Translocation (12;22) (p13;q11) in myeloproliferative disorders results in fusion of the ETS-like TEL gene on 12p13 to the MN1 gene on 22q11

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In myeloid and lymphoid leukemias recurrent chromosomal aberrations can be detected in chromosome region 12p13. We characterized the genes involved in t(12;22) (p13;q11) in two patients with myeloid leukemia and one with myelodysplastic syndrome (MDS). MN1, a gene on chromosome 22q11 was shown to be fused to TEL, a member of the family of ETS transcription factors on chromosome 12p13. The translocation results in transcription of the reciprocal fusion mRNAs, MN1-TEL and TEL-MN1, of which MN1-TEL is likely to encode an aberrant transcription factor containing the ETS DNA-binding domain of TEL. In addition to fusion of TEL to the PDGF receptor in t(5;12) in chronic myelomonocytic leukemia (CMMML), our data suggest that the involvement of this protein in myeloid leukemogenesis could be dual; its isolated protein-protein dimerization and DNA-binding domains may be crucial for the oncogenic activation of functionally different fusion proteins.

Keywords: translocation; TEL; MN1; myeloid leukemia; chromosome 12p

Introduction

The translocation (12;22)(p13;q11) has been associated with myeloid malignancies of different FAB subtypes. Originally it was found as an alternative translocation in chronic myeloid leukemia (CML) and it was proposed that the 22q− chromosome was an alternative Philadelphia chromosome (Engel et al., 1977; van der Blij-Philipsen et al., 1977; Verma and Dosik, 1979). However, later the same specific translocation was also found in rare cases of acute myeloid leukemia (AML) M1, M7, M4 and MDS (Hagemeier et al., 1981; Johanssosn et al., 1990; Callen et al., 1991; Mitelman, 1991; Kashimura and Minamihisamatsu, 1993). The chromosomal region 12p11-13 has been implicated in the genesis of 0.5–2% of different myeloid leukemias (Adriansen, 1992) and 10% of childhood acute lymphoblastic leukemias (ALL) (Raimondi et al., 1986). In the case of myeloid leukemias, this often involves balanced translocations with a collection of different partner chromosomes (Mitelman, 1991), which is also true for the specific sub-band 12p13, involved in t(12;22). In ALL the region is often deleted, but also balanced translocations have been described (Raimondi et al., 1986; Mitelman, 1991; Krance et al., 1992; van der Plas et al., 1995). With respect to the cytogenetic data, different loci, present on 12p11, 12p12 and 12p13, may be involved in these malignancies (Mitelman, 1991).

Our interest in this specific but rare translocation in myeloid malignancies was raised by the 22q11 breakpoint and its association with aspecific CML, which indicated a possible involvement of the BCR gene. However, initial mapping experiments with BCR cDNA probes excluded this possibility and the use of rodent/human hybrid cell lines, containing the segregated chromosomes of a patient with t(12;22) AML, suggested that the translocation breakpoint mapped telomeric from the BCR gene on chromosome 22 (DvdP, unpublished results). We set out to molecularly identify t(12;22) with the idea that it represented a crucial tumorigenic step in the genesis of myeloid malignancies and that the same locus on 12p13 may be involved in other chromosome translocations.

Using hybrid cell lines from a patient with t(12;22) AML-M4, we could map and clone the genomic chromosomal-breakpoint on 22q11. It was situated in the intron of MN1, a candidate gene for meningioma, encoding a protein with features characteristic of transcription factors (Lekanne Deprez et al., 1995, this issue). This allowed us to clone chromosome 12 sequences and to isolate fusion cDNAs, using a RT-PCR cloning procedure. The translocation results in the fusion of the MN1 gene to the recently identified TEL gene on 12p13, a member of the ETS family of transcription factors (Golub et al., 1994). The amino-terminal part of the TEL protein, containing a putative protein-protein dimerization domain, was found to be fused to the tyrosine kinase domain of the PDGF receptor by the t(5;12)(q34;p13) in chronic myelomonocytic leukemia (CMMML). In two out of three patients with different myeloid malignancies, carrying t(12;22), both the TEL-MN1 and MN1-TEL fusion mRNAs are expressed. In hybrid cell lines of the third patient, only expression of MN1-TEL mRNA could be found. This transcript will encode a fusion protein, containing an ETS DNA-binding domain, that may act as an altered transcription factor, similar to EWS-FLI1 in Ewing’s sarcoma (Bailly et al., 1994). Our data suggest that the involvement of TEL in human myeloid malignancies is dual. Distinct domains of the protein seem to be essential constituents of functionally different fusion proteins.
Fusion between MN1 and TEL in myeloproliferative disorders
A Buijs et al

Results

Localization of chromosomal breakpoint on 22q11

To localize the 22q11 breakpoint of t(12;22), probes of known localization in the 22q11-q13 region were hybridized to DNA of somatic cell hybrids, containing the segregated translocation chromosomes of a t(12;22) AML patient. To this end, bone marrow cells of a patient (pi) with t(12;22) AML were fused to the hamster cell line A3 using standard procedures (Geurts van Kessel et al., 1981). After selection, individual clones were picked and characterized cytogenetically, using Reverse banding techniques. The chromosomal content of the relevant hybrid cell lines is shown in Table 1. DNA of human thymus, hamster cell line A3

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<th>BCR</th>
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<th>D22S56</th>
<th>LIF</th>
<th>D22S15</th>
<th>1.8 kb</th>
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Chromosome: + chromosome seen in at least 5/10 metaphases. Marker: + positive hybridization signal; - no hybridization signal. *: the hybrid cell lines have additional human chromosomes not listed in this table.

Table 1

Hybrid cell lines containing segregated t(12;22) chromosomes scored for chromosome 22q-specific markers

Figure 1 (a) Schematic representation of human chromosome 22. Indicated are several loci with respect to t(12;22)(q13;q11). (b) Restriction map of MN1 on chromosome 22. Indicated below the map are probes E1.8, cDNA 2.2, E1.4, pUK4, cDNA 17.13' and D22S193 and cosmids clones 76A4 and 50B11. Localization of exons are indicated with black boxes. (c) Restriction map of TEL on chromosome 12. The t(12;22) breakpoints of patients 1, 2 and 3 are shown (p1, p2 and p3). Below the map, the phase contig is shown of which clones MR41 to MR40 were isolated by chromosome walking starting with chimeric phages containing the (12;22) breakpoint of p2. MR43 to MR47 were isolated by hybridization with TEL cDNA clone hpc7A to a genomic phage library. The cDNA hpc7A is depicted underneath the phase contig. The positions of the type I and II in t(12;22) and the t(5;12) fusions are indicated by arrows. Dashed lines indicate genomic fragments (black boxes) that cross-hybridize to hpc7A subfragments. These genomic fragments were delimited by various restriction enzyme sites, not shown in this map. HLH = Helix-Loop-Helix domain; DBD = ETS DNA-binding domain; E = EcoRI, S = SacI, B = BamHI, X = XhoI.
Figure 2. Localization of breakpoints on chromosome 22 in p1 and p2 (a) A Southern blot containing DNA of hamster cell line A3, hybrid cell lines with the segregated translocation chromosomes derived from bone marrow of p1, bone marrow of p2 (2) and human thymus DNA (N) was digested with EcoRI, BamHI, Bgl II or HindIII. Southern blots were hybridized with a 1.4 kb EcoRI-HindIII fragment, located at the 5' side of the 60 kb intron in MN1. Sizes of normal fragments are indicated (in kb) and arrowheads indicate aberrant fragments. (b) DNA of bone marrow from p2(2) and human thymus (N), digested with EcoRI, BamHI, Bgl II, HindIII or PstI was hybridized with 4.4 kb EcoRI fragment pUK4. Sizes of molecular weight markers are shown (in kb). Arrowheads indicate aberrant fragments shown in the middle of the figure.
As previously shown in Table 1, cDNA 17.1 3' hybridizes with DNA of chromosome 22 (Figure 1b). To analyse whether MNI was directly involved in t(12;22), we hybridized blots containing DNA of our hybrid cell lines with MNI cDNA probes 2.2 (1.5 kb) and 17.1 3' (1.4 kb), derived from the 5' and 3' exon, respectively (see Figure 1b). As shown in Table 1, cDNA 17.1 3' hybridizes with DNA derived from cell line A3JA 12A (#12,22q), but not with DNA derived from A3JA 1C (#12,12p+), while cDNA 2.2 hybridized with DNA derived from cell line A3JA 13B (#12p+). Thus the probes are separated by a physical distance of 100 kb (Figure 1b), spanning the translocation breakpoint on chromosome 22q11 of a meningioma associated balanced translocation t(4;22)(p16;q11), in a patient with sporadic meningiomas (Lekanne Deprez et al., 1991) (Lekanne Deprez et al., 1995, this issue). The physical distance between the probes is 100 kb (Figure 1b), spanning a region containing a gene called MNI, consisting of two large exons separated by an intron of 60 kb, oriented with its 3' end toward the centromere of chromosome 22 (Figure 1b). To analyse whether MNI was directly involved in t(12;22), we hybridized blots containing DNA of our hybrid cell lines with MNI cDNA probes 2.2 (1.5 kb) and 17.1 3' (1.4 kb), derived from the 5' and 3' exon, respectively (see Figure 1b). As shown in Table 1, cDNA 17.1 3' hybridizes with DNA derived from cell line A3JA 12A (#12,22q), but not with DNA derived from A3JA 1C (#12,12p+), while cDNA 2.2 hybridized with DNA derived from cell line A3JA 13B (#12p+). Thus the probes are separated by the translocation, indicating that MNI contains the 22q11 breakpoint of the t(12;22) in pl. To map the position of this breakpoint, genomic subfragments of cosmids 50B11 (see Figure 1b) were hybridized to Southern blots containing DNA of the hybrid of this patient digested with EcoRI, BamHI, BgII and HindIII. One of these, probe EH 1.4 (Figures 1b and 2a and b), detects a normal 7.7 kb EcoRI fragment in DNA of hybrid cell line A3JA 19A (#22), A3JA 2B (#22,12p+) from pl, in bone marrow DNA of p2 and normal human thymus DNA (Figure 3b), but an aberrant fragment of 5.7 kb in cell lines A3JA 1C (#12,12p+) and A3JA 2B. To exclude an EcoRI polymorphism, aberrant fragments were also detected in BamHI and BgII digested DNA of these cell lines (20 and 12 kb, respectively).

Figure 3  In situ hybridization and localization of the breakpoint on chromosome 12p13 in pl. (a) Chimeric TEL-MNI 12-22 phage clone MR2 from p2 was hybridized to metaphase chromosomes of normal human blood. Right panel shows position of chromosomes 12 and 22 with DAPI staining. Hybridization of MR2 to the short arm of chromosome 12 (strong signal) and the long arm of chromosome 22 (weak signal) is indicated with an arrow. Localization of chimeric phage clones MR 20 and MR2 on chromosomes 12 and 22 with DA PI staining. Hybridization of MR2 to the short arm of chromosome 12 (strong signal) and the long arm of chromosome 22 (weak signal) is indicated with an arrow. Localization of chimeric phage clones MR36 and MR2 and MR20 is indicated. Probes used to localize the 12p+ breakpoint of pl (1) are shown underneath.
A similar approach was followed to find the breakpoint in the MN1 gene in the bone marrow DNA of p2. The 4.4 kb EcoRI intronic fragment (pUK4; see Figure 1b and 2b) detects aberrant fragments of 20 kb and 2.2 kb in addition to the normal EcoRI fragment of 4.4 kb (Figure 2b). Aberrant fragments were also detected in BamHI, BglII, HindIII and PstI digested DNA of p2. These results indicate that the breakpoints on chromosome 22 in p1 and p2 are located in close proximity to one another at the 3′ side of the 60 kb intron of MN1.

Localization of the breakpoints on chromosome 12p
To identify the breakpoints of these patients on chromosome 12p13, a genomic λEMBL3 library (106 pfu) was constructed of bone marrow DNA from p2 (MERO 12/22 library). From the recombinant phages hybridizing with probe pUK4, eight out of 13 showed restriction patterns that in part diverged from the wild-type MN1 restriction map and contained EcoRI fragments of aberrant size. Of these phages, MR20 and MR2 appeared to contain the chromosome 12p13 and 22q11 specific breakpoints, respectively (Figure 3b). To verify whether MR2 indeed contained sequences derived from chromosome 12, the phage was used as probe in a fluorescent in situ hybridization experiment (FISH) on metaphase chromosomes from human peripheral blood. As shown in Figure 3a, MR2 clearly hybridized to chromosome 12 band p13, confirming its alleged chromosomal position. Since this phage clone also contains 0.7 kb of chromosome 22 sequences, a faint signal on chromosome 22 is visible. Similar data were obtained with phage MR20 (not shown). Mapping of phage clones MR2, MR20 and wild-type phages of a contig derived from this region (Figure 1c), revealed that the breakpoint of p2 is located in a 1.0 kb EcoRI–HindIII fragment (see Figure 1c). The breakpoint on chromosome 12 in p1 could be detected by Southern blot analysis of the hybrid cell lines using chromosome 12-specific probes (H3H3.0, H3H3.5, EE1.5 and EE1.0, Figure 3b). Of these, H3H3.5, EE1.5 and EE1.0 remain on the 12p+ chromosome, while H3H3.0 is translocated to the 22q– chromosome (not shown). A single copy 0.3 kb RsaI fragment (RR0.3, Figure 3b) from this region, detected aberrant fragments containing the chromosome 12p13 breakpoint in EcoRI, BamHI, BglII and HindIII digested DNA of hybrid cell lines A3JA 1C and A3JA 2B (Figure 3b). Exact mapping of the breakpoint showed that it is situated 13.5 kb telomeric from the 12p13 breakpoint in p2.

RACE-PCR cloning
Due to restricted amounts of patient material, we analysed the presence of aberrant mRNAs by RACE-PCR (rapid amplification of cDNA ends) (Frohman et al., 1988; Belyavsky et al., 1989). To analyse expression of 5′ chromosome 12/3′ MN1 chimeric mRNA, 1 μg of poly(A)+ RNA of p2 was primed with a 3′ MN1-specific antisense primer for first strand cDNA synthesis (Figure 4a, primer 1). This product was then amplified using a 5′ anchor primer and a nested 3′ MN1 primer (Figure 4a, primer 2). A PCR fragment of 440 bp (not shown) was subcloned and sequenced. The fragment contains 282 bp of new 5′ sequences spliced to the 3′ exon of MN1. In the reading frame of the MN1 moiety of the clone, the new sequence contains an open reading frame with a possible AUG start codon 163 bp 5′ of the MN1 sequence. Comparison of the predicted protein sequence with the recently published sequence of TEL (Golub et al., 1994), showed that they were identical. TEL is a member of the ETS family of transcription factors and contains, in addition to the carboxyl-terminal ETS DNA-binding domain, a putative helix–loop–helix (HLH) protein dimerization domain, located at the amino-terminal side of the protein. In t(5;12) in CMMML the HLH domain of TEL is fused to the kinase domain of PDGFRβ. However, in our case, the breakpoint in the TEL gene occurs at a position 5′ of the HLH coding sequence. Therefore the TEL-MN1 mRNA would encode a protein of 12 kDa containing the first 54 amino acids of TEL fused to the last 39 amino acids of MN1, not including the HLH domain of TEL.

Expression of the complementary MN1-TEL fusion mRNA, was tested by RT-PCR analysis using antisense TEL primers (Figure 4a, primers 4 and 5). Poly(A)+ RNA of p2 (1 μg) was used to perform a RT-PCR reaction with MN1 sense primer 3 and TEL antisense primer 4 (see Figure 4a). Part of this reaction was used for a second round of PCR with the same MN1 primer 3 and a nested antisense TEL primer 5 (see Figure 4a). After gel electrophoresis, a 550 bp product was visible (not shown), matching the size expected for the MN1-TEL fusion cDNA which was subsequently confirmed by sequence analysis. Therefore, the respective mRNA is calculated to encode a fusion product of 177 kDa, containing the first 1259 amino acids of MN1 fused to the last 397 amino acids of TEL, including the helix–loop–helix (HLH) protein dimerization domain (Figure 4a; type I).

Consistent expression of both fusion mRNAs was analysed in the other two t(12;22) patients, p1 and p3. RT-PCR analysis was done on 5 μg of total RNA of hybrid cell lines A3JA 2B (#12,22p+), A3JA 1C (#12,12p+) and A3JA 20A (#12,12p+) and A3JA 12C (#22q-, 12p+) for pi and on bone marrow RNA for p3. Unfortunately, RNA from hybrid cell lines had to be used for p1, since leukemic cells were no longer available. In all hybrid cell lines containing the 12p+ chromosome, the same 550 bp MN1-TEL cDNA was amplified, while in cell lines A3JA 14B (#12,22,22q−) and A3JA 12C (#12p+,22q−) no TEL-MN1 cDNA could be amplified. RT-PCR amplification of p3 RNA from bone marrow cells produced both a TEL-MN1 and a MN1-TEL-specific product, but the first appeared 165 bp longer and the second 165 bp shorter than the concomitant fusion cDNA products of p1 and p2. Cloning and sequence analysis of these amplified fragments showed that the breakpoint in the TEL coding sequence occurs 165 bp farther 3′, explaining the discrepancy in size of the amplified cDNA fragments. The breakpoint in the TEL coding sequence is located within the HLH region, indicating that neither of the predicted fusion proteins will contain the intact HLH domain of TEL. We designate the fusion in p1 and p2 as type I and p3 as type II (Figure 4a). Figure 4b shows the sequence around the breakpoint areas of both types of reciprocal fusion cDNAs, which in type I occurs at TEL codon 55 and...
in type II at codon 110. As a result the type II TEL-MN1 and MN1-TEL mRNAs would encode for 19 kDa and 170 kDa fusion proteins, respectively.

cDNA cloning and partial genomic organization of the TEL gene

A human placenta cDNA library of a complexity of $10^6$ pfu was hybridized with the 5' TEL-specific 246 bp EcoR1-XhoI fragment of the TEL-MN1 fusion cDNA fragment of p1. Many hybridizing plaques were isolated of which we analysed one with an insert of 1.7 kb (hpc7A) that contained the entire open reading frame of TEL as well as 5' and 3' UTRs of 106 bp and 199 bp, respectively. This cDNA clone was hybridized to the genomic MERO 12/22 EMBL3 library of p2 and out of 100 hybridizing plaques 20 were isolated and analysed. A partial physical map of TEL is shown in Figure 1c. Restriction analysis of phage MR44 revealed that it has overlap with MR40, which belongs to a phage contig of 100 kb that was isolated with the 12p13 breakpoint of p2 as a starting point. Subfragments of hpc7A were hybridized to the entire phage contig to identify fragment containing exons. The 541 bp XhoI-SacI TEL cDNA fragment hybridizes with the 2.2 kb XhoI-EcoRI fragment of MR43, the 12.5 kb SacI-EcoRI fragment of MR40, the 8 kb EcoRI fragment of MR44 and the 4.3 kb EcoRI fragment of MR46. Sequencing of the 2.2 kb XhoI-EcoRI fragment of MR43 showed that the exon/intron border coincides with the type I fusion point in the cDNA. The breakpoints in the TEL fusion cDNAs of our p1 and p3 and the published CMML patients (Golub et al., 1994) (i.e. 5', within, and 3' of the TEL HLH sequence, respectively) mark the borders of different TEL exons. Since the type II and t(5;12) CMML breakpoint map at least one exon apart and primer 4 (located between those breakpoints) hybridizes with the 8 kb EcoRI fragment in phage MR44 (Figure 1c), we deduce that the breakpoint of p3 is located within an intron between these exons within this fragment. From the hybridization pattern of 5' and

![Figure 4](image_url)

**Figure 4** Schematic representation of cDNAs and breakpoint regions in t(12;22) (a) cDNAs of normal MN1 and TEL and the reciprocal type I and II fusion products. ORFs of MN1 and TEL are shown in black and gray, respectively. Horizontally striped boxes indicate the HLH domain of TEL, whereas vertically striped boxes indicate the ETS-domain. Primers used in RT-PCR reactions are indicated above the MN1 and TEL cDNAs. (b) Sequences of reciprocal fusion cDNAs around the type I and II breakpoints. Amino acid sequences deduced from the nucleotide sequence are shown in one-letter code below. Numbers indicate in which codon of TEL and MN1 the fusion occurs for both types of translocations. The number on the right side indicates the length of the PCR fusion cDNAs with the different primer combinations.
3' genomic TEL fragments to the panel of hybrid cell lines of p1 we conclude that the gene is located in a 3' telomeric 3' centromeric orientation on 12p13. This orientation is in agreement with the orientation of the PDGFRβ (Morris et al., 1992) on chromosome 5q33 and MNL on chromosome 22q11 (Lekanne Deprez et al., 1995, this issue), predicting that fusion of these genes with TEL are the product of conventional balanced translocations.

Discussion

In this paper we show the existence of new leukemia specific fusion genes TEL-MNL and MNL-TEL as the result of the t(12;22) in different myeloid malignancies. The fact that in three out of three patients the same two genes are involved in the translocation provides compelling evidence for a direct role of the fusion products in the leukemic process. In contrast to most chromosome translocations both chimeric gene derivatives are expressed in patient bone marrow cells. However, the observation is not unique as it was consistently found in acute promyelocytic leukemia (APL) (Alcalay et al., 1992), where both PML-RARα and RARα-PML RNAs are expressed. In the hybrid cell lines of p1, containing chromosome 22q+, no TEL-MNL1 transcript could be amplified. Since the transcript was present in bone marrow cells of the two other patients, we suspect that its absence is due to silencing in the hybrid cell lines. Although we find both fusion RNAs using RT-PCR, we do not know their relative levels of expression in patient cells. Due to lack of patient material, we were not able to perform Northern blot hybridizations and we did not attempt to quantitate the RT-PCR approach.

The translocation involves the recently described ETS-related gene, TEL, and a gene with a possible role in meningioma, MNL. Golub et al. (1994) found TEL to be fused to the PDGFRβ gene in CML, a specific subtype of myelodysplastic syndrome (MDS). TEL mRNA encodes a typical ETS-related transcription factor belonging to the subgroup that have the DNA-binding domain at the carboxy-terminal end and a HLH protein-protein interaction domain at the amino-terminal side (Wasylyk et al., 1993). In CML, only the TEL-PDGFRβ fusion transcript is expressed, while the complementary fusion gene, which would contain the DNA-binding domain, is silent. Because the breakpoint occurs just 3' of the HLH-domain, Golub et al. (1994) suggested that, in addition to ectopic expression of the PDGFβ receptor by the TEL promotor, the putative dimerization motif may be important for ligand independent tyrosine kinase activation of the TEL-PDGFRβ fusion protein. In our fusion products, the breakpoint occurs just 5' or within the HLH region (depending on the type of fusion), suggesting that this dimerization domain is nonessential for the function of the fusion proteins in t(12;22) leukemic cells.

The possible function of the MNL1 protein is much less clear. As shown by Lekanne Deprez et al. (1995, this issue) the gene was cloned from a t(4;22) in an isolated case of meningioma and the encoded protein does not show significant homology to any known protein sequences. The only clue obtained so far is its alleged nuclear localization. This preliminary observation in combination with the glutamine/proline-rich amino-terminal sequence of the protein, a feature found in the activation domains of transcription factors (Gerber et al., 1994), may indicate a role in transcription activation. However, whether MNL1 would function as a classic DNA-binding transcription factor or as transcription factor associating protein remains to be determined.

As yet there are no clues whether TEL-MNL1, MNL-TEL or both are important for transformation of early myeloid precursors in t(12;22). Upon evaluation of their features we favor the latter product as a potential oncogene, since it has the structure of an altered transcription factor. It contains the carboxy-terminal part of the TEL protein providing the ETS DNA-binding domain linked to almost the entire MNL protein, including the glutamine/proline-rich stretches. This structure is highly reminiscent of the EWS-FLI1 and EWS-ERG1 fusion proteins in Ewing's sarcoma, where the glutamine/proline-rich amino-terminal part of the EWS protein is linked to the ETS domains of FLI1 or ERG (Delattre et al., 1992; Sorensen et al., 1994). It was shown that substitution of normal amino-terminal FLI1 by EWS sequences creates a transcription factor with much stronger transactivation potential (May et al., 1993; Baillie et al., 1994). Although it has not been analysed, it is reasonable to speculate that this is in part due to its glutamine/proline-rich sequences of EWS, making it plausible that MNL-TEL could have similar characteristics. In addition, fusion of MNL1 could enhance the DNA-binding affinity of the TEL moiety of the protein, by interference with a possible intra-molecular interaction in TEL that was shown to regulate DNA binding in several other ETS family members (Lim et al., 1992; Wasylyk et al., 1993). Both possibilities are currently under investigation.

Regulation of transcription activity of several members of the ETS family, like Elk1 and SAP in mouse (Janknecht et al., 1993; Marais et al., 1993; Hipskind et al., 1994) and pointed and yan in D. melanogaster (Brunner et al., 1994), is directed by phosphorylation by mitogen activated protein kinase (MAPK), that in turn is activated by several signal transduction pathways, one of which is the RAS pathway. It is interesting to note that TEL contains three candidate MAPK sites (Clark-Lewis et al., 1991; Gonzalez et al., 1991) at amino acid positions 20–23, 200–204 and 255–258 indicating that activity of the protein may be regulated by MAPK, via the RAS pathway. In this respect it is interesting that a number of t(12;22) patients, including p2, presents with a CML-like disease (Mitelman, 1991) while in CML tumorigenicity of the BCR-ABL protein in part depends upon continuous activation of the RAS signaling pathway (Pendergast et al., 1993). In much the same way, the supposedly activated tyrosine kinase of the TEL-PDGFRβ protein in CML would result in activation of the RAS pathway. This indicates that RAS activation (Sawyers and Denny, 1994) and thereby probably activation of members of the ETS family of transcription factors plays a central role in the genesis of myeloid leukemia. Also the description of an altered ERG gene in AML carrying a t(16;21) (Shimizu et al., 1993) could conform to this general
picture. Although TEL mRNA is ubiquitously expressed, it remains to be analysed whether the protein is a natural target of the RAS pathway. Alternatively, if TEL is not a downstream target of RAS, the activated MN1-TEL protein may feed into the same downstream processes by competition for DNA binding sites that would normally be occupied by RAS-responsive ETS proteins, activating genes essential for myeloid transformation.

To date, we have isolated 155 kb of genomic TEL sequences. Since the map of the large intron is incomplete and genomic DNA encoding the 5' and 3' UTRs still needs to be isolated, the gene may extend much farther. The involvement of TEL in t(5;12) (Golub et al., 1994) and t(12;22), in combination with detection of 12p12-13 aberrations in 0.5–2% of myeloid (Adriaansen, 1992) and 10% of pediatric lymphoid malignancies (Raimondi et al., 1986) malignancies make this gene a prime candidate for other chromosomal translocations or deletions involving this region. This possibility is currently under investigation. In contrast to most cases with chromosome 12p12-13 aberrations (Mitelman, 1991), t(12;22) is often the sole karyotypic abnormality, despite the variable phenotype of the leukemia. We favor the idea that additional, undetected mutations lead to diversification, while activation of TEL is only one of the necessary steps in malignant transformation. In this respect it is worthwhile to mention that p2 and p3 showed the translocation at relapse after treatment with cytostatic agents.

The most remarkable feature of TEL in these translocations seems that separate domains of the protein contribute to distinct fusion proteins that, although different in function, could lead to deregulation of the same downstream targets needed for transformation of myeloid progenitors.

**Materials and methods**

**Patients and cell lines**

Patient 1 (p1), a 19-year-old woman was diagnosed with AML-M4. Cytogenetic analysis on bone marrow showed a karyotype 47,XX,+8. Patient 2 (p2), a 58-year-old man, was diagnosed with Ph chromosome negative CML. Cytogenetic analysis of bone marrow showed a normal karyotype. Treatment with hydroxyurea was started and clinical status improved. However, 2 years later the patient relapsed with accelerated disease. Cytogenetic analysis of blood and bone marrow revealed a karyotype 46,XY, t(12;22)(p13;q11). Clinical and cytogenetic status has recently been described in detail (Geurts van Kessel et al., 1994). Patient 3 (p3), a 15-year-old boy, was diagnosed with myelodysplastic Syndrome (RAEB). At diagnosis the karyogram was normal. Antileukemic treatment was started. One year later cytogenetic analysis revealed a karyogram 46,XY, t(12;22)(p13;q11).

A hamster/human hybrid cell line panel was generated by fusion of hamster fibroblast cell line A3 with bone marrow cells of p1 using inactivated Sendai virus (Geurts van Kessel et al., 1981). Hybrid cell lines were cultured in Ham's F10 medium +10% FCS supplemented with 1×HAT. Cytogenetic analysis of patient material and hybrid cell lines was done according to standard methods (Hagemeijer et al., 1979).

**Southern blotting**

DNA of relevant hybrid cell lines was digested with various enzymes and electrophoresed on a 0.7% agarose gel. DNA was blotted onto nylon membranes (Hybond N+, Amer sham, UK). Filters were hybridized according to standard procedures (Sambrook et al., 1989) with probes labeled by random priming (Feinberg and Vogelstein, 1983). Filters were washed at a stringency of 0.3 × SSC at 65°C.

**Construction of genomic cEMBL3 library**

A genomic cEMBL3 library was constructed of 300μg DNA of bone marrow from p2 according to Frischauf et al. (1983). Recombinant phages (10^6) were plated and screened with a 4.4 kb EcoRI genomic fragment (pUK4) of MN1. Phages that showed a restriction pattern diverging from wild-type MN1 phages were considered as putative breakpoint containing recombinants and were analysed for chromosomal localization by FISH (Hagemeijer et al., 1993). A genomic map of chromosome the 12p13 region was generated by chromosome walking. Before hybridization, probes were competed with 100μg of human competitor DNA (Sigma, St. Louis, MO) for 1.5 h in 100μl 5 × SSC at 65°C.

**Fluorescent in situ hybridization**

FISH was done according to Hagemeijer et al. (1993). Chromosomes were identified by 4', 6'-diamino-2-phenyl indole (DAPI) banding, using a fluorescence microscope equipped with FITC and DAPI filters.

**RNA isolation**

Total RNA was isolated from bone marrow of patients or from A3JA hybrid cell lines using the acidic guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). Poly(A)^+ RNA was isolated using the PolyA Tract mRNA Isolation System III (Promega, Madison, WI).

**RACE cloning**

Poly(A)^+ RNA (1μg) of p2 was used to clone 5' TEL–3' MN1 fusion cDNA using the 5'-AmpLiFINDER™ RACE Kit (Clontech Laboratories, Palo Alto, CA). First strand cDNA was synthesized with MN1 antisense primer 1 (5'-AGTATTGGGCACGCCAGGAATG-3'). A nested antisense primer 2 (5'-CTTGAATTCCCAAATCTTGTGGGAG-3') was used to clone 5' TEL-3' of pUK4 genomic fragment (pUK4) of MN1. A human placenta cDNA library in XEMBL3 (Clontech Laboratories, Palo Alto, CA) was screened using a 5'-specific 246 bp EcoRI–XhoI cDNA fragment of p2, resulting in the isolation of a 1.7 kb TEL cDNA clone hpc7A.

**cDNA cloning**

A human placenta cDNA library in λgt11 (10^6 pfu) (Clontech Laboratories, Palo Alto, CA) was screened using a 5'-specific 246 bp EcoRI–XhoI cDNA fragment of p2, resulting in the isolation of a 1.7 kb TEL cDNA clone hpc7A.
RT-PCR

MN1-TEL and TEL-MN1 junctions were amplified using two sets of primers. 5 µg total RNA or 1 µg poly(A)+ RNA was used to synthesize first strand cDNA with MN1 antisense primer 1 and AMV Reverse Transcriptase (Promega, Madison, WI) at 42°C. PCR was performed using one cycle of denaturation (5 min, 94°C), annealing (2 min, 59°C) and extension (3 min, 72°C), followed by 24 cycles of denaturation (2 min, 94°C), annealing (2 min, 59°C) and extension (3 min, 72°C) with TEL sense primer 6 (5'-AGTGTAGCATTAAGCAGGAACG-3'). 2.5 µl of PCR reaction was used in a second round of PCR with TEL primer 2 and MN1 primer 1 (5'-TGATGACCTCGGCTG-3'), for 30 cycles of denaturation (5 min, 94°C), annealing (5 min, 47°C), and extension (3 min, 72°C), followed by 24 cycles of denaturation (2 min, 94°C), annealing (2 min, 57°C) and extension (3 min, 72°C). In case of MN1-TEL junction cDNA fragments TEL antisense primer 4 (5'-GTATGACCTCGGCTG-3') was used to synthesize first strand cDNA. PCR was performed using MN1 sense primer 3 (5'-TCCAGCTACAGAGGCA-3') for one cycle of denaturation (5 min, 94°C), annealing (5 min, 47°C) and extension (3 min, 72°C) followed by 30 cycles of denaturation (2 min, 94°C), annealing (2 min, 57°C) and extension (3 min, 72°C). 2.5 µl of this PCR reaction was used in a second amplification with nested antisense TEL primer 5, (5'-CCAGGTTGGAAGAATG-3'), for 30 cycles of denaturation (2 min, 94°C), annealing (2 min, 47°C) and extension (3 min, 72°C). 10 µl of the PCR reaction was electrophoresed on a 2% agarose gel and blotted onto nylon membranes (Hybond N+, Amersham, UK). Membranes were hybridized using either MN1 cDNA 2.2 probe and a 246 bp EcoRi-Xhol TEL-specific 5' cDNA fragment. PCR products were subcloned and sequenced.

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