the identity of its daughters accordingly. A similar cell lineage mechanism has been invoked to explain the formation of birth rank-specified progeny by the neuroblast stem cells of insects[15].

Specification of segmental identity is best understood in the *Drosophila* embryo, where individual segments become different through the regional activation of bithorax and antennapedia complex genes[16]. A homologous gene has been identified in the leech, and shown to have a segment-specific pattern of expression[17], but it is not yet clear what functional role such genes have in leech segmentation. Our present findings indicate that the segmental identity of leech blast cells is initially established through a process of temporal—rather than regional—specification, with this sequence of blast cell identities being translated into the anteroposterior sequence of segments by maintenance of birth rank along the blast cell chain. We would therefore predict that gene products that have a primary role in the specification of segmental identity are very probably expressed in the newly born blast cell and/or the parent teloblast, and that the temporal control of their expression may have a pivotal role in the differential specification of blast cells that are generated in succession. □

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1. French, V., *Development* **104** Suppl., 3-16 (1988).
2. Kimmel, C. B., Sepich, D. S. & Trevarrow, B. *Development* **104** Suppl., 197-208 (1988).
3. Stern, C. D., Fraser, S. E., Keynes, R. J. & Primmett, D. R. N. *Development* **104** Suppl., 231-244 (1988).
4. Patel, N. *et al. Cell* **58,** 955-968 (1989).
5. Weisblat, D. A. & Shankland, M. *Phil. Trans. R. Soc.* **312,** 39-56 (1985).
6. Masinovsky, B., Kempf, S. C., Callaway, J. C. & Willows, A. O. D. *J. comp. Neurol.* **273,** 500-512 (1988).
7. Shankland, M. & Martindale, M. Q. *Devl Biol.* **135,** 431-448 (1989).
8. Martindale, M. Q. & Shankland, M. *Devl Biol.* **123,** 85-96 (1990).
9. Blair, S. S., Martindale, M. Q. & Shankland, M. *J. Neurosci.* **10,** 3183-3193 (1990).
10. Bissen, S. T. & Weisblat, D. A. *J. Neurobiol.* **18,** 251-269 (1987).
11. Shankland, M. *Nature* **307,** 541-543 (1984).
12. Martindale, M. Q. & Shankland, M. *Devl Biol.* **125,** 290-300 (1988).
13. Loer, C. M., Jellies, J. & Kristan, W. B. *J. Neurosci.* **7,** 2630-2638 (1987).
14. Baptista, C. A. & Macagno, E. R. *J. Neurobiol.* **19,** 707-726 (1988).
15. Doe, C. Q. & Goodman, C. S. *Devl Biol.* **111,** 206-219 (1985).
16. Akam, M. *Development* **101,** 1-22 (1987).
17. Wysocka-Diller, J. W., Aisemberg, G. O., Baumgarten, M., Levine, M. & Macagno, E. R. *Nature* **341,** 760-763 (1989).

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Cloning of a gene that is rearranged in patients with choroideraemia

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CHOROIDERAEMIA (tapetochoroidal dystrophy, TCD), a common form of X-linked blindness[1], is characterized by progressive dystrophy of the choroid, retinal pigment epithelium and retina[2,3]. Previous studies have assigned the TCD gene to a small segment of the Xq21 band[4-6]. By making use of reverse genetics strategies we have isolated eight overlapping complementary DNA clones from the same chromosomal region. The corresponding gene is expressed in retina, choroid and retinal pigment epithelium. The cDNAs encompass an open reading frame of 948 base pairs that is structurally altered in eight TCD patients with deletions, and in a female patient with a balanced translocation involving Xq21. These findings provide strong evidence that we have cloned the gene underlying choroideraemia. Elucidation of its function should provide new insights into the molecular mechanisms responsible for this disorder and other hereditary retinopathies.

In previous studies we and others have shown that the TCD gene maps to an interval of the Xq21 band defined by the DNA markers DXS95, DXS165 and DXS233[7,8]. One of these loci, DXS165, is deleted in several patients with classical TCD[4-6]. As a prerequisite for the molecular characterization of the TCD gene region, deletion-breakpoint cloning[9], preparative field inversion gel electrophoresis[10], and walking and jumping techniques[5,11] were used to generate new DNA markers near the DXS165 locus. Using these new markers, a genomic segment of approximately 45 kilobases (kb) which overlaps most of the TCD-associated deletions (Fig. 1) could be cloned. From this segment, 15 single-copy sequences were isolated and screened for evolutionary conservation by hybridization to genomic DNA from various vertebrate species. With two of these DNAs, probes 398 and 413 (Fig. 1), specific hybridization signals were obtained with DNAs from several species including chicken (Fig. 3*a*). On northern blots, probe 398 yielded distinct signals in RNAs from retina and retinal cell lines (not shown). Screening of a human retinal cDNA library[12] resulted in the isolation of eight overlapping cDNA clones (T1-T8), which span a total of 4.5 kb but do not contain the 5′ and 3′ ends of the gene (Fig. 1). Clone T8 extends furthest upstream and contains an open reading frame (ORF) (Fig. 2*a*, ORFI) capable of encoding a polypeptide of 316 amino acids. Nuclease S1 analysis indicated that the insert of this clone is fully protected by messenger RNA from a retinal cell line up to about 55 nucleotides from its 5′ end (Fig. 2*b*). The sequence immediately upstream of position 56 (Fig. 2*a*; underlined) has a conspicuous resemblance to the consensus sequence for 3′ splice sites[13], which may indicate that these nucleotides originate from an intron extending further upstream. At position 61-63, there is a stop codon, and the first ATG codon of the ORF at position 127-129 may function as a translation start site[14].

None of the cDNA clones contains the poly(A) sequences normally indicative of the 3′ end of mRNAs. The complete sequence deduced from the combined cDNAs (not shown) contains a strikingly large 3′ untranslated region of 3.4 kb. In the middle of this region, starting at position 2,800 and adjacent to a sequence that could act as a 3′ splice site, we have found an ORF spanning 395 base pairs (bp), (Fig. 1; ORFII). The functional significance of this sequence remains to be elucidated.

To ascertain the relationship between the cloned cDNA and the TCD gene, genomic DNAs of eight patients with TCD who carried submicroscopic deletions of the Xq21 band[5,6] were re-examined with single-copy subclones of cDNA T1. In all patients the deletions had removed at least part of the long ORF that spans segments A to E of the consensus cDNA (Figs 1 and 3*b*). In addition, we could locate the position of the X-chromosomal breakpoint of a t(X; 13) translocation previously identified in a female with typical signs of TCD[5,15], to intron sequences that separate segments C and D of the cDNA (Fig. 3*c*). In combination, these data provide strong evidence that the cloned cDNA is indeed part of the choroideraemia gene.

RNA analyses have corroborated this conclusion. In choroid/retinal pigment epithelium, retina, two retinal cell lines (HER XC2 and HER RC2; ref. 16), and HeLa cells, all eight cDNA clones hybridize to an mRNA transcript of ~5,400 residues (Fig. 4*a*). Surprisingly, this mRNA is also expressed in Epstein-Barr virus-immortalized B cells, albeit at a significantly lower level. This finding enabled us to study mRNA patterns in patients with deletions of various size and should pave the way for the detection of point mutations in the TCD gene. As shown in Fig. 4*b*, with a probe from the 5′ end of the

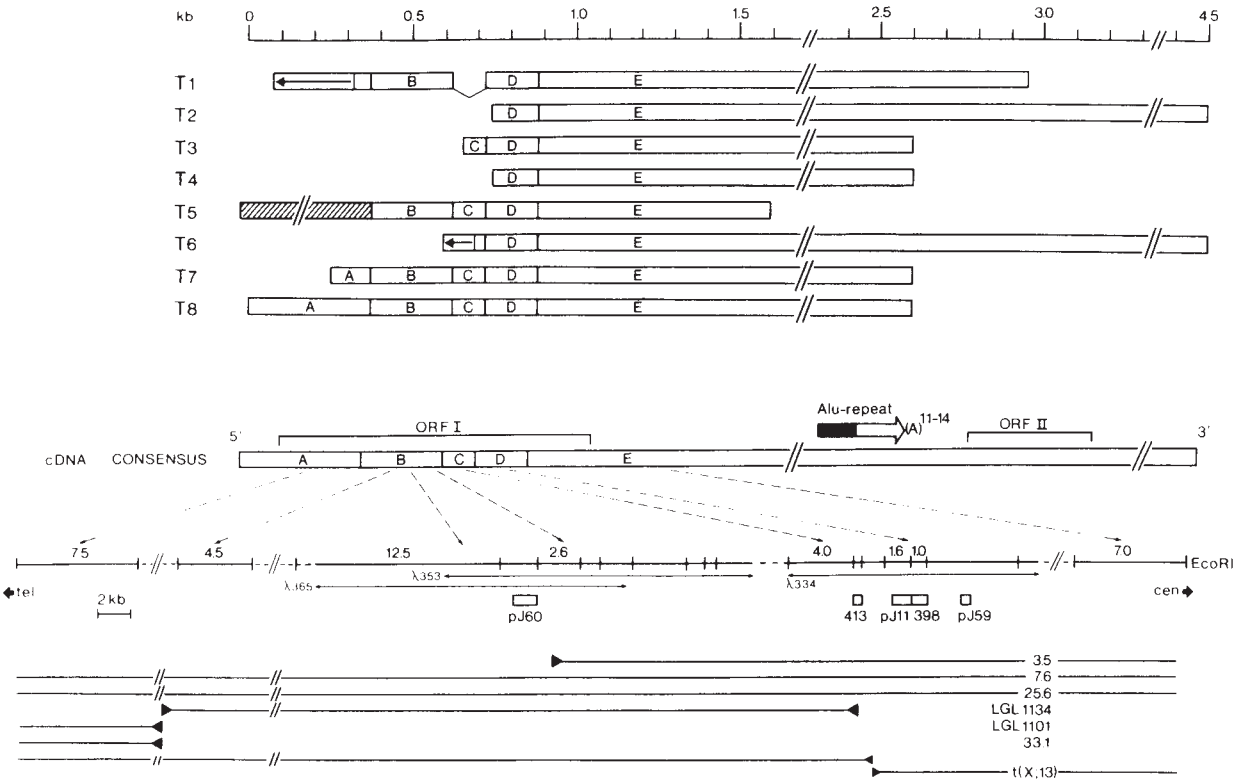

FIG. 1 Overlapping cDNA clones, their alignment with respect to genomic DNA fragments as well as deletion and translocation breakpoints in the Xq21 region. Top, the relative positions of the eight cDNAs shown were deduced from restriction enzyme mapping and sequence analysis. Genuine exon sequences in cDNA inserts are represented by open boxes. A–E denote distinct cDNA segments, each of which may be composed of several exons. Vertical bars represent exon–exon boundaries as inferred from comparison with genomic DNA sequences (clones pJ11 and 398 for defining 5′ and 3′ borders of segment D, respectively) or from comparisons between different cDNAs (T1, T5, T7, and T8 for both borders of segment C and the 3′-border of segment A). The cross-hatched box at the 5′ end of T5 probably represents an intron sequence, as a perfect splice acceptor splice site sequence[13] was identified directly upstream of segment B in this clone. Four clones (T3, T4, T7 and T8) with truncated 3′ ends probably originate from oligo(dT) primed reverse transcription starting at a poly(A) stretch located just 3′ of an internal Alu repeat, which is indicated by a boxed arrow. Clones T1 and T5 probably result from alternatively or aberrantly spliced precursor RNAs. Both the segment lacking from T1 (segment C) and the extra stretch identified at the 5′ end of T5 (cross-hatched segment) feature genuine splice site signals[13] at their borders. Arrows in clones T1 and T6 indicate sequences that are reversed with respect to the sequence determined for the combined cDNAs. These reversed segments contain short inverted repeats at their ends and probably originate from aberrant processing of stem-loop structures during reverse transcription of the mRNA. Bottom, the consensus cDNA as deduced from eight cDNA clones is shown in relation to a partial genomic map of the corresponding chromosome segments. Deletions are shown as horizontal bars between triangles. The X-chromosomal breakpoint in a female witn a balanced t(X;13) translocation[5,15] is indicated. The positions of the two longest ORFs in the cDNA are given. The partial *Eco*RI map was constructed by Southern analysis of *Eco*RI-digested genomic DNA, and by restriction analysis of three recombinant phages (λ334, λ353 and λ365). The chromosomal origin as well as the telomere–centromere orientation of the cDNA segments A–E is indicated. The outermost sections of the cDNA are homologous to *Eco*RI fragments that are still unlinked to the central region of the genomic map.

METHODS. Genomic DNA phage clones with human inserts were isolated employing jump clones pJ60, pJ11 and pJ59 (see ref. 5) using standard cloning methodology. Phage 353 and 365 originated from a total human DNA genomic library in phage EMBL3 (courtesy of G. Grosveld, Rotterdam). Phage 334 was isolated from a pool of recombinant λ DASH (Stratagene) phage containing 14.5 kb *Hin*dIII inserts enriched by preparative gel electrophoresis (for methods, see ref. 9). Plaque purification, DNA isolation, restriction enzyme mapping and subcloning of phage-insert fragments into pGEM 3/4 plasmid vectors (Promega Biotec) was done according to standard recombinant DNA procedures[23]. Single-copy *Eco*RI inserts identified in plasmids 398 and 413 were subsequently used as probes on zoo blots (see Fig. 3*a*) and for screening a λgt10 human retinal cDNA library[12]. Phage recombinants (10^6) were screened with a mixture of ^{32}P-labelled[24] 398 and 413 insert DNAs using standard procedures[23]. Eight positive cDNA clones, T1–T8, were plaque-purified. Insert DNA prepared from plate lysates of each of the eight recombinant phage was cleaved with *Eco*RI and subcloned in plasmid pGEM4. Alignment of various cDNAs was based on restriction mapping using restriction enzymes *Acc*I, *Bgl*II, *Eco*RI, *Hin*dIII, *Nco*I and *Pst*I and on sequence analysis determination (see Fig. 2). The region between nucleotide positions 1 and 1,000 shown at the top of the figure and a stretch of ~250 nucleotides at the 3′ ends were sequenced in all cDNA clones. Only for clone T6 was the insert completely sequenced.

cDNA (T1E0.5), no hybridization signals were seen on northern blots containing RNAs from lymphoblastoid cell lines of TCD patients 7.6 and 25.6. This is not unexpected as both deletions span the entire gene. In contrast, patient 3.5, who lacks the 3′ end of the gene, shows aberrant transcripts of about 4.5 and 5.5 kb. In this patient, two different novel sites may be used as polyadenylation signals.

Neither the nucleotide nor the predicted amino-acid sequence of the putative gene product revealed any significant homology to genes or protein sequences in the NBRF (December 1989), Swiss-Prot (January 1990) and EMBL (April 1990) databases. The protein carries potential phosphorylation sites for protein kinase C (residues 76, 122, 175, 178, 301) and for casein kinase (residues 13, 129, 178, 195, 239, 301), but no other sites associated with post-translational modification processes. Moreover, no topogenic sequences, no domains with known biological function and no enzymatic active sites could be determined using the PCGene PROSITE program. The C-terminal portion of the protein between residues 253 and 302 carries a so-called PEST region (PEST score of 7.3 according to Rogers *et al.*[17]). Proteins containing one or more PEST regions often exhibit intracellular half lives of less than 2 h, and several of these, such

a

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CGGAGAAGACCAAGGAGGGCGTCCTCTACGTCGGAAGCAAGACCTTGTTCATCAGGATAT    60

TAAGAGATCACATTTTATGAATATTTAAAGACTCAAAAATTAACCCCCAACCTCCAATAT   120
*
ATTGTCATGCATTCAATTGCAATGACATCAGAGACAGCCAGCAGCACCATAGATGGTCTC   180
      M  H  S  I  A  M  T  S  E  T  A  S  S  T  I  D  G  L     18

AAAGCTACCAAAAACTTTCTTCACTGTCTTGGGCGGTATGGCAACACTCCATTTTTGTTT  240
 K  A  T  K  N  F  L  H  C  L  G  R  Y  G  N  T  P  F  L  F    38

CCTTTATATGGCCAAGGAGAACTCCCCCAGTGTTTCTGCAGGATGTGTGCTGTGTTTGGT  300
 P  L  Y  G  Q  G  E  L  P  Q  C  F  C  R  M  C  A  V  F  G    58

GGAATTTATTGTCTTCGCCATTCAGTACAGTGCCTTGTAGTGGACAAAGAATCCAGAAAA  360
 G  I  Y  C  L  R  H  S  V  Q  C  L  V  V  D  K  E  S  R  K    78

TGTAAAGCAATTATAGATCAGTTTGGTCAGAGAATAATCTCTGAGCATTTCCTCGTGGAG  420
 C  K  A  I  I  D  Q  F  G  Q  R  I  I  S  E  H  F  L  V  E    98

GACAGTTACTTTCCTGAGAACATGTGCTCACGTGTGCAATACAGGCAGATCTCCAGGGCA  480
 D  S  Y  F  P  E  N  M  C  S  R  V  Q  Y  R  Q  I  S  R  A   118

GTGCTGATTACAGATAGATCTGTCCTAAAAACAGATTCAGATCAACAGATTTCCATTTTG  540
 V  L  I  T  D  R  S  V  L  K  T  D  S  D  Q  Q  I  S  I  L   138

ACAGTGCCAGCAGAGGAACCAGGAACTTTTGCTGTTCGGGTCATTGAGTTATGTTCTTCA  600
 T  V  P  A  E  E  P  G  T  F  A  V  R  V  I  E  L  C  S  S   158

ACGATGACATGCATGAAAGGCACCTATTTGGTTCATTTGACTTGCACATCTTCTAAAACA  660
 T  M  T  C  M  K  G  T  Y  L  V  H  L  T  C  T  S  S  K  T   178

GCAAGAGAAGATTTAGAATCAGTTGTGCAGAAATTGTTTGTTCCATATACTGAAATGGAG  720
 A  R  E  D  L  E  S  V  V  Q  K  L  F  V  P  Y  T  E  M  E   198

ATAGAAAATGAACAAGTAGAAAAGCCAAGAATTCTGTGGGCTCTTTACTTCAATATGAGA  780
 I  E  N  E  Q  V  E  K  P  R  I  L  W  A  L  Y  F  N  M  R   218

GATTCGTCAGACATCAGCAGGAGCTGTTATAATGATTTACCATCCAACGTTTATGTCTGC  840
 D  S  S  D  I  S  R  S  C  Y  N  D  L  P  S  N  V  Y  V  C   238

TCTGGCCCAGATTGTGGTTTAGGAAATGATAATGCAGTCAAACAGGCTGAAACACTTTTC  900
 S  G  P  D  C  G  L  G  N  D  N  A  V  K  Q  A  E  T  L  F   258

CAGGAAATCTGCCCCAATGAAGATTTCTGTCCCCCTCCACCAAATCCTGAAGACATTATC  960
 Q  E  I  C  P  N  E  D  F  C  P  P  P  P  N  P  E  D  I  I   278

CTTGATGGAGACAGTTTACAGCCAGAGGCTTCAGAATCCAGTGCCATACCAGAGGCTAAC 1020
 L  D  G  D  S  L  Q  P  E  A  S  E  S  S  A  I  P  E  A  N   298

TCGGAGACTTTCAAGGAAAGCACAAACCTTGGAAACCTAGAGGAGTCCTCTGAATAATGG 1080
 S  E  T  F  K  E  S  T  N  L  G  N  L  E  E  S  S  E  *      316

ATATACACCAAACTGGATACCCAACTTTGGAAATTCTGACTGGTCTCAGAGTCTACTTGA 1140

TAGAAGGACTGTTTGAGAAATGTTAGAAAGCAGCAGCAATTATAAGGCAAAATAGGTAAT 1200

AGAAATCCAAAAGGGGATTTTCCTTATAGAGGACATTCCAAGAACACACAACACTTATAA 1260

AGCACATTGACTTGCTCATTTTAAATACCAAACTTGTGTGACTAGCAGATGAAAATTATA 1320

AATCAATTGATTCTCAGGAATGTAACTGTGGATATGAAAGTGATCCTATGCATTGTTAAT 1380

AATTCCATGG                                                    1390
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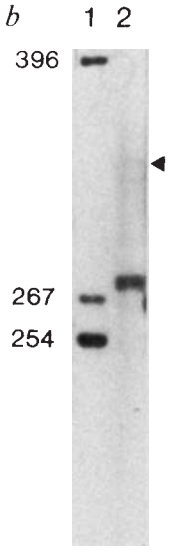

FIG. 2 Structure of the candidate TCD cDNA. *a*, Nucleotide sequence of the region containing the largest ORF and the deduced amino-acid sequence of the corresponding protein. The DNA sequence was derived through combination of several cDNAs and overlaps the ORF indicated in Fig. 1. Upper line, nucleotide sequence; lower line, amino-acid sequence (single-letter code) beginning at the putative methionine initiation codon (see text). A putative 3′ splice site is underlined. Asterisks indicate stop codons. *b*, Nuclease S1 protection assay using a fragment of clone T8. Lane 1, marker DNA is plasmid pGEM4 digested with *Hae*III, dephosphatased, and end-labelled with [γ-^{32}P]ATP. Lane 2, hybridization of the 5′ end of cDNA clone T8 to HER Xc2 mRNA, followed by nuclease S1 digestion, resulted in a protected DNA fragment of ~271 nucleotides.

METHODS. *a*, Inserts of cDNA phage T1–T8 (Fig. 1) were cleaved with restriction enzymes and the resulting fragments were recloned on ligation into the appropriate sites of the polylinker sequence of plasmid pGEM4 (Promega Biotec). Both strands of the relevant plasmid DNAs were sequenced with T7 or SP6 sequencing primers (Promega Biotec) following the procedures of Hattori and Sakaki[25]. *b*, A 330-nucleotide *Eco*RI–*Rsa*I fragment from clone T8 was 5′ end-labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase, hybridized with 25 μg total HER XC2 RNA[16] in 10 μl PIPES-formamide[23,26] at 42 °C for 20 h before digestion with S1 nuclease[26]. S1-resistant DNA fragments were resolved in a 6% (w/v) acrylamide–urea gel; autoradiography was for 4 days with one intensifying screen. Arrow indicates position of a 330-base fragment, corresponding to the size of the full-length probe.

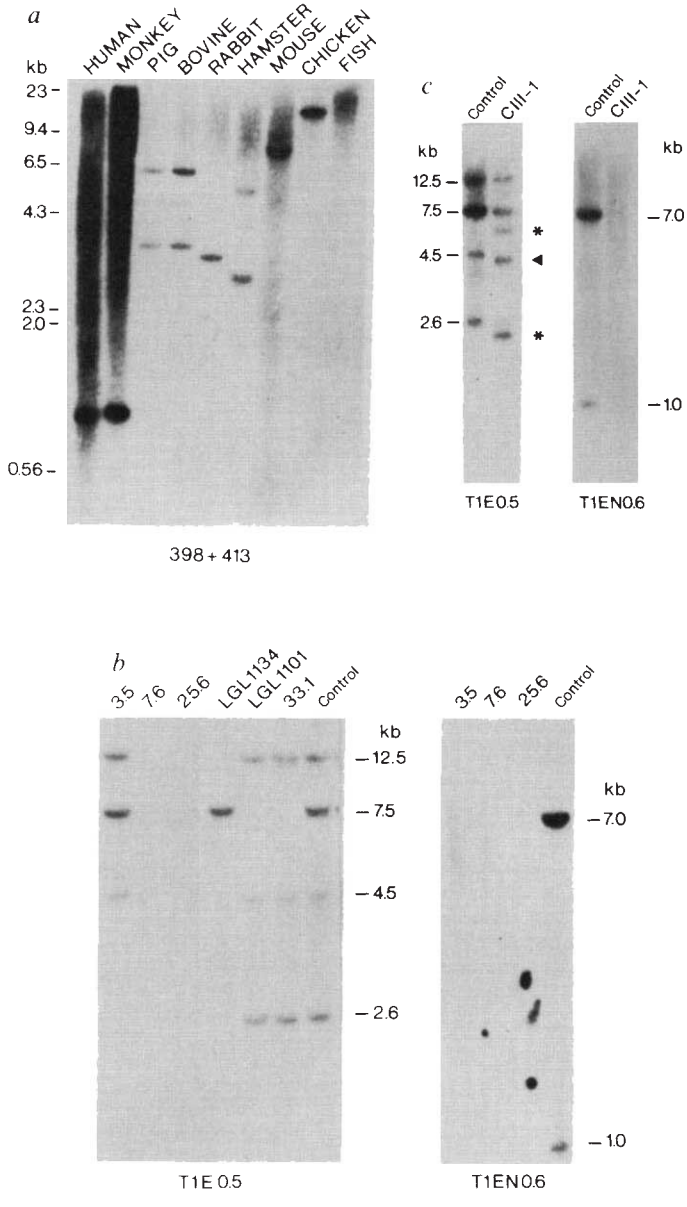

FIG. 3 Southern blot analysis of genomic and cDNA clones. *a*, A mixture of single-copy probes 413 (0.5 kb *Eco*RI) and 398 (1.0 kb *Eco*RI) shows cross-species homology to DNAs of various mammals, and to chicken. *b*, Probes T1E0.5 (segments A and B; see Fig. 1) and T1EN0.6 (segment D and part of segment E; see Fig. 1) detect deletions in male patients with TCD. Deletions in patients 7.6 and 25.6 span all cloned exons from the putative TCD cDNA. In the DNA of patient 3.5, the *Eco*RI bands of 2.6, 1.0/1.6, and 7.0 kb are deleted. The 1.6 kb *Eco*RI fragment contains only 27 bp of segment D, and is therefore not identified with probe T1EN0.6. Deletion LGL1134 spans segments B and C; deletions LGL1101 and 33.1 merely span segment A. *c*, The human–hamster cell hybrid CIII-1 contains the der(13) chromosome from a female with a balanced t(X; 13) translocation associated with TCD[5,15]. This cell line, which contains the Xq21.2-qter part of the human X chromosome, carries all genomic fragments detected by clone T1E0.5, and lacks the two *Eco*RI fragments detected by T1EN0.6. The 4.5-kb *Eco*RI band in control male DNA (control) is represented by a polymorphic variant of 4.2 kb in cell line CIII-1, which comigrates with a hamster band (triangle; F.P.M.C., unpublished results). The extra fragments detected by T1E0.5 in CIII-1 DNA are hamster-specific (see asterisks).

METHODS. Genomic DNAs (10 μg of each) were digested with *Eco*RI. The fragments were subjected to electrophoresis and blotted to GeneScreenPlus (NEN) as described[7]. Isolation and radiolabelling of plasmid inserts was done as described[24]. The blot in *a* was hybridized in 0.5 M NaH_2PO_4 pH6.8, 7% (w/v) SDS, 1 mM EDTA, 50 μg ml^{-1} sonicated, denatured herring sperm DNA, at 50 °C, for 18 h. Washing was in 40 mM NaH_2PO_4, 1% (w/v) SDS at 60 °C for 1 h. The hybridization and washing procedures for *b* and *c* have been published elsewhere[7].

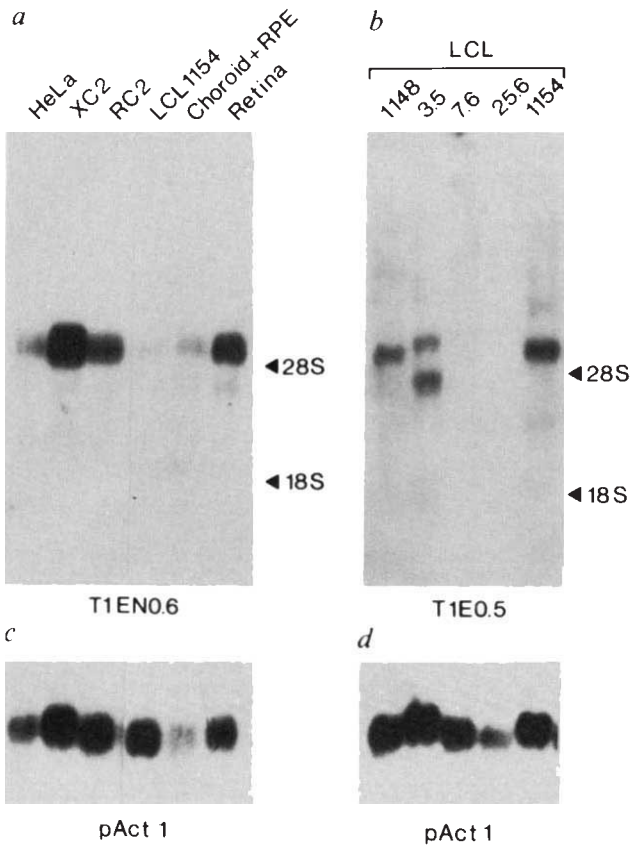

FIG. 4 Northern blot analysis of RNA from several human cell lines and tissues using cDNA clone T1. *a*, Blot containing RNA from HeLa, two retinal cell lines (HER RC2 and HER XC2; ref. 16), an EBV-immortalized lymphoblastoid cell line (LCL1154), as well as human choroid/retinal pigment epithelium and retina. *b*, Blot containing RNA from lymphoblastoid cell lines from patients with different microdeletions encompassing the TCD locus (Fig. 1, and refs 5 and 6), and from two male controls (LCL1148 and LCL1154). *c*, *d*, Hybridization of a hamster actin cDNA clone (pAct-1; ref. 27) to the same blots as in *a* and *b*, respectively. The positions of the 18S and 28S ribosomal RNA bands are indicated.

METHODS. Total cellular RNA was isolated from cells and tissues using the modified LiCl–urea extraction procedure[28,29]. Approximately 10 μg of RNA was dissolved in 30 μl of 50% (v/v) formamide, 2.2 M formaldehyde, 20 mM MOPS, pH 7.0 buffer (4-morpholine-propanesulphonic acid, 5 mM Na-acetate, 1 mM EDTA), loaded onto a 1% agarose gel containing 2.2 M formaldehyde in 20 mM MOPS buffer, and resolved by electrophoresis at 1 V cm^{-1} for 16 h. The gel was rinsed extensively with sterile water for 15 min and RNA blotted to a GeneScreenPlus membrane (NEN) in 10 × SSC. After baking for 2 h at 80 °C, blots were hybridized with random-primed probes. Prehybridization and hybridization was done in 5 × SSPE, 10% (w/v) dextransulphate, 1% (w/v) SDS, and 100 μg ml^{-1} sonicated, denatured herring sperm DNA at 60 °C for 4 and 16 h respectively. Washing was done at the same temperature in 2 × SSC, 1% (w/v) SDS for 40 min.

as Fos, Myc and E1A, are important transcriptional regulators[17,18].

Recently, photoreceptor-specific genes have been implicated in autosomal dominant retinitis pigmentosa[19] and two different forms of retinal degeneration of the mouse[20,21]. It is of note, therefore, that the expression of the putative TCD gene is not confined to the eye. This finding is not unprecedented, however. For example, gyrate atrophy, a choroidal disease with clinical similarity to TCD, is caused by a deficiency of an ubiquitously expressed protein, the enzyme ornithine aminotransferase[22]. By analogy, this may indicate that TCD also is due to a generalized metabolic defect. As this is the first candidate gene for any of the X-linked forms of chorioretinal degeneration, elucidation of its structure and function may provide deeper insight into the molecular mechanisms underlying degenerative processes of the retina, retinal pigment epithelium and choroid. □

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1. Heckenlively, J. R. *Retinitis pigmentosa* (Lippincott, Philadelphia, 1988).
2. Goedbloed, J. *Ophthalmologica* **104,** 308–315 (1942).
3. Waardenburg, P. J. *Acta ophthalmol.* **20,** 235–274 (1942).
4. Cremers, F. P. M. *et al. Clin. Genet.* **32,** 421–423 (1987).
5. Cremers, F. P. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 7510–7514 (1989).
6. Cremers, F. P. M. *et al. Am. J. hum. Genet.* (in the press).
7. Cremers, F. P. M. *et al. Genomics* **4,** 41–46 (1989).
8. Merry, D. E. *et al. Am. J. hum. Genet.* **45,** 530–540 (1989).
9. Cremers, F. P. M. *et al. Hum. Genet.* (in the press).
10. van de Pol, T. J. R., Cremers, F. P. M., Brohet, R. M., Wieringa, B. & Ropers, H.-H. *Nucleic Acids Res.* **18,** 725–731 (1990).
11. Merry, D. E. *et al. Genomics* **6,** 609–615 (1990).
12. Nathans, J., Thomas, D. & Hogness, D. S. *Science* **232,** 193–202 (1986).
13. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seiler, S. & Sharp, P. A. *A. Rev. Biochem.* **55,** 1119–1150 (1986).
14. Kozak, M. *J. molec. Biol.* **196,** 947–950 (1987).
15. Siu, V. M., Gonder, J. R., Jung, J. H., Sergovich, F. R. & Flintoff, W. F. *Hum. Genet.* **84,** 459–464 (1990).
16. Vaessen, R. T. M. J., Houweling, A., Israel, A., Kourilsky, P. & van der Eb, A. J. *EMBO J.* **5,** 335–341 (1986).
17. Rogers, S., Wells, R. & Rechsteiner, M. *Science* **234,** 364–368 (1986).
18. Rechsteiner, M., Rogers, S. & Rote, K. *Trends Biochem. Sci.* **12,** 390–394 (1987).
19. Dryja, T. P. *et al. Nature* **343,** 364–366 (1990).
20. Travis, G. H., Brennan, M. B., Danielson, P. E., Kozak, C. A. & Sutcliffe, J. G. *Nature* **338,** 70–73 (1989).
21. Bowes, C., Danciger, M., Kozak, C. A. & Farber, D. B. *Proc. natn. Acad. Sci. U.S.A.* **86,** 9722–9726 (1989).
22. Valle, D. & Simell, O. in *The Metabolic Basis of Inherited Disease* (eds. Stanbury, J. B., Wijngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S.) 382–401 (McGraw-Hill, New York, 1983).
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).
24. Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137,** 266–267 (1984).
25. Hattori, M. & Sakaki, Y. *Analyt. Biochem.* **152,** 232–238 (1986).
26. Weaver, R. F. & Weissman, C. *Nucleic Acids Res.* **7,** 1175–1193 (1979).
27. Dodemont, H. J. *et al. EMBO J.* **1,** 167–171 (1982).
28. Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107,** 303–314 (1980).
29. LeMeur, M. *et al. Cell* **23,** 561–571 (1981).

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Retinal degeneration in the *rd* mouse is caused by a defect in the β subunit of rod cGMP-phosphodiesterase

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MICE homozygous for the *rd* mutation display hereditary retinal degeneration and the classic *rd* lines serve as a model for human retinitis pigmentosa[1,2]. In affected animals the retinal rod photoreceptor cells begin degenerating at about postnatal day 8, and by four weeks no photoreceptors are left[3]. Degeneration is preceded by accumulation of cyclic GMP in the retina[4] and is correlated with deficient activity of the rod photoreceptor cGMP-phosphodiesterase[5]. We have recently isolated a candidate complementary DNA for the *rd* gene[6] from a mouse retinal library and completed the characterization of cDNAs encoding all subunits of bovine photoreceptor phosphodiesterase[7]. The candidate cDNA shows strong homology with a cDNA encoding the bovine phosphodiesterase β subunit. Here we present evidence that the candidate cDNA is the murine homologue of bovine phosphodiesterase β cDNA. We conclude that the mouse *rd* locus encodes the rod photoreceptor cGMP-phosphodiesterase β subunit.

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