligomers is significantly weaker than that of TEL oligomers (data not shown). It would be interesting to analyze whether the conserved amino-terminal domain of YAN is important for its function in the Sevenless signaling pathway and, if so, whether it can be substituted by that of TEL. Like YAN, TEL contains several potential MAP kinase phosphorylation sites and is a phosphoprotein *in vivo* (Poirel *et al.*, 1997). It is possible, therefore, that both YAN and TEL normally function as signal-regulated transcriptional repressors and that this activity depends upon their oligomerization properties.

The result of the t(5;12) chromosomal translocation in the leukemic cells of CMML is the expression of a fusion protein in which the extracellular ligand binding domain of PDGFRβ is substituted by the first 154 amino acid residues of TEL. Our results show that deletion of the PDGFRβ extracellular domain (mutant TP-5) is not sufficient to explain the constitutive autophosphorylation and the mitogenic properties of TEL–PDGFRβ. They support instead the notion that these properties are critically dependent upon TEL-mediated homotypic oligomerization of TEL–PDGFRβ. TEL–PDGFRβ forms oligomers in

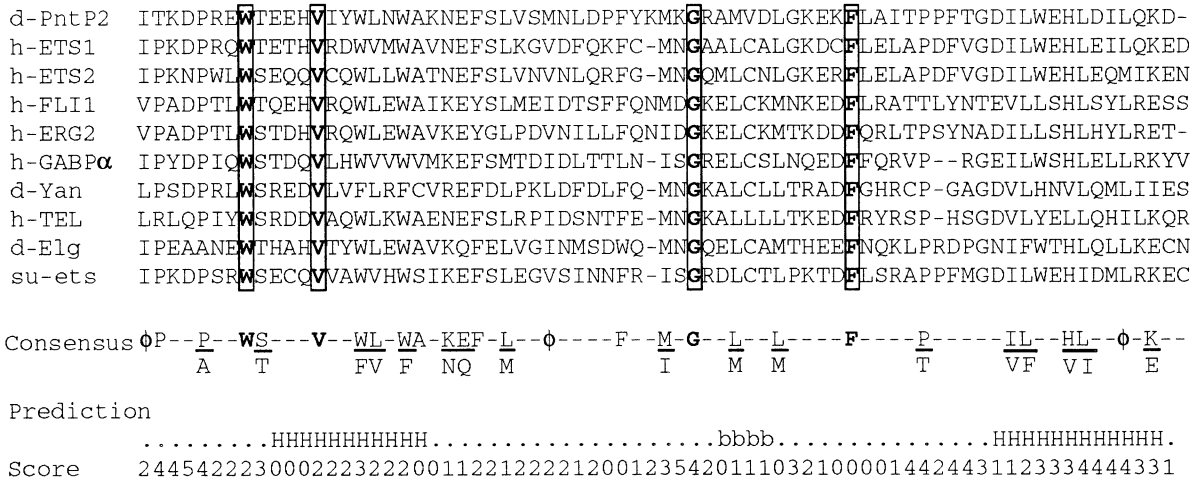


**Fig. 9.** Multiple sequence alignment of the amino-terminal conserved domain of human and invertebrate ETS proteins. The CLUSTAL W program (Thompson *et al.*, 1994) was used to align the amino acid sequences (single letter code) of human ETS-1, ETS-2, FLI-1, ERG-2, GABPα and TEL; *D.melanogaster* PNTP2, YAN/POK and ELG; and sea urchin *S.purpuratus* ETS protein (NCBI, accession No. L19541). Invariant residues are boxed and positions displaying conservative substitution are indicated in the consensus lane. The secondary structure prediction were made using the SIMPA 95 program (Levin and Garnier, 1988; J.Levin, in preparation). The prediction method is based on a search for local similarity between the test sequence and a database of known structures and has a prediction accuracy of 68% (Levin *et al.*, 1993). By using the extra information available in a multiple alignment, which assumes that equivalent positions have the same secondary structure, the prediction accuracy rises to 72% for a three state prediction (helix, strand and coil). A scale from 0 to 6 indicates the strength of the prediction.

stably transfected Ba/F3 cells (data not shown), and transformation by TEL–PDGFRβ is likely to be linked to the intermolecular cross-phosphorylation of each subunit in the oligomer on specific tyrosine residues, resulting in further activation of the catalytic activity of TEL–PDGFRβ and in the generation of specific binding sites for critical substrates containing SH2 domains. The protein kinase activity of TEL–PDGFRβ is essential to its mitogenic properties since a tyrphostin inhibitor specific for the PDGF receptor kinase specifically inhibits the IL-3-independent proliferation in Ba/F3 cells (A.Ostman, J.Ghysdael and C.H.Heldin, in preparation). In that respect, activation of TEL–PDGFRβ appears to recapitulate in a constitutive manner the activation of PDGFRβ which follows ligand binding (Bishayee *et al.*, 1989; Heldin *et al.*, 1989). It is also reminiscent of the oncogenic activation of other receptor-type protein kinases as the result of constitutive oligomerization either by mutation, as in the case of c-ErbB-2/Neu (Bargmann *et al.*, 1986; Weiner *et al.*, 1989), or by fusion to unrelated oligomerization domains including the TPR sequences of TPR–MET and TPR–TRK (Greco *et al.*, 1992; Rodrigues and Park, 1993) or the regulatory subunit of cAMP-dependent protein kinase in PTC-RET (Grieco *et al.*, 1990; Bongarzone *et al.*, 1993).

Several observations suggest that PDGFRβ signaling could play a role in hematopoietic cell proliferation. First, low level expression of PDGFRβ has been observed in murine myeloblastic, mast and multipotential cell lines (de Parseval *et al.*, 1993) and during the differentiation of primary monocytes and of cell lines THP1 and HL60 (Pantazis *et al.*, 1991; Inaba *et al.*, 1993). Second, PDGF treatment in several of these situations has been shown to induce a mitogenic response in both immature and mature cells (de Parseval *et al.*, 1993; Inaba *et al.*, 1993). It is possible, therefore, that the mitogenic properties of TEL–PDGFRβ might be mimicking, in a constitutive manner, normal PDGFRβ signaling. The situation is probably more complex since our immunofluorescence studies (Figure 7C) and subcellular fractionation experiments (data not shown) show that TEL–PDGFRβ is essentially cytoplasmic and is therefore likely to access or phosphorylate protein substrates other than those normally involved in PDGFRβ signaling.

Recent evidence has shown that optimal proliferation of Ba/F3 cells in response to cytokine requires at least three distinct and independent signaling pathways (Miyazaki *et al.*, 1995). Constitutive activation of any of two of these pathways is sufficient to induce cytokine-independent proliferation of Ba/F3 cells, albeit at a reduced rate. Expression of TEL–PDGFRβ in Ba/F3 cells was found to induce their proliferation at a lower rate as compared with Ba/F3 cells maintained in IL-3, suggesting that TEL–PDGFRβ only activates a subset of the signaling pathways required for maximal proliferation. TEL–PDGFRβ-expressing Ba/F3 cells could, therefore, provide a useful system to identify other pathways which can cooperate with TEL–PDGFRβ-dependent signaling to induce proliferation. Identification of the components of these pathways could help to identify the molecular events which cooperate with TEL–PDGFRβ in human leukemias.

## Materials and methods

### *Construction of recombinants*

To generate the HA epitope-tagged version of TP-0, the cDNA encoding TEL–PDGFRβ (Golub *et al.*, 1994) was inserted into *Eco*RI- and *Hin*dIII-restricted M13mp18. The single-stranded M13 recombinant DNA was used as substrate for oligonucleotide site-directed mutagenesis to create a *Sal*I restriction enzyme site immediately downstream of the initiator methionine codon (mutagenic primer M1). The insert was recovered from the corresponding replicative form (RF) by digestion with *Sal*I and *Hin*dIII and subcloned between the *Xho*I and *Hin*dIII sites of the previously described SV40 early promoter-based expression plasmid ΔEB-HA (Rabault and Ghysdael, 1994). A pBluescript KS(+) allowing the T3-driven expression of the 3′-truncated HA-TP-0 (t) was obtained by digestion of ΔEB-HA-TP-0 with *Eco*RI and *Apa*I and subcloning into the *Eco*RI- and *Apa*I-restricted pBluescript KS(+). This truncated form of TP-0 only included the 133 amino-terminal residues

of the PDGFRβ-derived domain of TEL–PDGFRβ. Mutant TP-1 was constructed from the same M13 DNA template using the mutagenic primer M2 to create a *Bgl*II restriction enzyme site at nucleotide position 382 of TEL. These mutations resulted in a His→Gln substitution at codon 119 of TEL. The RF containing the mutagenized insert was digested with *Eco*RI and *Apa*I and subcloned into *Eco*RI- and *Apa*I-restricted pBluescript KS(+) to generate TP-1 (t). Mutant TP-2 was created by site-directed mutagenesis of the M13 DNA template containing TP-1 to create a *Bam*HI restriction enzyme site at nucleotide position 179 using the mutagenic primer M3. These mutations resulted in Ala→Gly and His→Ile substitutions at codons 52 and 53 of TEL, respectively. The substitution in TP-2 did not affect its oligomerization properties as compared with TP-0 (data not shown). The RF containing the mutagenized insert was digested with *Eco*RI and *Apa*I and subcloned into *Eco*RI- and *Apa*I-restricted pBluescript KS(+) to generate TP-2 (t). Mutant HA-TP-3 (t) was obtained by substitution of the *Eco*RI–*Bam*HI fragment of pBS-TP-2 (t) with the *Eco*RI–*Bgl*II fragment of ΔEB-HA. Mutant HA-TP-4 (t) was obtained by substitution of the *Eco*RI–*Bgl*II fragment of pBS-TP-2 (t) with the *Eco*RI–*Bgl*II fragment of ΔEB-HA. Mutant HA-TP-5 (t) was obtained by insertion of a *Bgl*II-bordered insert obtained by PCR amplification of PDGFRβ sequences encoding amino acid residues 527–660 into *Bgl*II-restricted ΔEB-HA. The amplimers used for PCR amplification were 5′-GAAGATTCTTCGGGCCCAA-GGTGACTCATGATCTCT-3′ (5′ amplimer) and 5′-GGAGATCTTCT-CCTTGCCCTTTAAGGTGGTGGTG-3′ (3′ amplimer). The HA-tagged TP-5 (t) was subcloned via *Eco*RI and *Apa*I into *Eco*RI- and *Apa*I-restricted pBluescript SK(+). To generate TP-6 (t), the *Eco*RI–*Bgl*II fragment obtained from the 5′ part of a chicken c-*ets1* cDNA (Boulukos *et al.*, 1988) was inserted into the *Eco*RI- and *Bgl*II-restricted pBS-TP-1 (t). Mutants TP-7 (t), TP-8 (t) and TP-9 (t) were obtained by substitution of the *Bam*HI–*Bgl*II fragment of TP-2 (t) by fragments bordered by in-frame *Bam*HI (5′ side) and *Bgl*II (3′ side) generated by PCR amplification of the chicken ETS-1 cDNA (Boulukos *et al.*, 1988), human ERG-2 cDNA (Reddy *et al.*, 1987 a generous gift of Dr E.Reddy) and mouse GABPα cDNA (LaMarco *et al.*, 1991 a generous gift of Drs Brown and S.McKnight), respectively. The amplimers used for PCR amplification were: ETS-1 5′, 5′-CAGGATCCTCCCCAAAGATCCCCAGCAGTG-3′; ETS-1 3′, 5′-GGAGATCTTCTCCAGGTGTTCCCAAAGGATATC-3′; ERG 5′, 5′-CAGGATCCTGCCAGCAGATCCTACGCTATGGAGT-3′; ERG 3′, 5′-GGAGATCTGGTGGAGATGTGAG AGAAGGATG-TCG-3′; GABPα 5′, 5′-CAGGATCCTCCCCTATGATCCTATACGC-TGG-3′; GABPα 3′, 5′-GGAGATCTGCTCCAGATGACTCCAAAGA-ATTTCTCC-3′. Mutant TP-10 (t) was obtained by *Bam*HI and *Bgl*II digestion of pBS-TP-2 (t) followed by religation. To generate mutant TP-11 (t), the *Bgl*II–*Apa*I fragment of pBS-TP-2 (t) was substituted by the corresponding fragment of pBS-HA-TP-5 (t). Mutant HA-TP-12 (t) was constructed by substitution of the *Bgl*II–*Apa*I fragment of pBS-HA-TP-3 (t) by the *Bgl*II–*Apa*I fragment of HA-TP-5 (t). Mutant TP-13 (t) was constructed by substitution of the *Bgl*II–*Apa*I fragment of pBS-TP-2 (t) with the *Bgl*II–*Apa*I fragment of pBS-HA-TP-5 (t). All mutants were also constructed so as to contain the full-length PDGFRβ-derived region of TEL–PDGFRβ by insertion of the *Eco*RI–*Sac*II fragment obtained from each mutant into the *Eco*RI- and *Sac*II-restricted HA-TP-0.

The HA-TP-0 and HA-TP-12 were digested with *Eco*RI and *Sac*I, and subcloned into *Eco*RI- and *Sac*I-restricted ΔEB-HA to generate ΔEB-HA-TP-0 (tt) and ΔEB-HA-TP-12 (tt) respectively. pBS SK+ allowing the T3-driven expression of chicken ETS-1 was constructed by inserting the chicken c-*ets1* cDNA insert (Boulukos *et al.*, 1988) at the *Eco*RI of pBS SK+. pBS SK+ allowing expression of HA-TEL was obtained by subcloning the *Eco*RI–*Hin*dIII HA-TEL fragment of ΔEB-HA-TEL encoding the full-length human TEL fused to the HA epitope at its amino-terminus will be described elsewhere. To generate TEL(1–119)/ETS-1(130–441) and HA-TEL(54–119)/ETS-1(130–441), the *Xba*I and *Bgl*II fragments of TP-2 (t) and HA-TP-12 (t) were subcloned into a *Xba*I- and *Bgl*II-restricted pBS SK+ ETS-1, respectively. HA-ETS-1(130–441) was obtained by substitution of the *Xba*I and *Bgl*II fragment of pBS SK+ ETS-1 with the *Xba*I and *Bgl*II HA fragment of HA-TP-0 (t). The eukaryotic expression vectors for these mutants were obtained by *Eco*RI digestion of the corresponding pBS SK+ plasmids and subcloning at the *Eco*RI site of the SV40 early promoter-based expression plasmid ΔEB (Boulukos *et al.*, 1989). To construct the pBabeNeo derivatives, pBS SK+ encoding TP-0, HA-TP-5, TP-6 and TP-10 were digested with *Eco*RI and *Sal*I and the resulting fragments were subcloned into the *Eco*RI- and *Sal*I-restricted pBabeNeo (Morgenstern and Land, 1990). To obtain the GST–TEL (54–119) fusion protein, the cDNA corresponding to the *Bam*HI–*Bgl*II fragment of the TP-2 (t) construct was subcloned at the *Bam*HI site of the pGEX-3X vector (Pharmacia Biotech). The resulting plasmid encoded a protein containing a GST sequence fused to amino acid residues 54–119 of TEL. The sequences of fragments obtained by oligonucleotide site-directed mutagenesis and PCR amplification were checked by sequencing using the dideoxy sequencing method. The sequences of the mutagenic primers used in site-directed mutagenesis were as follows: M1, 5′-GGAGTCTCA-GACATGGTCGACAGCGAGAGAGATCAGG-3′; M2, 5′-CGAGGTT-TCCTCTGCTTCAAGATCTGCTGAAGGAGTTCATAGAGC-3′; M3, 5′-CCAGTAAATTGGC TGCAAGCGCAGGAT CCCAGGCAGGCG-GATCGAGTCTTCC-3′.

### *Transient transfection and immunoprecipitation analyses*

HeLa cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS). For transient expression studies, $7\times10^5$ cells were seeded in 100 mm Petri dishes and transfected 24 h after with 5 μg of the SV40 early promoter-based ΔEB expression plasmid encoding the indicated proteins, using the calcium phosphate co-precipitation method. After overnight incubation, culture media were changed and cells incubated for an additional 36 h before being processed for metabolic labeling.

Ba/F3 cells were maintained in RPMI-1640 medium containing 10% FCS and 5% conditionned medium from the WEHI-3B cell line as a source of IL-3. Cells were transfected by electroporation as described previously (Dusanter-Fourt *et al.*, 1994).

For metabolic labeling with radioactive amino acids, transiently transfected HeLa and stably transfected Ba/F3 cells ($10^7$ cells) were rinsed once in methionine- and cysteine-free Eagle's medium (Flow Laboratories) and incubated for the indicated times in the same medium supplemented with 1% dialyzed FCS and 500 μCi/ml of a L-[$^{35}$S]methionine and L-[$^{35}$S]cysteine mixture (Promix, Amersham). Cells were washed twice in phosphate-buffered saline, lysed in 3 ml of RIPA buffer [Tris–HCl 10 mM, pH 7.4; NaCl 0.1 M; EDTA 0.001 M; Triton X-100 1%; sodium deoxycholate 0.5%; sodium dodecylsulfate 0.1%; aprotinin (Sigma) 1%; phenylmethylsulfonyl fluoride (PMSF) 100 μg/ml and leupeptin (Sigma) 0.1%] and centrifuged at 100 000 *g* for 30 min.

Immunoprecipitation analyses were performed using a rabbit antiserum to the DNA binding domain of ETS-1 (Ghysdael *et al.*, 1986), a rabbit antiserum to the DNA binding domain of TEL (Poirel *et al*., 1997), a monoclonal antibody specific for the influenza HA (12CA5, Boehringer Mannheim) and a rabbit antiserum specific for amino acids 1013–1025 of human PDGFRβ receptor (Upstate Biotechnology Inc.; Claesson-Welsh *et al.*, 1988).

Immunoprecipitates were analyzed by denaturing polyacrylamide gel electrophoresis in the presence of SDS followed by fluorography of the dried gel (Amplify, Amersham).

### *In vitro translation*

The cDNAs encoding TEL, ETS-1, ERG-2, GABPα and TEL–PDGFRβ (Golub *et al.*, 1994) and their derivatives were subcloned into pBluescript SK (+) (Stratagene). *In vitro* T3-driven transcription and translation were performed in micrococcal nuclease-treated reticulocyte lysates using the TNT-coupled transcription/translation kit (Promega) using L-[$^{35}$S]methionine as radioactive tracer amino acid. An aliquot (2 μl) of the programed lysate was analyzed directly by polyacrylamide gel electrophoresis, the remainder of the lysate was diluted in 10 volumes of RIPA buffer, centrifuged at 15 000 *g* for 10 min and aliquots of the supernatant were subjected to immunoprecipitation analyses.

Immunoblotting analyses were performed after electrophoretic transfer of proteins separated by SDS–PAGE on nitrocellulose membrane and incubation in the presence of 1 μg/ml of the 4G10 anti-phosphotyrosine monoclonal antibody (Upstate Biotechnology Inc.) or a 1/100 dilution of the rabbit anti-human PDGFRβ. Bands were visualized using the ECL chemiluminescence kit (Amersham).

### *In vitro binding experiments on glutathione–agarose beads*

For GST fusion protein overexpression, *Escherichia coli* Dh5α cells transformed with the indicated plasmid were induced with 0.1 mM IPTG for 1 h at 37°C. Proteins were extracted as described by Frangioni and Neel (1993). Protein association experiments were performed on glutathione–agarose beads using GST fusion proteins.

For binding experiments, 10 μl of a standard *in vitro* translation reaction was incubated overnight at 4°C with 10 μl of beads in the presence of 1 mg/ml bovine serum albumin and 1 mM PMSF. After centrifugation, the supernatant (unbound fraction) was removed and the beads were washed three times with RIPA buffer. The bound proteins were eluted from the beads by boiling in SDS–PAGE sample buffer. Equivalent amounts of the unbound, wash and elution fractions were

analyzed by polyacrylamide gels electrophoresis. After staining with Coomassie blue to detect GST fusion proteins, gels were treated with Amplify (Amersham Corp.), dried and analyzed by fluorography.

### *Immunofluorescence analyses*

Immunofluorescence analyses were performed on HeLa cells transfected with 1 μg of either the ΔEB-TP-0 or ΔEB-TP-10 expression plasmids, or ΔEB as control. Cells were fixed in 4% paraformaldehyde, permeabilized using 0.2% Triton X-100 and analyzed by indirect immunonofluorescence as previously described (Bailly *et al.*, 1994).

## Acknowledgements

The authors thank D.Bohmann, T.A.Brown, G.Gilliland, C.H.Heldin, G.Mavrothalassitis, S.L.McKnight, P.Mayeux and E.Reddy for reagents; M.Yaniv and C.H.Heldin for helpful discussions; M.Pironin for expert technical assistance; F.Arnouilh for patient typing; and G.Calothy and F.Lamballe for critical reading of the manuscript. C.J., C.C., A.B. and C.T.Q. are predoctoral fellows of the French MESR; C.O. holds a post-doctoral fellowship from the EEC. This work was supported by funds from the Centre National de la Recherche Scientifique, Institut Curie, Ligue Nationale contre le Cancer (Axe Oncogénèse et Réseaux de Signalisation), Association pour la Recherche sur le Cancer, Fondation contre la Leucémie and EEC (Biomed, Contract BMH4-CT96-1355).

## References

Bailly,R., Bosselut,R., Zucman,J., Cormier,F., Delattre,O., Roussel,M., Thomas,G. and Ghysdael,J. (1994) DNA-binding and transcriptional activation properties of the EWS–FLI-1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. *Mol. Cell. Biol.*, **14**, 3230–3241.

Bargmann,C.I., Hung,M.C. and Weinberg,R.A. (1986) Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185. *Cell*, **45**, 649–657.

Ben-David,Y., Giddens,E., Letwin,K. and Bernstein,A. (1991) Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the *ets* gene family, *Fli-1*, closely linked to c-*ets-1*. *Genes Dev.*, **5**, 908–918.

Bishayee,S., Majumdar,S., Khire,J. and Das,M. (1989) Ligand-induced dimerization of the platelet-derived growth factor receptor. Monomer–dimer interconversion occurs independently of receptor phosphorylation. *J. Biol. Chem.*, **264**, 11699–11705.

Bongarzone,I., Monzini,N., Borrello,M.G., Carcano,C., Ferraresi,G., Arighi,E., Mondellini,P., Della Porta,G. and Pierotti,M.A. (1993) Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI alpha of cyclic AMP-dependent protein kinase A. *Mol. Cell. Biol.*, **13**, 358–366.

Bories,J.C., Willerford,D.M., Grevin,D., Davidson,L., Camus,A., Martin,P., Stehelin,D. and Alt,F.W. (1995) Increased T-cell apoptosis and terminal B-cell differentiation induced by inactivation of the *Ets-1* proto-oncogene. *Nature*, **377**, 635–638.

Boulukos,K., Pognonec,P., Begue,A., Galibert,F., Gesquiere,J., Stehelin,D. and Ghysdael,J. (1988) Identification in chickens of an evolutionarily conserved cellular *ets-2* gene (c-*ets-2*) encoding nuclear proteins related to the products of the c-*ets* proto-oncogene. *EMBO J.*, **7**, 697–705.

Boulukos,K., Pognonec,P., Rabault,B., Begue,A. and Ghysdael,J. (1989) Definition of an Ets1 protein domain required for nuclear localization in cells and DNA-binding activity *in vitro*. *Mol. Cell. Biol.*, **9**, 5718–5721.

Brown,T.A. and McKnight,S.L. (1992) Specificities of protein–protein and protein–DNA interaction of GABP alpha and two newly defined ets-related proteins. *Genes Dev.*, **6**, 2502–2512.

Brunner,D., Ducker,K., Oellers,N., Hafen,E., Scholz,H. and Klambt,C. (1994) The ETS domain protein pointed-P2 is a target of MAP kinase in the sevenless signal transduction pathway. *Nature*, **370**, 386–389.

Buijs,A. *et al*. (1995) Translocation (12;22) (p13;q11) in myelo-proliferative disorders results in fusion of the ETS-like *TEL* gene on 12p13 to the *MN1* gene on 22q11. *Oncogene*, **10**, 1511–1519.

Claesson-Welsh,L., Eriksson,A., Moren,A., Severinsson,L., Ek,B., Ostman,A., Betsholtz,C. and Heldin,C.H. (1988) cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. *Mol. Cell. Biol.*, **8**, 3476–3486.

Daley,G.Q. and Baltimore,D. (1988) Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. *Proc. Natl Acad. Sci. USA*, **85**, 9312–9316.

Daley,G.Q., Van Etten,R.A., Jackson,P.K., Bernards,A. and Baltimore,D. (1992) Nonmyristoylated Abl proteins transform a factor-dependent hematopoietic cell line. *Mol. Cell. Biol.*, **12**, 1864–1871.

Dalton,S. and Treisman,R. (1992) Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element. *Cell*, **68**, 597–612.

Damen,J., Wakao,H., Miyajima,A., Krosl,J., Humphries,K., Cutler,R. and Krystal,G. (1995) Tyrosine 343 in the erythropoietin receptor positively regulates erythropoietin-induced cell proliferation and Stat 5 activation. *EMBO J.*, **14**, 5557–5568.

de Parseval,N., Fichelson,S., Mayeux,P., Gisselbrecht,S. and Sola,B. (1993) Expression of functional beta-platelet-derived growth factor receptors on hematopoietic cell lines. *Cytokine*, **5**, 8–15.

Delattre,O. *et al*. (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature*, **359**, 162–165.

Delgado,M., Hallier,M., Meneceur,P., Tavitian,A. and Moreau-Gachelin,F. (1994) Inhibition of Friend cells proliferation by spi-1 antisense oligodeoxynucleotides. *Oncogene*, **9**, 1723–1727.

Donaldson,L.W., Petersen,J.M., Graves,B.J. and McIntosh,L.P. (1994) Secondary structure of the ETS domain places murine Ets-1 in the superfamily of winged helix–turn–helix DNA-binding proteins. *Biochemistry*, **33**, 13509–13516.

Donaldson,L.W., Petersen,J.M., Graves,B.J. and McIntosh,L.P. (1996) Solution structure of the ETS domain from murine Ets-1: a winged helix–turn–helix DNA binding motif. *EMBO J.*, **15**, 125–134.

Dusanter-Fourt,I. *et al*. (1994) Identification of JAK protein tyrosine kinases as signaling molecules for prolactin. Functional analysis of prolactin receptor and prolactin–erythropoietin receptor chimera expressed in lymphoid cells. *EMBO J.*, **13**, 2583–2591.

Frangioni,J.V. and Neel,B.G. (1993) Solubiliziation and purification of enzymatically active glutathione *S*-transferase (pGex) fusion proteins. *Anal. Biochem.*, **210**, 179–187.

Gegonne,A., Punyammalee,B., Rabault,B., Bosselut,R., Seneca,S., Crabeel,M. and Ghysdael,J. (1992) Analysis of the DNA binding and transcriptional activation properties of the Ets1 oncoprotein. *New Biol.*, **4**, 512–519.

Gegonne,A., Bosselut,R., Bailly,R.A. and Ghysdael,J. (1993) Synergistic activation of the HTLV1 LTR Ets-responsive region by transcription factors Ets1 and Sp1. *EMBO J.*, **12**, 1169–1178.

Ghysdael,J., Gegonne,A., Pognonec,P., Boulukos,K., Leprince,D., Dernis,D., Lagrou,C. and Stehelin,D. (1986) Identification in chicken macrophages of a set of proteins related to, but distinct from, the chicken cellular c-*ets*-encoded protein p54c-ets. *EMBO J.*, **5**, 2251–2256.

Giese,K., Kingsley,C., Kirshner,J.R. and Grosschedl,R. (1995) Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein–protein interactions. *Genes Dev.*, **9**, 995–1008.

Gobert,S. *et al*. (1995) Tyrosine phosphorylation of the erythropoietin receptor: role for differentiation and mitogenic signal transduction. *Blood*, **86**, 598–606.

Golub,T.R., Barker,G.F., Lovett,M. and Gilliland,D.G. (1994) Fusion of PDGF receptor beta to a novel *ets*-like gene, *tel*, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell*, **77**, 307–316.

Golub,T.R. *et al*. (1995) Fusion of the *TEL* gene on 12p13 to the *AML1* gene on 21q22 in acute lymphoblastic leukemia. *Proc. Natl Acad. Sci. USA*, **92**, 4917–4921.

Greco,A., Pierotti,M.A., Bongarzone,I., Pagliardini,S., Lanzi,C. and Della Porta,G. (1992) *TRK-T1* is a novel oncogene formed by the fusion of *TPR* and *TRK* genes in human papillary thyroid carcinomas. *Oncogene*, **7**, 237–242.

Grieco,M. *et al*. (1990) PTC is a novel rearranged form of the *ret* proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell*, **60**, 557–563.

Gugneja,S., Virbasius,J.V. and Scarpulla,R.C. (1995) Four structurally distinct, non-DNA-binding subunits of human nuclear respiratory factor 2 share a conserved transcriptional activation domain. *Mol. Cell. Biol.*, **15**, 102–111.

Heldin,C.H. (1995) Dimerization of cell surface receptors in signal transduction. *Cell*, **80**, 213–223.

Heldin,C.H., Ernlund,A., Rorsman,C. and Ronnstrand,L. (1989)

Dimerization of B-type platelet-derived growth factor receptors occurs after ligand binding and is closely associated with receptor kinase activation. *J. Biol. Chem.*, **264**, 8905–8912.

Hiebert,S.W. *et al*. (1996) The t(12;21) translocation converts AML-1B from an activator to a repressor of transcription. *Mol. Cell. Biol.*, **15**, 1786–1796.

Hill,C.S. and Treisman,R. (1995) Differential activation of c-fos promoter elements by serum, lysophosphatidic acid, G proteins and polypeptide growth factors. *EMBO J.*, **14**, 5037–5047.

Hipskind,R., Rao,V., Mueller,C., Reddy,E. and Nordheim,A. (1991) Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. *Nature*, **354**, 531–534.

Ichikawa,H., Shimizu,K., Hayashi,Y. and Ohki,M. (1994) An RNA-binding protein gene, *TLS/FUS*, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. *Cancer Res.*, **54**, 2865–2868.

Inaba,T. *et al*. (1993) Expression of platelet-derived growth factor beta receptor on human monocyte-derived macrophages and effects of platelet-derived growth factor BB dimer on the cellular function. *J. Biol. Chem.*, **268**, 24353–24360.

Janknecht,R., Ernst,W.H. and Nordheim,A. (1995) SAP1α is a nuclear target of signaling cascades involving ERKs. *Oncogene*, **10**, 1209–1216.

Jeon,I.S., Davis,J.N., Braun,B.S., Sublett,J.E., Roussel,M.F., Denny,C.T. and Shapiro,D.N. (1995) A variant Ewing's sarcoma translocation (7; 22) fuses the *EWS* gene to the *ETS* gene *ETV1*. *Oncogene*, **10**, 1229–1234.

John,S., Reeves,R.B., Lin,J.X., Child,R., Leiden,J.M., Thompson,C.B. and Leonard,W.J. (1995) Regulation of cell-type-specific interleukin-2 receptor alpha-chain gene expression: potential role of physical interactions between Elf-1, HMG-I(Y), and NF-kappa B family proteins. *Mol. Cell. Biol.*, **15**, 1786–1796.

Klambt,C. (1993) The *Drosophila* gene *pointed* encodes two ETS-like proteins which are involved in the development of the midline glial cells. *Development*, **117**, 163–176.

Kodandapani,R., Pio,F., Ni,C., Picciali,G., Klemsz,M., McKercher,S., Maki,R.A. and Ely,K.R. (1996) A new pattern for helix-turn-helix recognition revealed by the PU.1 ETS domain–DNA complex. *Nature*, **380**, 456–460.

LaMarco,K., Thompson,C.C., Byers,B.P., Walton,E.M. and McKnight, S.L. (1991) Identification of Ets- and notch-related subunits in GA binding protein. *Science*, **253**, 789–792.

Leprince,D., Gegonne,A., Coll,J., de Taisne,C., Schneeberger,A., Lagrou,C. and Stehelin,D. (1983) A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. *Nature*, **306**, 395–397.

Levin,J.M. and Garnier,J. (1988) Improvements in a secondary structure prediction method based on a search for local sequence homologies and its use as a model building tool. *Biochim. Biophys. Acta*, **955**, 283–295.

Levin,J.M., Pascarella,S., Argos,P. and Garnier,J. (1993) Quantification of secondary structure prediction improvement using multiple alignments. *Protein Engng*, **6**, 849–854.

Liang,H., Mao,X., Olejniczak,E.T., Nettesheim,D.G., Yu,L., Meadows, R.P., Thompson,C.B. and Fesik,S.W. (1994) Solution structure of the ets domain of Fli-1 when bound to DNA. *Nature Struct. Biol.*, **1**, 871–875.

Ma,P.C., Rould,M.A., Weintraub,H. and Pabo,C.O. (1994) Crystal structure of MyoD bHLH domain–DNA complex: perspectives on DNA recognition and implications for transcriptional activation. *Cell*, **77**, 451–459.

Mathey-Prevot,B., Nabel,G., Palacios,R. and Baltimore,D. (1986) Abelson virus abrogation of interleukin-3 dependence in a lymphoid cell line. *Mol. Cell. Biol.*, **6**, 4133–4135.

May,W.A., Gishizky,M.L., Lessnick,S.L., Lunsford,L.B., Lewis,B.C., Delattre,O., Zucman,J., Thomas,G. and Denny,C.T. (1993a) Ewing sarcoma 11;22 translocation produces a chimeric transcription factor that requires the DNA-binding domain encoded by FLI1 for transformation. *Proc. Natl Acad. Sci. USA*, **90**, 5752–5756.

May,W.A., Lessnick,S.L., Braun,B.S., Klemsz,M., Lewis,B.C., Lunsford, L.B., Hromas,R. and Denny,C.T. (1993b) The Ewing's sarcoma *EWS/FLI-1* fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than *FLI-1*. *Mol. Cell. Biol.*, **13**, 7393–7398.

McWhirter,J.R. and Wang,J.Y. (1993) An actin-binding function contributes to transformation by the Bcr–Abl oncoprotein of Philadelphia chromosome-positive human leukemias. *EMBO J.*, **12**, 1533–1546.

McWhirter,J.R., Galasso,D.L. and Wang,J.Y. (1993) A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr–Abl oncoproteins. *Mol. Cell. Biol.*, **13**, 7587–7595.

Metz,T. and Graf,T. (1991a) Fusion of the nuclear oncoproteins v-Myb and v-Ets is required for the leukemogenicity of E26 virus. *Cell*, **66**, 95–105.

Metz,T. and Graf,T. (1991b) v-*myb* and v-*ets* transform chicken erythroid cells and cooperate both in *trans* and in *cis* to induce distinct differentiation phenotypes. *Genes Dev.*, **5**, 369–380.

Miyazaki,T., Liu,Z.J., Kawahara,A., Minami,Y., Yamada,K., Tsujimoto,Y., Barsoumian,E.L., Permutter,R.M. and Taniguchi,T. (1995) Three distinct IL-2 signaling pathways mediated by *bcl-2*, c-*myc*, and *lck* cooperate in hematopoietic cell proliferation. *Cell*, **81**, 223–231.

Moreau-Gachelin,F., Tavitian,A. and Tambourin,P. (1988) *Spi-1* is a putative oncogene in virally induced murine erythroleukaemias. *Nature*, **331**, 277–280.

Morgenstern,J.P. and Land,H. (1990) Advanced mammalian gene transfer: high titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. *Nucleic Acids Res.*, **18**, 3587–3596.

Muthusamy,N., Barton,K. and Leiden,J.M. (1995) Defective activation and survival of T cells lacking the Ets-1 transcription factor. *Nature*, **377**, 639–642.

Nunn,M.F., Seeburg,P.H., Moscovici,C. and Duesberg,P.H. (1983) Tripartite structure of the avian erythroblastosis virus E26 transforming gene. *Nature*, **306**, 391–395.

Nye,J.A., Petersen,J.M., Gunther,C.V., Jonsen,M.D. and Graves,B.J. (1992) Interaction of murine ets-1 with GGA-binding sites establishes the ETS domain as a new DNA-binding motif. *Genes Dev.*, **6**, 975–990.

O'Neil,E., Rebay,I., Tjian,R. and Rubin,G. (1994) The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. *Cell*, **78**, 137–147.

Palacios,R. and Steinmetz,M. (1985) IL-3-dependent mouse clones that express B-220 surface antigen, contain Ig genes in germ-line configuration, and generate B lymphocytes *in vivo*. *Cell*, **41**, 727–734.

Pantazis,P., Kharbanda,S., Goustin,A.S., Galanopoulos,T. and Kufe,D. (1991) Coexpression of the genes for platelet-derived growth factor B-chain receptor and macrophage colony-stimulating factor 1 receptor during monocytic differentiation. *Proc. Natl Acad. Sci. USA*, **88**, 2481–2485.

Papadopoulos,P., Ridge,S.A., Boucher,C.A., Stocking,C. and Wiedemann,L.M. (1995) The novel activation of *ABL* by fusion to an ets-related gene, *TEL*. *Cancer. Res.*, **55**, 34–38.

Poirel,H. *et al*. (1997) The TEL gene products: nuclear proteins with DNA-binding properties. *Oncogene*, in press.

Pongubala,J.M., Nagulapalli,S., Klemsz,M.J., McKercher,S.R., Maki,R.A. and Atchison,M.L. (1992) PU.1 recruits a second nuclear factor to a site important for immunoglobulin kappa 3′ enhancer activity. *Mol. Cell. Biol.*, **12**, 368–378.

Prasad,D.D., Ouchida,M., Lee,L., Rao,V.N. and Reddy,E.S. (1994) TLS/FUS fusion domain of TLS/FUS–erg chimeric protein resulting from the t(16;21) chromosomal translocation in human myeloid leukemia functions as a transcriptional activation domain. *Oncogene*, **9**, 3717–3729.

Rabault,B. and Ghysdael,J. (1994) Calcium-induced phosphorylation of ETS1 inhibits its specific DNA binding activity. *J. Biol. Chem.*, **269**, 28143–28151.

Rabault,B., Roussel,M.F., Tran Quang,C. and Ghysdael,J. (1996) Phosphorylation of Ets1 regulates the complementation of a CSF-1 receptor impaired in mitogenesis. *Oncogene*, **13**, 877–881.

Rao,V.N., Ohno,T., Prasad,D.D., Bhattacharya,G. and Reddy,E.S. (1993) Analysis of the DNA-binding and transcriptional activation functions of human Fli-1 protein. *Oncogene*, **8**, 2167–2173.

Rebay,I. and Rubin,G.M. (1995) Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell*, **81**, 857–866.

Reddy,E.S., Rao,V.N. and Papas,T.S. (1987) The *erg* gene: a human gene related to the *ets* oncogene. *Proc. Natl Acad. Sci. USA*, **84**, 6131–6135.

Rodrigues,G.A. and Park,M. (1993) Dimerization mediated through a leucine zipper activates the oncogenic potential of the met receptor tyrosine kinase. *Mol. Cell. Biol.*, **13**, 6711–6722.

Romana,S.P., Poirel,H., Leconiat,M., Flexor,M.A., Mauchauffe,M., Jonveaux,P., Macintyre,E.A., Berger,R. and Bernard,O.A. (1995) High

frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood*, **86**, 4263–4269.
Schneikert,J., Lutz,Y. and Wasylyk,B. (1992) Two independent activation domains in c-Ets-1 and c-Ets-2 located in non-conserved sequences of the *ets* gene family. *Oncogene*, **7**, 249–256.
Schuetze,S., Stenberg,P.E. and Kabat,D. (1993) The Ets-related transcription factor PU.1 immortalizes erythroblasts. *Mol. Cell. Biol.*, **13**, 5670–5678.
Scott,E.W., Simon,C., Anastasi,J. and Singh,H. (1994) Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Structural basis for DNA bending by the architectural transcription factor LEF-1. *Science*, **265**, 1573–1577.
Seth,A. and Papas,T.S. (1990) The c-*ets-1* proto-oncogene has oncogenic activity and is positively autoregulated. *Oncogene*, **5**, 1761–1767.
Shimizu,K., Ichikawa,H., Tojo,A., Kaneko,Y., Maseki,N., Hayashi,Y., Ohira,M., Asano,S. and Ohki,M. (1993) An *ets*-related gene, *ERG*, is rearranged in human myeloid leukemia with t(16;21) chromosomal translocation. *Proc. Natl Acad. Sci. USA*, **90**, 10280–10284.
Sieweke,M.H., Tekotte,H., Frampton,J. and Graf,T. (1996) MafB is an interaction partner and repressor of Ets-1 that inhibits erythroid differentiation. *Cell*, **85**, 49–60.
Thompson,C.C., Brown,T.A. and McKnight,S.L. (1991) Convergence of Ets- and notch-related structural motifs in a heteromeric DNA binding complex. *Science*, **253**, 762–768.
Thompson,J.D., Higgins,D.G. and Gibson,T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.
Tran Quang,C., Pironin,M., Von Lindern,M., Beug,H. and Ghysdael,J. (1995) Spi-1 and mutant p53 regulate different aspects of the proliferation and differentiation of primary erythroid progenitors. *Oncogene*, **11**, 1229–1239.
Virbasius,J.V., Virbasius,C.A. and Scarpulla,R.C. (1993) Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. *Genes Dev.*, **7**, 380–392.
Wassarman,D.A., Therrien,M. and Rubin,G.M. (1995) The Ras signaling pathway in *Drosophila*. *Curr. Opin. Genet. Dev.*, **5**, 44–50.
Wasylyk,B., Wasylyk,C., Flores,P., Begue,A., Leprince,D. and Stehelin,D. (1990) The c-*ets* proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature*, **346**, 191–193.
Wasylyk,B., Hahn,S.L. and Giovane,A. (1993) The Ets family of transcription factors. *Eur. J. Biochem.*, **211**, 7–18.
Watanabe,H., Sawada,J., Yano,K., Yamaguchi,K., Goto,M. and Handa,H. (1993) cDNA cloning of transcription factor E4TF1 subunits with Ets and notch motifs. *Mol. Cell. Biol.*, **13**, 1385–1391.
Weiner,D.B., Liu,J., Cohen,J.A., Williams,W.V. and Greene,M.I. (1989) A point mutation in the *neu* oncogene mimics ligand induction of receptor aggregation. *Nature*, **339**, 230–231.
Williams,L.T. (1989) Signal transduction by the platelet-derived growth factor receptor. *Science*, **243**, 1564–1570.
Woods,D., Ghysdael,J. and Owen,M. (1992) Identification of nucleotide preferences in DNA sequences recognised specifically by c-Ets-1 protein. *Nucleic Acids Res.*, **20**, 699–704.
Wotton,D., Prosser,H.M. and Owen,M.J. (1993) Regulation of human T cell receptor beta gene expression by Ets-1. *Leukemia*, **7**, S55–60.
Yang,B.S., Hauser,C.A., Henkel,G., Colman,M.S., Van Beveren,C., Stacey,K.J., Hume,D.A., Maki,R.A. and Ostrowski,M.C. (1996) Ras-mediated phosphorylation of a conserved threonine residue enhances the transactivation activities of c-Ets1 and c-Ets2. *Mol. Cell. Biol.*, **16**, 538–547.
Zucman,J. *et al.* (1993) Combinatorial generation of variable fusion proteins in the Ewing family of tumours. *EMBO J.*, **12**, 4481–4487.

*Received on June 13, 1996; revised on September 18, 1996*