The EMBO Journal vol.12 no.3 pp.1161 – 1167, 1993

Fusion between a novel *Krüppel*-like zinc finger gene and the retinoic acid receptor-α locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia

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Communicated by M.F.Greaves

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We have identified a unique case of acute promyelocytic leukaemia (APL) with a t(11;17) reciprocal chromosomal translocation involving the retinoic acid receptor α (RARα) and a previously uncharacterized zinc finger gene. As a result of this translocation, mRNAs containing the coding sequences of the new gene, fused in-frame either upstream of the RARα B region or downstream from the unique A1 and A2 regions of the two major RARα isoforms, are expressed from the rearranged alleles. The above gene, which we have termed PLZF (for *p*romyelocytic *l*eukaemia *z*inc *f*inger), encodes a potential transcription factor containing nine zinc finger motifs related to the *Drosophila* gap gene *Krüppel* and is expressed as at least two isoforms which differ in the sequences encoding the N-terminal region of the protein. Within the haematopoietic system the PLZF mRNAs were detected in the bone marrow, early myeloid cell lines and peripheral blood mononuclear cells, but not in lymphoid cell lines or tissues. In addition, the PLZF mRNA levels were down-regulated in NB-4 and HL-60 promyelocytic cell lines in response to retinoic acid-induced granulocytic differentiation and were very low in mature granulocytes. Our results demonstrate for the first time the association of a variant chromosomal translocation involving the RARα gene with APL, further implicating the RARα in leukaemogenesis and also suggesting an important role for PLZF as well as retinoic acid and its receptors in myeloid maturation.

Key words: differentiation/gene expression/haematopoiesis/myeloid

Introduction

Chromosomal abnormalities are common in human cancer (Heim and Mitelman, 1987; Mitelman, 1991). Molecular studies of translocations frequently associated with a given haematopoeietic malignancy have led to the identification of a number of fused genes whose components are often transcription factors (Cleary, 1991; Rabbitts, 1991; Sawyers *et al.*, 1991). For example, the t(1;19) translocation associated with childhood pre-B cell acute lymphoblastic leukaemia (ALL) fuses the helix−loop−helix transcription factor encoded by the E2A gene to a homeobox gene, PBX1 (Kamps *et al.*, 1990; Nourse *et al.*, 1990). Recently the E2A gene has also been shown to be involved in a t(17;19) translocation which results in expression of a chimeric gene consisting of the E2A sequences fused upstream to sequences from a previously unidentified gene called HLF (Inaba *et al.*, 1992). A similar situation occurs in a subset of acute myeloid leukaemias (AMLs) with a characteristic t(6;9) translocation, which fuses DEK and CAN genes, and expression of the *dek-can* fusion mRNA (Lindern *et al.*, 1992a). Recently, fusion between CAN and a novel gene named SET, with subsequent expression of the fusion *set-can* mRNA, has also been reported (Lindern *et al.*, 1992b).

Acute promyelocytic leukaemia (APL), an M3 subtype of AML by FAB criteria (Bennett *et al.*, 1976), represents ~10% of all cases of AML. This disease is consistently associated with a t(15;17)(q22:q12−21) reciprocal chromosomal translocation (Larson *et al.*, 1984) and represents the first example of a human cancer successfully treated with differentiation therapy using *all-trans* retinoic acid (RA) (Degos, 1992; and references within). Studies have shown that a t(15;17) reciprocal chromosomal translocation fuses the retinoic acid receptor α (RARα) and PML genes (Borrow *et al.*, 1990; de Thé *et al.*, 1990; Alcalay *et al.*, 1991), resulting in expression of chimeric PML-RARα and RARα-PML gene products (de Thé *et al.*, 1991; Kakizuka *et al.*, 1991; Pandolfi *et al.*, 1991; Alcalay *et al.*, 1992; Chang *et al.*, 1992; Kastner *et al.*, 1992; Pandolfi *et al.*, 1992) whose molecular mechanism of action remains not understood. The PML gene belongs to a newly discovered gene family (Freemont *et al.*, 1991) which encodes nuclear proteins with a characteristic zinc-finger coiled-coil domain (Freemont *et al.*, 1992). Although the function of the PML protein is not known, its structural features strongly suggest that it binds DNA and that it may be a transcription factor.

Variant translocations have also been reported in APL (Mitelman, 1991). These translocations often involve chromosomes 15, 17 and a third chromosome. In some instances, two-way variant translocations may occur which rearrange the chromosome 17 to a chromosome other than 15. Molecular studies on these variant translocations may identify as yet unknown genes and help to understand better the molecular mechanism(s) underlying the development of APL. Therefore, we examined a series of 32 patients with APL for novel chromosomal abnormalities (Chen *et al.*, 1992). We now report a discovery of a new C_2H_2 zinc finger gene which is involved in a translocation with the RARα locus in a unique case of APL and characterize its pattern of expression in various human cell lines and tissues.

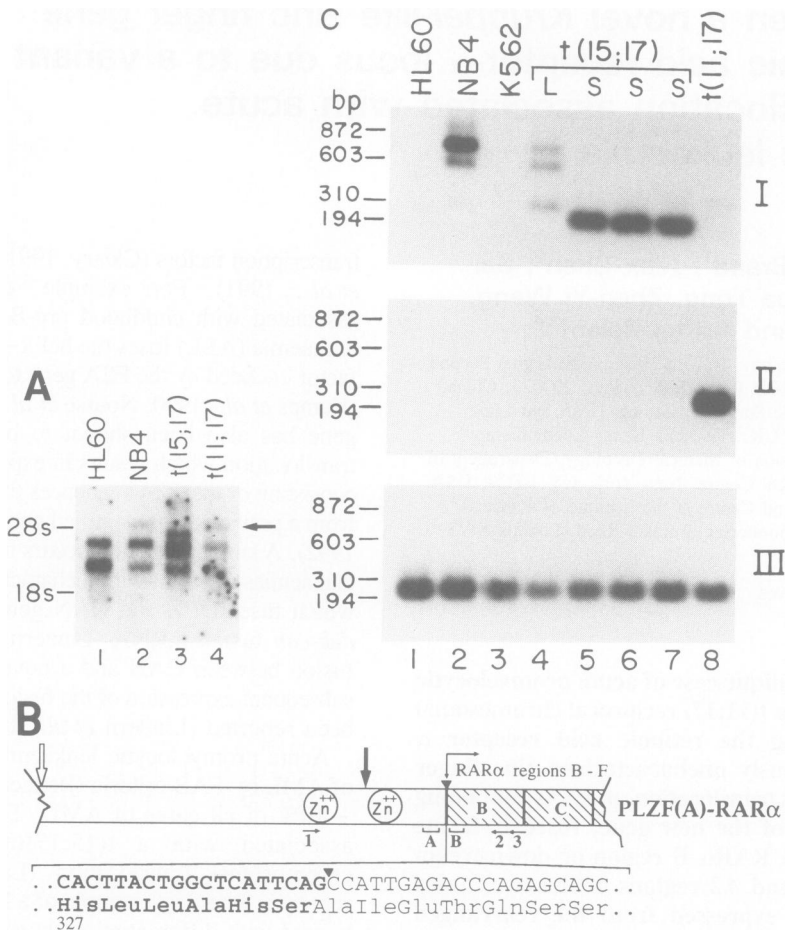

Fig. 1. Expression of the PLZF-RARα mRNA is restricted to the t(11;17)-positive sample. **A.** Northern blot showing abnormal RARα transcript detected in BM of a patient with t(11;17)-positive APL (lane 4, indicated with an arrow). RARα mRNA profiles of HL-60 cells, t(15;17)-positive NB-4 cells and cells derived from BM of a t(15;17)-positive APL patient are shown in lanes 1–3, respectively. **B.** Schematic representation of PLZF(A)-RARα partial cDNA. PLZF(A) is represented as an open box with circled Zn^{2+} symbols indicating the zinc fingers. The junction between the PLZF and RARα sequences is indicated by a bold arrowhead. The broken arrow points in the direction of regions B–F which represent the functional domains of the RAR (see Chambon *et al.*, 1991 for review). The RARα portion of the chimeric cDNA appears as a hatched box and only regions B and C are indicated. The DNA and amino acid sequences spanning the junction between PLZF and RARα (in bold and normal characters, respectively) are shown underneath. The open and bold vertical arrows indicate the 5′ end of the longest PLZF(A)-RARα cDNA (isolated by anchored PCR) and the position of a unique *Sac*I restriction enzyme site, respectively. Horizontal arrows and bars indicate approximate position of oligonucleotide primers and probes. **C.** RT/PCR analysis of the PLZF-RARα chimeric mRNA expression in APL. Analysis was performed on total RNA samples (2.5 μg) derived from t(15;17) APL patients expressing the PML-RARα long (L, lane 4) and short (S, lanes 5, 6 and 7) isoforms, as well as the t(11;17)-positive APL patient (lane 8), using primer pairs specific for PML-RARα (panel I), PLZF-RARα (panel II, oligonucleotides 1 and 2 in B) and RARα1 (panel III) sequences. NB-4 cell mRNA was used as a positive control for PML-RARα expression (lane 2). Poly(A)$^+$ (1 μg) and total (2.5 μg) RNAs derived from both t(15;17)- and t(11;17)-negative HL-60 and K562 cell lines were used as negative controls (lanes 1 and 3). Amplified cDNAs were hybridized with a ^{32}P-labelled RARα B region-specific oligonucleotide (probe B in Figure 1B). Molecular standards are shown in bp on the left.

Results

Detection and isolation of a novel fusion cDNA containing the RARα sequences

In our recent study (Chen *et al.*, 1992) one patient with APL had a variant reciprocal chromosomal translocation t(11;17)(q23-q21.1) and a normal chromosome 15. Southern analysis showed that the breakpoint on chromosome 17q was within the third intron of the RARα gene (not shown), ~2 kb downstream of the exon encoding the A2 region of the human RARα2 isoform and its RA-responsive promoter (Leroy *et al.*, 1991a). No rearrangement of the PML gene in this patient was seen with Southern analysis, nor were PML-RARα mRNAs detected by 'nested' reverse transcriptase/polymerase chain reaction (RT/PCR) analysis (not shown and see below). Northern analysis, however, revealed an additional, abnormally migrating RARα transcript (Figure 1A, compare lanes 1 and 4).

In order to identify the possible heterologous sequences linked upstream to the B–F regions of RARα, anchored PCR was used with 'nested' 3′ oligonucleotide primers complementary to the RARα B region sequence. A novel chimeric cDNA was cloned which contained 605 nucleotides (nt) of previously uncharacterized sequence (see Figure 1B for schematic representation). This sequence, which has been mapped to chromosome 11q23 by *in situ* hybridization (Z.Chen, R.Berger, S.Waxman and A.Zelent, unpublished results), possessed a single open reading frame (ORF) in phase with that of RARα and contained two *Krüppel* (*Kr*)-like cysteine/histidine (C_2H_2) zinc fingers immediately upstream of the RARα B region. The expression of the fusion mRNA was restricted to the patient with t(11;17) and

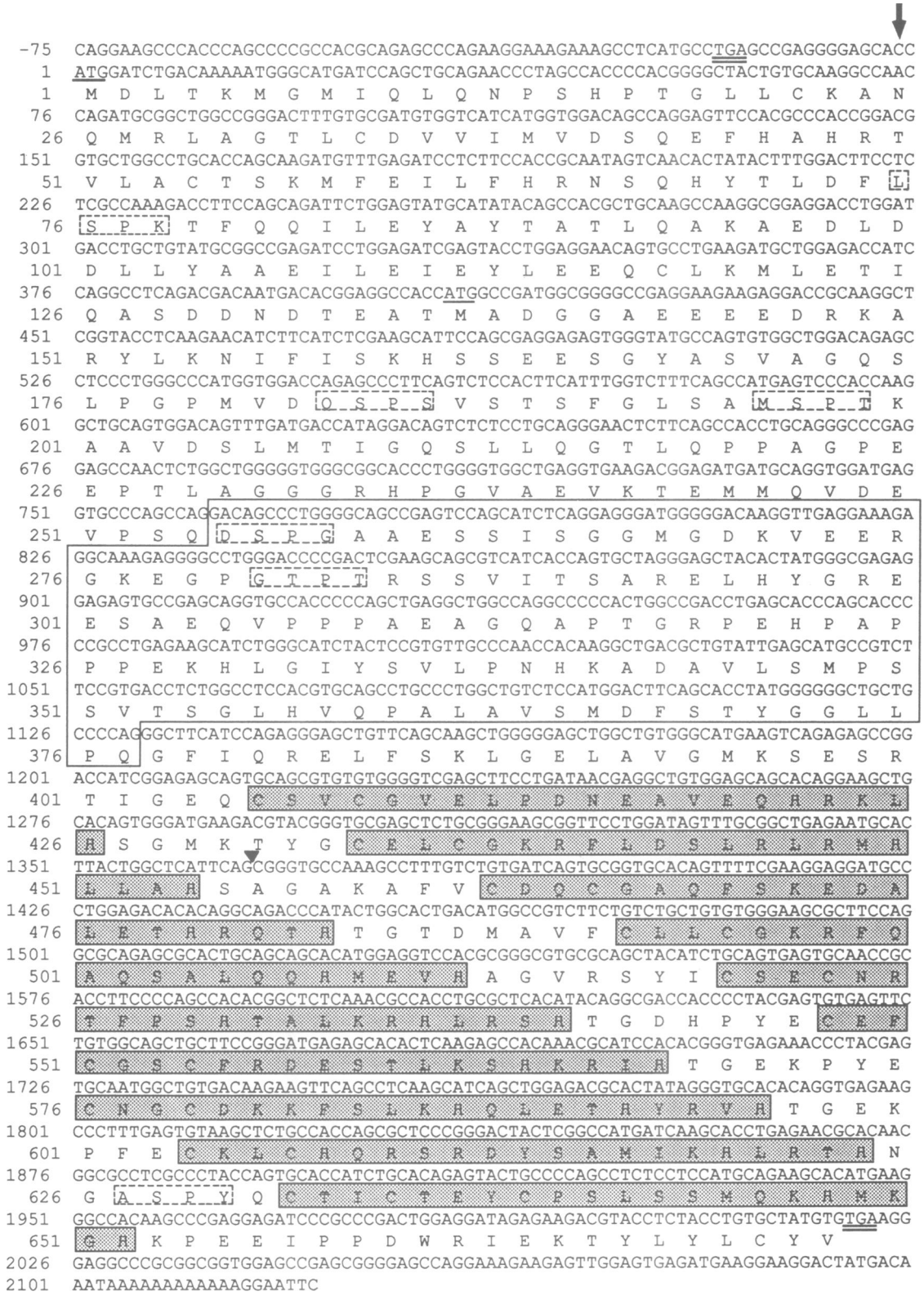

Fig. 2. Nucleotide and deduced amino acid sequence of a partial PLZF(B) cDNA. The DNA and deduced protein sequences are shown and numbered on the left. The initiation and termination codons are underlined with single and double lines, respectively. The sequences of nine zinc fingers are shaded. Six motifs resembling '$x^{S}/_{T}Px$' proline-dependent phosphorylation targets are delineated with a discontinuous line. The putative exon that was not present in the anchored PCR-derived rearranged PLZF(A) cDNA is boxed. The arrowhead denotes the junction between two PLZF exons flanking the translocation breakpoint. The vertical arrow indicates the 5′-most nucleotide of the isolated PLZF(B) cDNA clone. Sequence corresponding to nt −75 to −3 was derived from three independent cDNA clones obtained by anchored PCR as described (see Materials and methods).

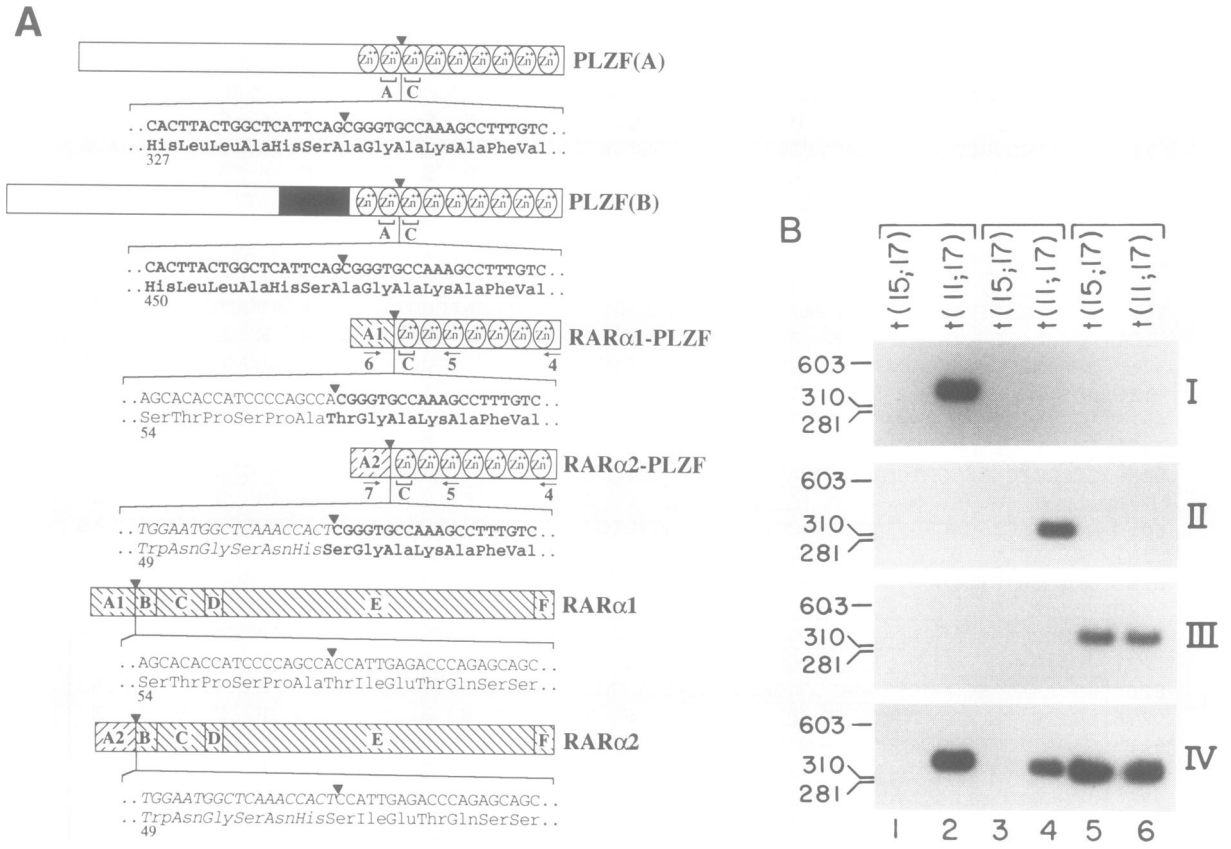

Fig. 3. Expression of RARα1- and RARα2-PLZF fusion mRNAs. **A**. Schematic representation of protein coding regions for the two PLZF(A) and (B) and RARα1 and α2 cDNA isoforms, as well as various RARα and PLZF fusion cDNAs. The black box represents a putative alternatively spliced exon present exclusively in PLZF(B) cDNA. RARα1 and α2 are designated with hatched boxes subdivided into their conserved (B–F) and divergent A1(α1) and A2(α2) functional domains. Other symbols are as described in Figure 1B. Nucleic acid and deduced amino acid sequences spanning the junctions (indicated with a bold arrowhead) between the RARα and PLZF cDNAs, as well as the A and B regions of the two RARα isoforms, are shown underneath each diagram. In the diagrams of PLZF(A) and (B), the junction between two exons flanking the translocation breakpoint is indicated. The numbers correspond to published amino acid sequence for RARα1, the PLZF(B) (Figure 2) and human RARα2 A2 region sequence (our unpublished results). Sequences unique to the RARα2 and PLZF are in italics and bold, respectively. **B**. RT/PCR analysis showing specific expression of RARα1-PLZF and RARα2-PLZF reciprocal chimeric cDNAs in t(11,17)-positive APL (lanes 2 and 4), but not in the t(15;17)-positive NB-4 cell line (lanes 1 and 3). Expression of wild-type PLZF was detected in both samples (lanes 5 and 6). The amplification primers were specific for RARα1-PLZF (lanes 1 and 2, primer pair 5+6 in Figure 3A), RARα2-PLZF (lanes 3 and 4, primer pair 5+7 in Figure 3A) and PLZF (lanes 5 and 6, primers 5 and 1 as shown in Figure 3A and 1A, respectively). Exact sequences of all the PLZF oligonucleotide probes and primers, as well as the human RARα2 sequence, are available on request. Southern blots of amplified cDNAs were hybridized with ^{32}P-labelled oligonucleotide probes corresponding to unique A1 region sequence of RARα1 (panel I), A2 region of RARα2 (panel II), PLZF cDNA sequences derived from exons lying upstream (probe A) and downstream (probe C) of the chromosomal breakpoint (panels III and IV, respectively). Molecular size markers are as shown in Figure 1C.

was absent from t(15;17) positive APLs, the NB-4 cell line which contains the t(15;17) translocation (Lanotte *et al.*, 1991), or control RNAs from HL-60 (Collins, 1987) and K562 (Lozzio and Lozzio, 1975) cells (Figure 1C, lanes 1–8, compare panels I and II). Expression of a fusion mRNA containing the entire ORF was confirmed by RT/PCR and DNA sequencing (not shown).

Cloning of the cDNA derived from the wild-type zinc finger gene identified by the t(11;17) translocation

Using a ^{32}P-labelled probe containing only the novel sequences derived from the fusion cDNA, we screened a human ventricular muscle cDNA library, as *Kr*-related genes have previously been isolated from heart cDNA libraries (N.J.Brand, unpublished results), and cloned a 2.1 kbp cDNA which we called PLZF(B) (see below). DNA sequencing of this clone revealed a 2019 bp ORF beginning with an initiation codon within a near-perfect Kozak sequence (Kozak, 1983) at nt 1 (Figure 2) and encoding a 673 amino acid protein of M_r 74 340. The partial 5′-untranslated region (5′-UTR) sequence, which was obtained by anchored PCR (see legend to Figure 2 for details), contains a TGA termination codon located 18 bp upstream of and in-frame with the initiator ATG. *In vitro* transcription/translation from this cDNA produced only a single band of approximate M_r 80 000 (J.Licht, S.Waxman and A.Zelent, unpublished results), despite the presence of an additional ATG initiation codon surrounded by a consensus Kozak sequence (nt 405 in Figure 2). The PLZF(B) sequence differs within the ORF from that found in fusion with RARα, which we will refer to as PLZF(A), by the insertion of an additional 123 codons (see Figure 3A for schematic representations). This sequence possibly corresponds to an alternatively spliced exon (nt 759–1127, boxed in Figure 2), included in the PLZF(B) transcript but omitted from PLZF(A). Both PLZF isoforms were expressed in the patient with t(11;17) as well as HL-60 and NB-4 cells (not shown).

The deduced PLZF(B) amino acid sequence revealed nine

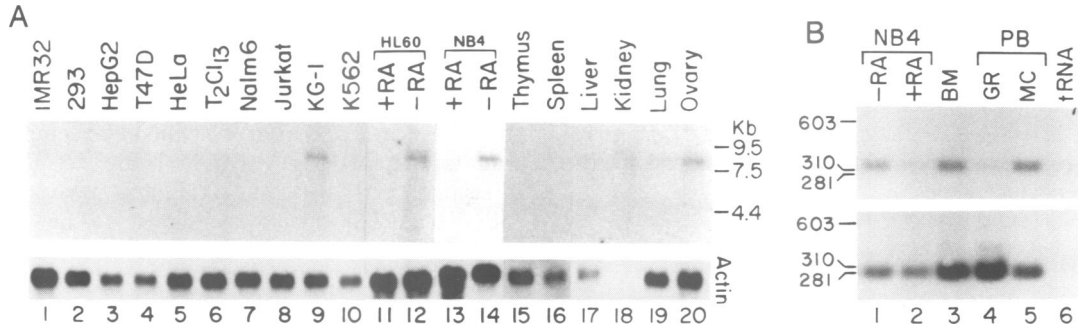

Fig. 4. Analysis of PLZF gene expression. **A.** Northern blot analysis of PLZF mRNAs from various human cell lines and tissues as indicated. Lanes 11+12 and 13+14 show PLZF expression in RA-treated (for 48 h at 5×10^{-7} M) and untreated HL-60 and NB-4 cells respectively. Lanes 1–6 contained RNAs derived from neuroblastoma cells, adenovirus-transformed 293 cells, hepatoma cells, breast carcinoma cells, cervical carcinoma cells and teratocarcinoma cells, respectively. In all cases 3 μg of poly(A)$^+$ RNA was analysed and all lanes correspond to a 5 day exposure at −80°C. The bottom panel indicates results obtained when the same blot was rehybridized with an actin cDNA probe (Zelent *et al.*, 1991). The positions of molecular size markers (in kb) are indicated on the right. **B.** RT/PCR analysis of PLZF gene expression in BM (lane 3), PB granulocytes (lane 4) and mononuclear cells (lane 5). RNA derived from RA-treated (lane 2) and untreated (lane 1) NB-4 cells serves as a control for negative and positive results, respectively. Negative control using tRNA is also shown (lane 6). In all cases 2.5 μg of RNA was used for each reverse transcription reaction. Lower panel corresponds to a positive control for amplification using RARα1 cDNA sequences obtained with the same reverse transcription reaction as the PLZF cDNAs. Molecular standards are shown on the left (in bp).

repetitive zinc finger motifs contained within a single domain in the C-terminal half of the protein. Comparison of PLZF(B) sequence against SwissProt and GenBank/EMBL data bases using the FASTA and TFASTA algorithms (Devereux *et al.*, 1984) revealed similarities, which were restricted to the zinc finger encoding region, between the PLZF(B) and other members of the *kr* family of transcription factors (Chowdhury *et al.*, 1987; Gaul and Jäckle, 1987; and references therein). Among the sequences which received the best scores (53.9% identity in 970 bp overlap) was a recently described myeloid-specific zinc finger gene called MZF-1 (Hromas *et al.*, 1991). No identity was seen between PLZF(B) and any other sequence in the data bases. The N-terminal sequence of the putative PLZF protein shows clusters of negatively charged residues, proline-rich regions, and six potential proline-dependent (xSPx or xTPx, Vulliet *et al.*, 1989) phosphorylation sites. Note that the above described motifs have been shown to comprise regulatory domains in other transcription factors (for reviews see Ptashne, 1988; and Mitchell and Tjian, 1989). Within the same family, Krox-20 contains N-terminal stretches of acidic amino acids and proline-rich sequences, for example (Chavrier *et al.*, 1990). Thus it is likely that the PLZF(B) protein functions as a transcriptional regulator. In this respect it is worth noting that the putative alternatively spliced exon unique to the PLZF(B) encodes 16 out of 39 total proline residues. This suggests that PLZF(A) and (B) may have distinct regulatory properties.

Expression of the RARα-PLZF chimeric gene

The positions of the translocation breakpoints within the RARα and PLZF genes (data not shown) suggested that in addition to the PLZF(A)-RARα hybrid mRNA, which is expressed from the PLZF gene promoter from the derivative 11q+ chromosome, both reciprocal RARα1-PLZF and RARα2-PLZF mRNAs are expressed from the derivative 17q- chromosome. We used RT/PCR with RNA from our index patient and oligonucleotide primers complementary to either hRARα1 or hRARα2 5′ sequences, combined with a primer specific for the 3′ end of the PLZF cDNA, to demonstrate their expression (Figure 3B, lanes 2 and 4). Sequencing of the amplified cDNAs showed that the junctions between the RARα and PLZF sequences correspond precisely to their exonic boundaries (Leroy *et al.*, 1991b; and data not shown). Note that no hybrid expression was detected in NB-4 cells that lack t(11;17) (Figure 3B, lanes 1 and 3). Using oligonucleotide primers 5′ and 3′ to the coding sequences of RARα and PLZF, respectively, RT/PCR indicated that full length RARα1- and RARα2-PLZF fusion mRNAs were expressed in the patient (not shown).

Expression of the wild-type PLZF gene is tissue specific and is regulated by RA

Northern blot analysis showed that, within the haematopoietic system, the PLZF gene expression is restricted to the myeloid lineage [KG-1 (Koeffler and Golde, 1978), HL-60 and NB-4 cells] and is down-regulated upon RA-induced granulocytic differentiation of HL-60 and NB-4 promyelocytic cells (Figure 4A, lanes 9 and 11–14). Expression was not detected in pre-B (Nalm6, a cell line derived from a patient with common ALL) or pre-T (Jurkat, Weiss *et al.*, 1984) lymphocytes, nor in lymphoid tissues such as thymus or spleen (Figure 4A, lanes 7–8 and 15–16, respectively). RT/PCR analysis showed that PLZF is expressed in normal human bone marrow (BM) as well as normal peripheral blood mononuclear cells (PBMC) (Figure 4B, upper panel, lanes 3 and 5). Since lymphoid tissues were negative, it is likely that the signal from PBMC derives from monocytes/macrophages. Expression of the PLZF gene was just detectable in mature granulocytes (Figure 4B, lane 4), corroborating the hypothesis that its down-regulation may be an important step in granulopoiesis. The faint RT/PCR signal present in lane 4 may reflect a very low level of expression or may derive from an incomplete separation of the granulocytic and the mononuclear cell fractions during the isolation procedure. Note that RNA derived from the NB-4 cells treated with RA for 48 h, negative for PLZF by Northern analysis, was also faintly positive by RT/PCR assay (Figure 4B, lane 2), indicating the more sensitive nature of this technique.

It is worth noting that other members of this gene family are expressed in haematopoietic cells (Morishita *et al.*, 1988; Pannuti *et al.*, 1988; Hromas *et al.*, 1991). Expression of

both Evi-1 (Morishita *et al.*, 1992) and MZF-1 (Bavisotto *et al.*, 1991) has been shown to play a role in granulopoiesis. Among adult tissues, PLZF expression was also detected in ovary, and to a lesser degree in kidney and lung (Figure 4A, lanes 18–20), suggesting that the PLZF gene may play a role in the development and/or maintenance of other differentiated tissues.

Discussion

Although translocations involving C_2H_2 zinc finger genes have been identified in human cancer (Solomon *et al.*, 1991; El-Baradi and Pieler, 1991; and references therein), they have never previously been associated with the expression of a chimeric gene product. In one instance, retroviral insertion near the murine *Evi-1* gene resulted in deregulation of its expression and myeloid leukaemia (Morishita *et al.*, 1988). Our results demonstrate for the first time a potential oncogenic fusion between a putative transcription factor of the *Krüppel* family and RARα which is a member of the steroid/thyroid hormone receptor superfamily of ligand-inducible *trans*-acting factors (Evans, 1988; Green and Chambon, 1988; Beato, 1989). This study represents also the first example of the RARα gene involvement in a translocation with a gene other than PML and association of such a translocation with APL. Noteworthy are the parallels between the RARα and E2A and CAN genes, which have also been associated with variant translocations and expressions of different chimeric gene products containing either E2A or CAN sequences (see Introduction).

The putative PLZF-RARα and PML-RARα proteins contain identical portions of the RARα and differ from each other in the N-terminal sequences located upstream of the A/B region junction. The PLZF and PML sequences in the above chimeras are not related to each other except for the fact that both contain potential DNA binding elements (zinc fingers) and sequences implicated in regulation of transcription (proline-rich regions). The fact that the RARα is the common target in both types of translocation associated with APL, and that the same functional domains of the receptors are present in both chimeric proteins, strongly implicates expression of an abnormal fusion receptor gene product in the aetiology of this disease. It is possible that the PLZF-RARα, like PML-RARα, can antagonize the action of normal RARα during differentiation of promyelocytes. The therapeutic effect of RA in APL patients may be due to pharmacological levels of RA which, in contrast to its physiological levels, are able to render the chimeric receptor functionally normal. Alternatively, continuous expression of the putative RARα1- and RARα2-PLZF proteins from the chimeric RARα-PLZF gene may substitute for the wild-type PLZF function, which is down-regulated during granulopoiesis (see Results), and cause a differentiation arrest at the level of the promyelocyte. Note that both RARα-PLZF chimeric mRNAs encode nearly the entire putative DNA binding domain of the PLZF protein which may still recognize its cognate response element(s). Further functional studies of the above chimeric molecules, as well as PML-RARα, will clarify their role in the pathogenesis of APL.

Northern and RT/PCR analyses showed that the PLZF gene is expressed in a cell type and tissue specific manner. Within the haematopoietic system the PLZF expression was lineage and differentiation stage specific, which is in contrast to the much less restricted pattern of the PML gene expression (data not shown). Sensitivity of the PLZF gene expression to RA corroborates the idea that the PLZF and retinoid receptor genes may be important components of the regulatory pathway which leads to the myeloid differentiation. It is worth noting that the expression of the MZF-1 gene, which was shown to be required for granulopoiesis (Bavisotto *et al.*, 1991), is also RA sensitive (Hromas *et al.*, 1991). However, in contrast to the PLZF gene, its expression is up-regulated by RA and is required during granulocytic differentiation. The above observations suggest a possibility of cross-regulatory interaction between the PLZF and MZF-1 genes. It will be of great interest to determine what genes are regulated by the putative PLZF proteins, and in turn, what cell type and developmental stage specific *trans*-acting factors are controlling the expression of the PLZF gene. In this respect it is important to note the cross-regulatory interactions which exist between the homeotic and *Kr*-related genes (Chavrier *et al.*, 1990). Future studies should also elucidate exactly what role(s) the PLZF gene plays in haematopoiesis and perhaps vertebrate development in general.

Materials and methods

RNA isolation

Peripheral blood granulocytes and mononuclear cells were separated by centrifugation through Polyprep™ (NYCOMED AS). Cells were either used immediately for RNA preparation or snap-frozen in liquid nitrogen and stored at −80°C until further use. Total RNA was prepared using either the guanidine/CsCl gradient technique (Chirgwin *et al.*, 1974), or one step acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi, 1987) when dealing with relatively small numbers of cells. Polyadenylated [poly(A)+] RNA was isolated as previously described (Aviv and Leder, 1972) by one cycle of chromatography on oligo(dT)–cellulose (Collaborative Research).

Anchored PCR and RT/PCR analysis

Anchored PCR was carried out essentially as described before by Zelent *et al.* (1991). The 5′ primers were as described by Loh *et al.* (1989). The 3′ nested oligonucleotide primers used for the two consecutive rounds of PCR were oligonucleotides 2 and 3 (Figure 1B). The oligonucleotide primer used for reverse transcription was located just downstream of primer 3. Amplified cDNAs were subcloned using the TA cloning system (Invitrogen). Four clones corresponding to PLZF-RARα were found. The longest isolated PLZF(A)-RARα clone (650 nt) was sequenced using dideoxy sequencing (Ausubel *et al.*, 1987). The same anchored PCR protocol was used to obtain the very 5′ sequence of the PLZF cDNA. Sequences of 3′ oligonucleotide primers used for reverse transcription of 1 μg of HL-60 cell mRNA, and two consecutive rounds of PCR corresponded to nt 226–256, and 116–135 and 98–114, respectively (see Figure 2). For RT/PCR all cDNAs were synthesized as before (Chen *et al.*, 1992) using either PLZF-specific oligonucleotide primer 4 (Figure 3A), or RARα-specific oligonucleotide primer 3 (Figure 1B). PCR amplification was carried out under conditions identical to those described by Chen *et al.* (1992) except that one round of 35 cycles was carried out using an annealing step at 59°C for 1 min and denaturation and extension for 25 s at 95°C and 3 min at 72°C, respectively, except that in the first cycle denaturation was performed for 1 min at 98°C. Note that only 25 cycles of amplification were performed to obtain the data shown in Figure 4B. Amplified cDNAs were detected after transfer to nitrocellulose with ^{32}P-labelled oligonucleotide probes as indicated (see legends to figures). Sequences of the RARα- and PML-specific oligonucleotide primers, PCR conditions and Southern analysis of amplified cDNAs have been described previously (Chen *et al.*, 1992). All oligonucleotide probes used in this study were labelled by standard techniques (Ausubel *et al.*, 1987) to a specific activity of ~10^8 c.p.m./μg and were used in hybridization at 10^6 c.p.m./ml under previously described conditions (Zelent *et al.*, 1991; Chen *et al.*, 1992). Exposure times were in the range 0.5–1 h at room temperature using Kodak XAR-5 film.

cDNA cloning

Unique sequences corresponding to the PLZF(A) cDNA (between two arrows in Figure 1B) were ^{32}P-labelled and used to screen $\sim 1.5 \times 10^6$ recombinant plaques from a human adult ventricular muscle cDNA library, prepared in λZAPII (Stratagene) by combined random priming and oligo(dT)-tailing of mRNA. The screening conditions and subsequent isolation and characterization of the clones followed standard protocols (Ausubel *et al.*, 1987). Six clones were isolated; one of these, which contained a 2.1 kbp insert, was selected for further study and its nucleotide sequence was determined from both strands using dideoxy sequencing.

Northern analysis

Northern blotting was as described by Zelent *et al.* (1991). A fragment of the PLZF cDNA (nt 1–1305 in Figure 2), or entire human RARα cDNA (Petkovich *et al.*, 1987) was ^{32}P-labelled to a specific activity of $\sim 10^9$ c.p.m./μg of DNA by random priming (Ausubel *et al.*, 1987) and used as a probe at concentration of 2.5×10^6 c.p.m./ml. Hybridization and washing conditions were as before (Zelent *et al.*, 1991). Blots were exposed for 5 days at −80°C using two intensifying screens and Kodak XAR-5 film.

Acknowledgements

We thank Profs. M.F.Greaves, C.Marshall and J.Licht, and Dr L.Wiedemann for helpful discussion and critical reading of the manuscript. We are grateful to Prof. H.-Q.Yu, Dr M.Lanotte and Dr P.Barton for providing the patient's sample, the NB-4 cell line and the cDNA library, respectively. N.J.B. thanks Dr P.Barton, Prof. M.Yacoub and the British Heart Foundation for their support. This research was supported by The Leukaemia Research Fund of Great Britain, and also by The Samuel Waxman Cancer Research Foundation, Charles H.Revson Foundation, The Chinese Foundation for High Technology (863), The Chemotherapy Foundation and The Herman Goldman Foundation.

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Received on October 22, 1992; revised on November 30, 1992

Note added in proof

The nucleotide sequence data reported here have been deposited in the EMBL/GenBank/DDBJ Sequence Databases under the accession number Z19002. The data by Z.Chen *et al.* listed as unpublished is now in press (Chen,S.-J., Zelent,A., Tong,J.-H., Yu,H.-Q., Wang,Z.-Y., Derre,J., Berger,R., Waxman,S. and Chen,Z. (1993) *J. Clin. Invest.*).