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Chromosomal Localization of DBL[1] Oncogene Sequences

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The DBL oncogene was generated by rearrangements involving three discontinuous regions of the human genome. Analyses of panels of human × rodent somatic cell hybrids demonstrated that the DBL proto-oncogene located on the X chromosome (just proximal or distal to bands q26–27.2) underwent recombination at its 5′ and 3′ ends with sequences derived from chromosomes 3 (p13q–ter) and 16 (p13–q22), respectively. DBL was localized to chromosome Xq27–q28 by *in situ* hybridization. Another oncogene, MCF2, was previously shown to contain sequences derived from Xq27 as well. Comparison of the restriction maps and nucleotide sequences of genomic and cDNA clones representing the chromosome X-specific sequences of the DBL oncogene and MCF2, taken together with their chromosomal localization, indicates that these oncogenes were derived from the same genetic locus.

INTRODUCTION

The DBL oncogene was initially isolated from a human *d*iffuse poorly differentiated *B*-cell *l*ymphoma by DNA transfection of mouse NIH/3T3 cells (Eva and Aaronson, 1985). A subsequent independent isolate was obtained following transfection of NIH/3T3 cells with DNA of a human *n*odular, *p*oorly *d*ifferentiated *l*ymphoma (designated NPDL–*dbl* by the authors) (Eva *et al.*, 1987). Comparative restriction enzyme analyses of the DBL and NPDL–*dbl* oncogenes in transfected NIH/3T3 cells and of related sequences in normal human placenta DNA (Eva *et al.*, 1987) revealed that the generation of these oncogenes involved recombination between at least two sets of human sequences derived from noncontiguous loci. In the case of the DBL oncogene, recombination occurred within the 5′ and 3′ regions of the DBL proto-oncogene, whereas only a 5′ rearrangement led to the generation of NPDL–*dbl*. In NIH/3T3 transfectants we have shown that the DBL oncogene-specific transcript is derived from 5′ and middle sets of sequences, but not from the 3′ set. Examination of the DNA of the tumor cells from which DBL and NPDL-*dbl* oncogenes were derived did not reveal alteration of DBL-related sequences and led to the conclusion that transforming activity was acquired as result of the transfection process (Eva *et al.*, 1987).

Nucleotide sequence analyses of cDNAs representing DBL oncogene and proto-oncogene transcripts showed that the rearrangement resulted in the loss of the amino-terminal 497 codons of DBL and acquisition of new sequences including a promoter region and an amino terminus of 50 amino acids derived from an unidentified gene (Eva *et al.*, 1988; Ron *et al.*, 1988). A molecular clone of the NPDL–*dbl* oncogene has not yet been isolated. Thus, we do not have more detailed knowledge of its structure.

The DBL cDNA is capable of inducing transformation of NIH/3T3 cells provided that its expression is driven by a strong promoter; however, its transforming activity is 20–50 times lower than that of the DBL oncogene (Ron *et al.*, 1988). Truncating either the DBL oncogene or the DBL cDNAs at a point corresponding to the recombination site in the oncogene (that is, codons 1–497 and 1–50 in the DBL and DBL oncogene sequences, respectively) generates molecules with transforming activities equivalent to that of the parental oncogene when each of these molecules is placed under the transcriptional control of the same promoter (Ron *et al.*, 1989, in press).

The predicted amino acid sequence of DBL is not significantly similar to that of any known oncogene, is markedly hydrophilic, and does not possess features characteristic of integral membrane proteins. In transfected mouse cells the 66-kDa DBL oncogene protein is distributed approximately equally between soluble and membrane fractions of the cytoplasm and is not detected in the nucleus (Srivastava *et al.*, 1986). Thus, DBL is considered to represent a unique class of human

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. J03639.

[1] HGM symbol.

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transforming gene. In order to understand better its possible role in human malignancies and the events leading to the activation of DBL, we have determined the chromosomal location of DBL sequences in the human genome.

MATERIALS AND METHODS

Cell Hybrids, Molecular Clones, DNA Probes, and Southern Analysis

Construction and karyotypic and biochemical analyses of human × mouse and human × hamster somatic cell hybrids retaining varying numbers of human chromosomes have been reported in detail (McBride *et al.*, 1982a,b,c). Genomic (Eva and Aaronson, 1985) and cDNA (Eva *et al.*, 1988) clones of the DBL oncogene have been described and they are shown diagrammatically, along with the hybridization probes used in this study, in Fig. 1. The DBL oncogene was isolated by cloning DNA of an NIH/3T3 transfectant into a cosmid vector; this cosmid clone transforms NIH/3T3 cells. A restriction map of this clone is shown in Fig. 1A along with the regions used as hybridization probes. This clone has been described in more detail previously (Eva *et al.*, 1987). Two DBL oncogene cDNA clones were also used as probes. The cDNAs were derived from poly(A)$^+$ RNA isolated from NIH/3T3 transfectants (Eva *et al.*, 1988). Sequences in clone 1b1-1 were derived solely from the middle region of the DBL oncogene, indicated by the solid line.

Isolation and purification of molecularly cloned DNA fragments and high-molecular-weight eucaryotic cell DNA were performed according to standard techniques. The hybridization conditions used for analyzing Southern blots of somatic cell hybrid DNA have been described (Jaiswal *et al.*, 1988). Briefly, nylon membranes to which DNA fragments had been transferred were hybridized with ^{32}P-labeled probes at 42°C for 24–48 h in a solution containing 50% formamide and 10% dextran sulfate and were washed at 55°C in 0.15 *M* NaCl, 0.0015 *M* sodium citrate, pH 7.0. In some cases, after autoradiography, the bound probe was removed from the blot with 0.4 *M* NaOH at 42°C and the membrane was rehybridized with another probe.

In Situ Hybridization and Autoradiography

Peripheral blood lymphocytes from a normal male subject (46;XY) were cultured for 96 h in RPMI medium supplemented with 15% fetal bovine serum, phytohemagglutinin, and antibiotics. Air-dried chromosome preparations were obtained from methotrexate-synchronized cultures as previously described (Popescu *et al.*, 1985). Radiolabeled probes (sp act, $\sim 1/5 \times 10^7$ cpm/μg) were prepared by nick translation of plasmid DNAs using all four ^{3}H-labeled deoxynucleoside triphosphates and were hybridized to RNase-treated and formamide-denatured chromosomes for 20 h at 40°C. Hybridized chromosome preparations were washed in 50% formamide, 2× SSC at 39°C and then with an alcohol series and covered with a 50% solution of NTB-2 nuclear track emulsion (Kodak, Rochester, NY). The slides were stored desiccated at 4°C for 14 days and then developed. Grains situated on nonoverlapping chromosomal regions were counted and their specific locations determined on G-banded chromosomes as previously described (Popescu *et al.*, 1985; Tronick *et al.*, 1985).

RESULTS

Somatic Cell Hybrids

We initially employed genomic probes (lacking repetitive sequences) representing transcribed regions of the DBL oncogene isolated by cloning DNA of a third-cycle NIH/3T3 transfectant (Eva *et al.*, 1987). One probe was derived from the 5′ region, 2.4–3.5 kb on the map of the cosmid clone (probe a in Fig. 1A). The second probe corresponds to 13–14.1 kb on the map (probe b in Fig. 1A). Each probe detected the 3.0-kb DBL-specific mRNA in NIH/3T3 transfectants (Eva *et al.*, 1987). These probes were hybridized to several restriction enzyme digests of DNAs isolated from a series of human–rodent somatic cell hybrids containing different human chromosomes (McBride *et al.*, 1982a,b,c). Probe a detected a 6-kb *Eco*RI DNA fragment that could be localized to chromosome 3 (Table 1). Two of the hybrids examined contained spontaneous breaks involving this chromosome. Both of these cellular DNAs hybridized with the 5′ probe and contained the human α2-HS-glycoprotein sequence (3q27–q29), but they did not contain the RAF1 oncogene sequence (3p25) or express aminoacylase 1 (3p21). In addition, one of these hybrids retained the functional glutathione peroxidase 1 gene (3p13–q12), whereas the other did not. This indicates that the probe a sequences representing 5′ DBL oncogene-transcribed sequences were located on the human chromosome 3 long arm or proximal short arm (3p13–qter).

In contrast, probe b (Fig. 1) detected a human-specific 3.3-kb *Eco*RI fragment in different hybrid cell lines of this same series and could be shown instead to be present on the human X chromosome (Table 1). A cDNA clone isolated from a third-cycle NIH/3T3 cell transfectant, designated 1b1-1 (Eva *et al.*, 1988), and derived entirely from the set of DBL coding sequences that are not rearranged with respect to those found in normal human placental DNA (Eva *et al.*, 1987) (that is, only DNA fragments from within the region between

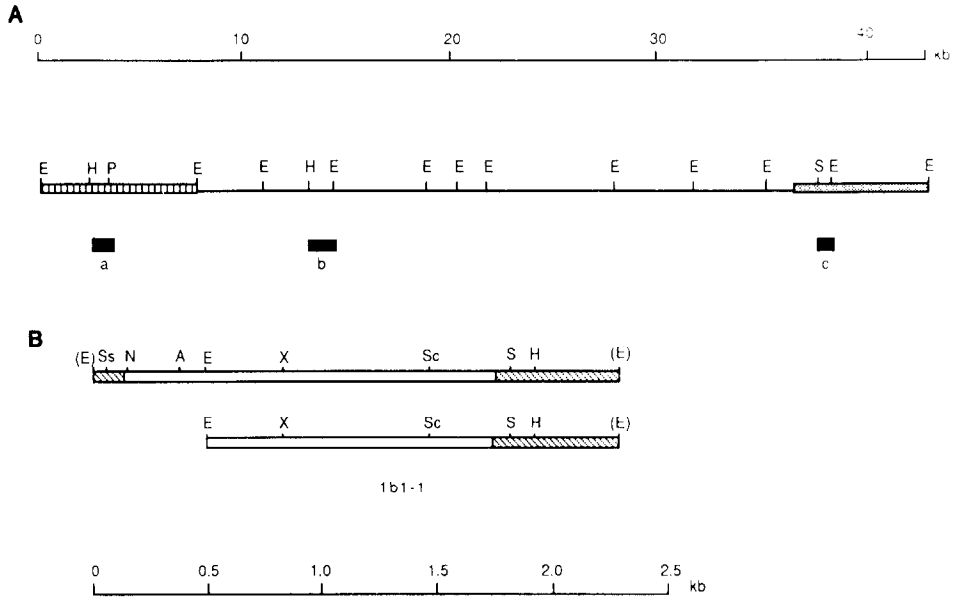

FIG. 1. Physical maps of DBL oncogene genomic and cDNA clones and probes utilized for chromosomal localization. The genomic map (**A**) represents a biologically active cosmid clone (4). The boxes at each end of the map represent the regions acquired as a result of rearrangements of DBL proto-oncogene sequences. The region in common with DBL is indicated by the thin line. The unique sequence probes (**a, b, c**) are indicated by black boxes. A more detailed version of the map has been published (5). DBL oncogene cDNA probes are shown in **B.** Either a full-length cDNA clone (upper diagram) containing both chromosome 3 and X sequences or a probe representing chromosome X sequences only (designated 1b1-1) was employed. 5′ and 3′ untranslated regions are indicated by the diagonal lines, and the coding sequence is denoted by the white rectangle. The nucleotide sequence of this cDNA has been reported (6).

10.8 and 22.6 on the cosmid map) was also used as a probe (Fig. 1A). The results were identical to those obtained with the probe derived from the cosmid clone that was localized to chromosome X. Thus, the cDNA probe identified the same 3.3-kb *Eco*RI band as well as additional fragments of 3.5, 3.8, 4.5, and 6.2 kb. These bands are present within a contiguous region of the DBL oncogene cosmid clone (Eva *et al.*, 1987) (Fig. 1A).

These sequences could all be regionally localized to the distal long arm of the human X chromosome (Xq13–qter) by analysis of hybrids containing only a portion of this chromosome. A hybrid retaining only the human X-chromosome long arm, which had translocated to a mouse chromosome, retained clone 1b1-1 sequences. We also examined a series of hybrids isolated after fusion of human parental cells containing a reciprocal X;14 translocation (Xq13;14q32) (McBride *et al.*, 1987). Fifteen independent hybrids contained both the Xq13–qter translocation chromosome and the clone 1b1-1 sequences. In contrast, these sequences were not present in any of the lines that had lost this translocation chromosome after back selection and were not detected in three hybrids that retained the reciprocal translocation chromosome containing Xpter–q13.

DNAs were also isolated from mouse cell lines retaining only small fragments of human X chromosome after chromosome-mediated gene transfer. One of these lines (A9S2), which contained the distal third of the X-chromosome long arm, also contained clone 1b1-1 sequences (i.e., all five hybridizing bands). These sequences were not detected in either line that retained smaller portions of Xq in the immediate vicinity of the hypoxanthine phosphoribosyltransferase (HPRT) locus. These results indicated that the DBL-related sequences were located just proximal or distal to the HPRT locus (Xq26–27.2).

Comparative restriction enzyme mapping showed that in the DBL oncogene cosmid clone the 3′-terminal 6 kb and sequences upstream to them are not contiguous within normal human placenta DNA. These 3′-terminal sequences were not found to be detectably transcribed in transfectants (Eva *et al.*, 1987). Probe c was derived from within this region (37.9 to 38.8 kb on the map, Fig. 1A) and was used to analyze DNAs of the rodent–human hybrid cell lines. A 4.6-kb human-specific *Eco*RI fragment was detected by this probe and could be localized to chromosome 16 (Table 1). Thus, this sequence was detected in all hybrids containing an intact human chromosome 16, but there was discordant segregation with all other human chromosomes. The sequence was also found in a human–hamster hybrid and five subclones of another hybrid, all of which expressed diaphorase (NADH/NADPH–cytochrome b5 reductase; DIA4) (16q12–q22) but

TABLE 1

Segregation of DBL Sequences with Specific Human Chromosomes

Human chromosome	% Discordancy		
	Probe a	Probe b/1b1-1	Probe c
1	26	47	26
2	30	53	42
3	0	41	50
4	23	41	49
5	28	50	32
6	42	43	34
7	51	41	47
8	37	47	27
9	35	51	36
10	25	43	45
11	32	43	32
12	33	40	29
13	42	43	50
14	56	44	29
15	49	43	52
16	35	43	1
17	47	46	45
18	42	51	49
19	19	41	35
20	35	49	36
21	33	28	50
22	32	51	47
X	33	0	36

Note. Chromosomal mapping of regions of DBL oncogene sequences was based on Southern analysis of size-fractionated *Eco*RI digests of human–hamster and human–mouse somatic cell hybrid DNAs with ^{32}P-labeled cloned probes. Probe a [2.4–3.5 kb on the map (Fig. 1)] detected a 6.0-kbp *Eco*RI fragment in human DNA which was easily resolved from 12.5- and 15-kb cross-hybridizing sequences in the hamster and mouse DNAs, respectively. Probe b [13–14.1 kb on the map (Fig. 1)] identified a single 3.5-kb *Eco*RI fragment in human DNA. The cDNA (cl 1b1-1) identified this same fragment, as well as 3.8-, 4.5-, and 6.2-kb *Eco*RI bands in human DNA. These four bands all segregated concordantly in hybrid cells. Under the hybridization conditions employed, neither probe b nor clone 1b1-1 detected related sequences in hamster or mouse DNAs. Probe c [37.9 to 38.3 kb on the map (Fig. 1)] detected a 4.6-kbp *Eco*RI fragment in human DNA. Detection of each of these hybridizing sequences is correlated with the presence or absence of each human chromosome in the somatic cell hybrids. Discordancy indicates the presence of hybridizing sequences in the absence of the chromosome or absence of the hybridizing bands despite the presence of the chromosome; the sum of these numbers divided by the total hybrids examined (×100) represents percentage discordancy. There were 57 somatic cell hybrids (21 positive) examined with probe a and 68 (41 positive) with probe b. The human, mouse, and Chinese hamster parental cells and the procedures for fusion, isolation, and characterization of the hybrids have been described (16–18).

not phosphoglycolate phosphatase (PGP) (16p13.31–p13.12). It was also present in six hybrids that expressed the PGP marker but no DIA4. The active metallothionein gene cluster 16q21–q21.1 was present in both groups of hybrids. These results permitted regional localization of the probe c sequence to 16p13–q22.

In Situ Hybridization

We attempted to map DBL sequences more precisely by *in situ* hybridization. A probe derived from a cDNA clone (Eva *et al.*, 1988) containing sequences derived from both chromosomes 3 and X (Fig. 1B) was hybridized to prometaphase and metaphase chromosome spreads. Following autoradiography, the grains were localized on photomicrographs of chromosomes before and after G-banding. Of total 175 grains localized, 27% were on the long arm of chromosome X. The majority of grains on chromosome X clustered on the terminal region of the long arm at bands q26–28 (Fig. 2). Similarly, in experiments utilizing the genomic probe b specific for chromosome X, the only significant accumulation of grains, 16 of a total of 105 (18%), was observed at Xq26–28 (data not shown). Therefore we assigned DBL to band q27–28.

Chromosome 3, as expected from the somatic cell hybrid data, was found to also harbor DBL-related sequences (Fig. 3). There were 32 grains (18%) on the long arm of chromosome 3, 21 of which (67%) were at bands 3q13–23 and 8 of which (25%) were at bands 3q25–27 (Fig. 3). The remaining grains were randomly distributed over the other chromosomes. There is as yet no explanation for the bimodal distribution of grains but it could be due to the hybridization conditions employed. Representative chromosome spreads are shown in Fig. 4.

DISCUSSION

DNA transfection experiments have uncovered several examples of human genes whose oncogenic potential is activated by DNA rearrangements. These proto-oncogenes include members of the tyrosine-specific [MET (Park *et al.*, 1986), MCF3/ROS1 (Birchmeier *et al.*, 1986), RET (Takahashi *et al.*, 1985), TRK (Martin-Zanca *et al.*, 1986; Kozma *et al.*, 1988)], and serine/threonine-specific [RAF1 (Shimizu *et al.*, 1985; Fukui *et al.*, 1987; Stanton and Cooper, 1987)] protein kinase families, the fibroblast growth factor family [HSTF1 (Delli-Bovi *et al.*, 1987; Sakamoto *et al.*, 1986)], and an angiotensin receptor [MAS1 (Young *et al.*, 1986; Jackson *et al.*, 1988)]. The functions of some [DBL, MCF2, and *tre* (Huebner *et al.*, 1988)] have not yet been established. In many cases, including that of DBL, the rearrangements most likely occurred as a result of the gene transfer process. It should be noted, however, that the possibility that these genes were rearranged in a small fraction of the original tumor cell population still exists.

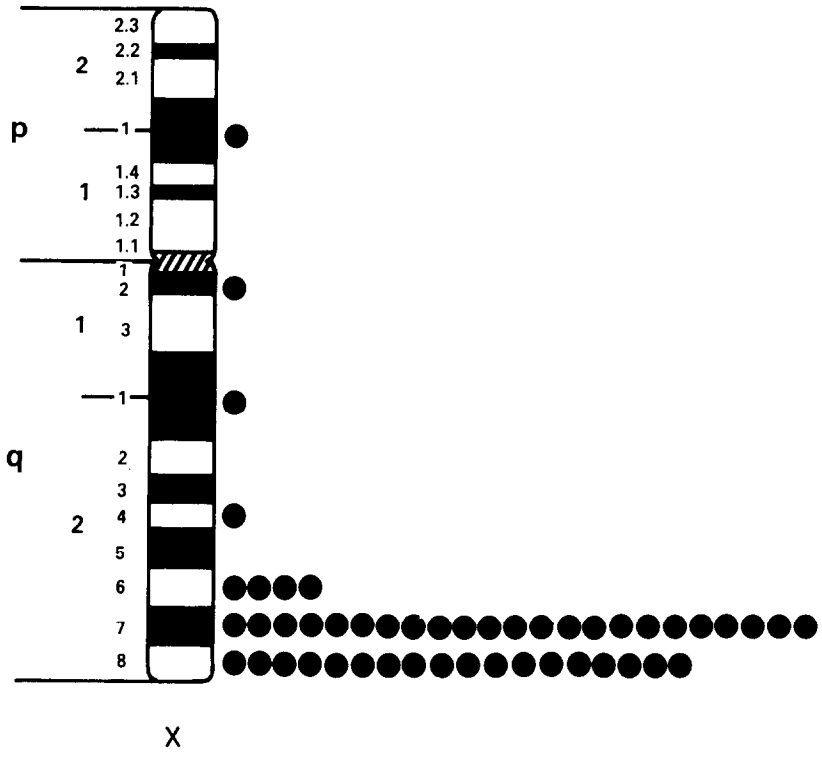

FIG. 2. Regional localization of DBL sequences to the human X chromosome, bands q27–q28. The dots indicate hybridization signals (grains). The 1b1-1 cDNA probe was used.

We have demonstrated that DBL can be activated as a transforming gene for NIH/3T3 cells either by expression at high levels (Ron *et al.*, 1988) or by deletion of its first 497 codons (Ron *et al.*, 1989, in press). These studies demonstrate that in DBL the chromosome 3-derived sequences provided transcriptional and translational elements necessary for the expression of a truncated, activated form of the proto-oncogene,

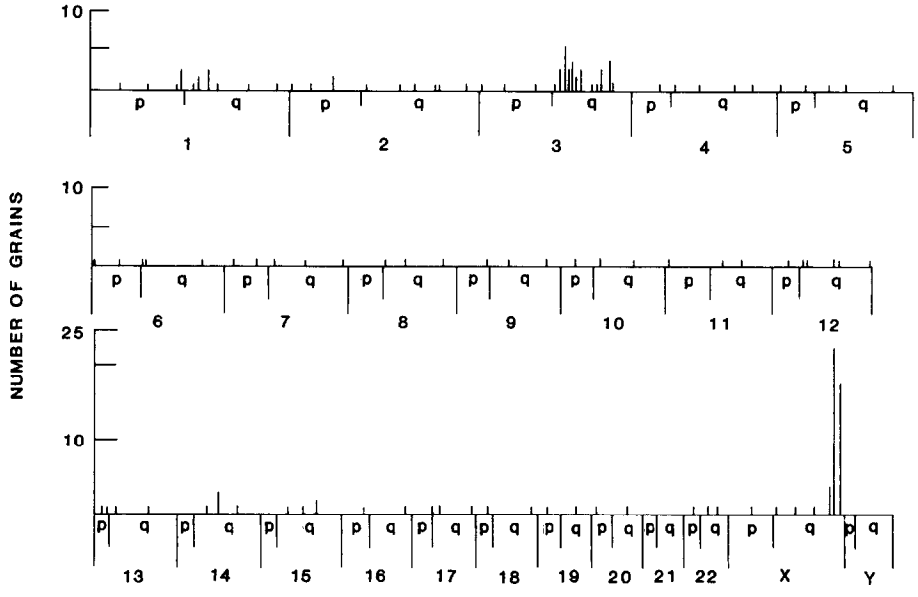

FIG. 3. Chromosomal localization of DBL sequences using a DBL oncogene cDNA probe containing sequences derived from chromosomes 3 and X.

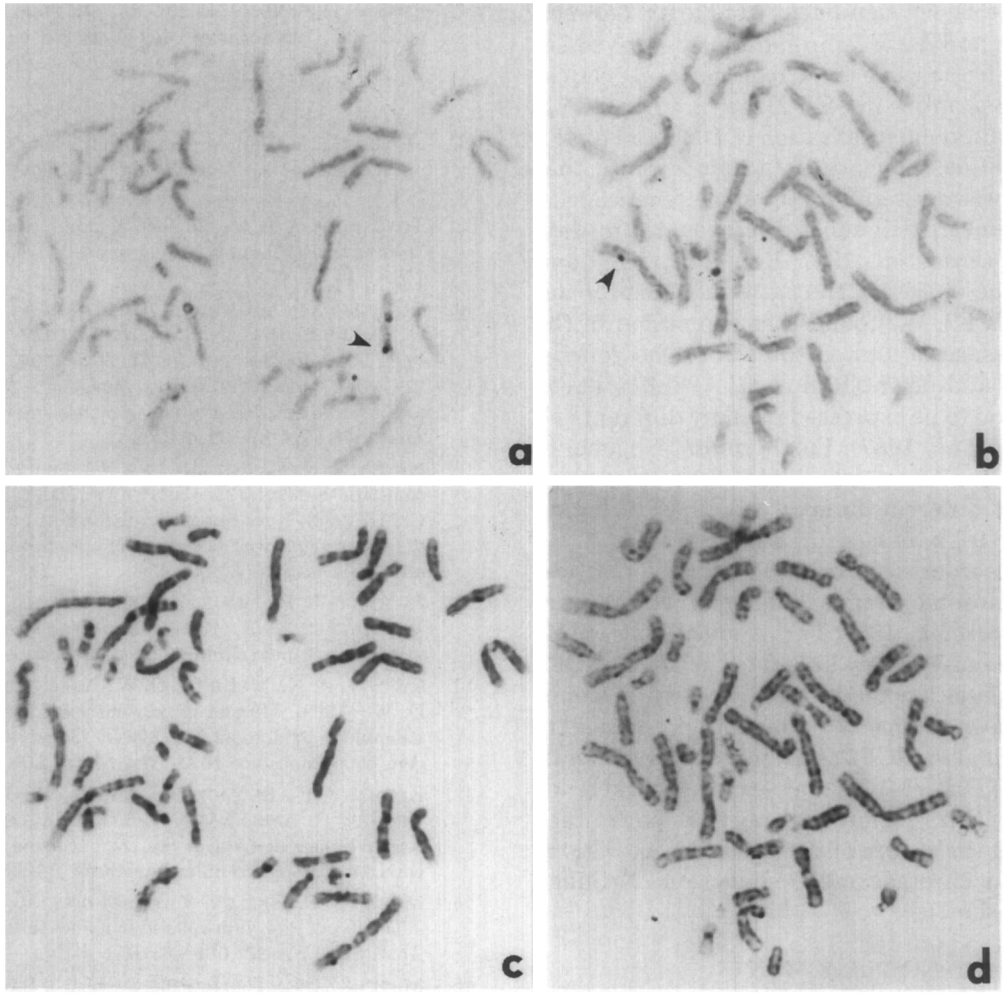

FIG. 4. Human metaphases from normal peripheral leukocyte cultures after *in situ* hybridization with a DBL oncogene cDNA probe, exhibiting label at the terminus of the long arm of a submetacentric chromosome (**a**) and a metacentric (**b**). After G-banding, the labeled chromosomes are identifiable as chromosomes X (**c**) and 3 (**d**) where DBL sequences were localized.

whereas chromosome 16 sequences played no role in transforming activity. The sequences derived from chromosomes 3 and 16 flanked a contiguous region of approximately 25 kb in length derived from DBL which could be localized to the human X chromosome, bands q27–28.

Three other sequences related to oncogenes have been localized to chromosome X. The pseudogene HRASP (Miyoshi *et al.*, 1984), which is a homolog of the viral Harvey RAS oncogene, was mapped to Xcen–q26 (O'Brien *et al.*, 1983). The ARAF1 gene (Huebner *et al.*, 1986; Mark *et al.*, 1986), which is related to the transforming gene of the 3611 murine sarcoma virus (Rapp *et al.*, 1983), was localized to Xp11.2–11.4 (Huebner *et al.*, 1986; Mark *et al.*, 1986; Popescu and Mark, in press). The MCF2 oncogene (Fasano *et al.*, 1984) was isolated by DNA transfection from the human mammary carcinoma cell line MCF-7 and was found to reside at Xq27 (Noguchi *et al.*, 1987). Thus, DBL maps to the same locus as MCF2. Comparison of the restriction map of DBL (Eva *et al.*, 1987, 1988) to that subsequently published for the transforming region of MCF2 (Noguchi *et al.*, 1987) suggested that they may be the same gene. More recently, Noguchi *et al.* (1988) published the nucleotide sequences of partial cDNA clones of MCF2 and its normal counterpart. Comparison of the MCF2 sequence with our previously published sequence data showed only 4 differences in the 3′ 1285 nucleotides of DBL and MCF2 coding sequences. The sequences diverge (going upstream) at the point of recombination between chromosome 3 and DBL proto-oncogene sequences.

Interestingly, the 5′ rearrangements in DBL and MCF2 oncogenes both involved chromosome 3, and, in each case, chromosome 3 sequences contributed the amino terminus of the resulting transforming proteins.

However, there is no significant similarity between DBL oncogene and MCF2 chromosome 3-derived sequences. In each oncogene these regions are G · C rich; however, in DBL, unlike in MCF2, they are noncoding. Thus, the chromosomal localization of DBL and MCF2 and the nucleotide sequence data demonstrate that each oncogene was derived from the same genetic locus. There are, however, still some discrepancies in data reported by us (Eva *et al.*, 1987; Ron *et al.*, 1988) and by Noguchi *et al.* (1987) on the patterns of expression of DBL and MCF2. We found that expression of the 5-kb DBL message is limited to cells of the gonads, adrenal gland, and brain (Ron *et al.*, 1988), whereas MCF2 was found to be expressed in many different cell types (Nguyen *et al.*, 1987). Furthermore, Noguchi *et al.*, (1987) detected 2.7- and 2.3-kb MCF2-related transcripts in four different human cell lines including MCF-7 and a 3.9-kb message in human testis.

Of clinical disorders that map to the distal long arm of the X chromosome, fragile X-linked mental retardation (Patterson *et al.*, 1987; Turner and Jacobs, 1984) (Xq27.3) is the most closely linked to the DBL/MCF2 locus. The coagulation factor IX, involved in hemophilia B, has been mapped to a region approximately 30–80 kb proximal to MCF2 (Anson *et al.*, 1988). Thus, the positions of DBL/MCF2 may serve as markers for disorders of the Xq27 region and may be important for further defining the nature of the molecular alterations associated with chromosome fragility and X-linked mental retardation.

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Note added in proof. Since this manuscript was accepted for publication, the International Workshop on Human Gene Mapping retained MCF2 as the gene symbol for DBL.

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