Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6568–6572, September 1986
Genetics

The human c-*ros* gene (*ROS*) is located at chromosome region 6q16→6q22

(*ros* oncogene/UR2 avian sarcoma virus/gene mapping/*in situ* hybridization)

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Communicated by Peter C. Nowell, May 27, 1986

ABSTRACT **The human homolog, c-*ros*, of the transforming gene, v-*ros*, of the avian sarcoma virus, UR2, has been isolated from a human genomic library. A single-copy fragment from the human c-*ros* genomic clone has been used to map the human c-*ros* homolog (*ROS*) to human chromosome region 6q16→6q22 by somatic cell hybrid analysis and chromosomal *in situ* hybridization. Thus, the c-*ros* gene joins the c-*myb* oncogene, which is distal to the c-*ros* gene on the long arm of human chromosome 6, as a candidate for involvement in chromosome 6q deletions and rearrangements seen in various malignancies.**

The demonstration that the c-*myc* and c-*abl* oncogenes are directly involved (1–5) in characteristic chromosomal translocations that are specific to Burkitt lymphoma and chronic myelogenous leukemia (6–12) has spurred the search for oncogenes that may be located at other characteristic chromosomal breakpoints observed in human malignancies; the search has indeed pinpointed other oncogenes that are near to, or directly involved in, specific chromosomal breaks (13, 14).

The goal of the present study was to isolate a molecular clone for the human homolog of v-*ros*, the transforming gene (15, 16) of the avian sarcoma virus, UR2 (17), in order to determine the location of the c-*ros* gene in the human genome as a prelude to assessment of possible involvement of this gene in human malignancy.

The avian UR2 virus induces sarcomas *in vivo* and transforms chicken embryo fibroblasts *in vitro* to a distinctive elongated morphology, but these cells retain a high level of cytoskeletal organization (17, 18). The fused *gag–ros* sequences in UR2 code for a 68,000 molecular weight polyprotein, P68, which was found to be associated with a tyrosine-specific protein kinase activity (16). The kinase domain of P68 shares amino acid sequence homology with the intracellular domains of human epidermal growth factor receptor and insulin receptor (19–22) and is a membrane-associated protein (52). The *ros* oncogene is a member of the *src* gene family that also includes *fps/fes, yes, fms, abl, erb B*, and *fgr* genes (for review see ref. 23). The proteins encoded by these genes have a high degree of amino acid sequence homology and are all associated with tyrosine-specific kinase activities (ref. 23) that are believed to be essential to their mechanisms of cell transformation. The *src*-related cellular oncogenes are expressed at low levels in normal cells (24), and some cellular homologs of *src*-related oncogenes are associated with tyrosine-specific protein kinase activities (25–29). Evidence is accumulating that shows these proteins to be involved in the control of cell proliferation and differentiation. For example, the cellular homologs of v-*erbB* and v-*fms* are the receptors for epidermal growth factor (19) and the macrophage growth factor known as colony-stimulating factor 1 (30), respectively. Because of the importance of this family of genes in normal cell function and in malignancy, it is of great interest to isolate, characterize, and chromosomally localize each member of this oncogene family.

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MATERIALS AND METHODS

Cloned DNA Probes. A full-length UR2 DNA clone, pKD6, which is a permuted clone derived from the original UR2 cloned genome (31), was cleaved with restriction endonucleases *Sma* I and *Hin*dIII, and a 2.1-kilobase (kb) DNA fragment was isolated that contained the entire v-*ros* sequence plus a small amount of *gag* sequence at the 5′ end and *env* sequence at the 3′ end. This fragment was radiolabeled (32) and used to screen a human genomic library for the human c-*ros* gene.

For comparative regional localization of the human c-*ros* gene, we have also used DNA probes for two other oncogenes located on human chromosome 6, a human c-*myb* cDNA probe (D. Wilcox and C.M.C., unpublished data) and a human *pim-1* genomic clone (33). The human c-*myb* gene has been mapped to the long arm of chromosome 6 (34–36), and human *pim-1*, a putative oncogene, has been mapped to chromosome region 6p12–6p21 (33). Probes were labeled to 1×10^8 cpm/0.1 μg by nick-translation using deoxynucleotide[α-^{32}P]triphosphates (37).

Cells. Isolation, propagation, and characterization of parental cells and somatic-cell hybrids used in this study have been described (33, 38–41). Hybrids were characterized for expression of enzymes and retention of DNA markers assigned to each human chromosome; some hybrid clones were karyotyped by trypsin/Giemsa and/or G-11 banding methods as described (38).

For regional localization of the c-*ros* gene, mouse–human hybrids that retained a partial human chromosome 6 were used. Hybrid clone 56-47c122 retains a t(6;17)(6pter→6p21::17p13→17qter) translocation chromosome (42) and hybrid clone MCP-6 retains a t(6;X)(6p21→6qter::Xq13→Xqter) translocation chromosome (43).

Southern Blot Analysis. DNAs from human peripheral blood lymphocytes and cell lines, mouse cell lines, and mouse–human hybrid cell lines were prepared by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNAs were digested with an excess of appropriate restriction enzymes, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose or nylon filters as described by Southern (44). Hybridization with ^{32}P-labeled

Abbreviations: kb, kilobase; kbp, kilobase pairs.

nick-translated probes (37) was carried out at 68°C in 4× SSC/1× Denhardt's solution containing 0.2 mg of sonicated salmon sperm DNA per ml (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; 1× Denhardt's solution is 0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll). Filters were washed in 0.1× SSC/0.1% $NaDodSO_4$ at 68°C; hybridized filters were exposed to Kodak XAR film at −70°C in the presence of an intensifying screen for 12–72 hr.

Chromosomal *in Situ* Hybridization. Total 5.5-kilobase pair (kbp) human c-*ros*-containing plasmid, phrosHi6 (described in *Results*), was nick-translated with all four ^{3}H-labeled deoxynucleotide triphosphates (39) and hybridized to human metaphase preparations from peripheral blood cells of a normal male. The techniques used for *in situ* hybridization were essentially as described by Harper and Saunders (45). Human metaphases were hybridized in 50% formamide/2× SSC/10% dextran sulfate at 37°C for 15 hr. A 200-fold excess of sonicated salmon sperm DNA was included as carrier. The slides were thoroughly washed in 50% formamide/2× SSC at 39°C, exposed to Kodak NTB2 nuclear track emulsion for 10 days at 4°C, and developed with Kodak Dektol at 15°C. The slides were stained in a mixture of six parts borate buffer (pH 9.2) to one part of Wright's/Giemsa stain solution (2.4 g of Wright's stain per liter/1.4 g of Giemsa stain per liter in methanol) (46).

Human Genomic Library. DNA from the peripheral blood cells of a donor with acute promyelocytic leukemia (APL tumor C, ref. 41) was partially digested with restriction enzyme *Sau* 3A and fractionated by sucrose gradient velocity sedimentation. The 15- to 23-kbp DNA fragments were pooled and ligated with the EMBL 3A phage vector and packaged *in vitro*. The karyotype of the APL tumor C cells appeared normal (41) except for the characteristic t(15;17)(q21;q22) translocation chromosome seen in many APLs (47).

RESULTS

The Human c-*ros* Molecular Clone. The structure of the genome of UR2, the v-*ros*-containing retrovirus, has been elucidated, and the v-*ros* gene has been sequenced (31). In cloning the closed circular proviral UR2 DNA, Neckameyer and Wang (31) inserted the UR2 genome into pBR322 at the *Eco*RI site that interrupts the v-*ros* gene; thus, we have used a circularly permuted construct, pKD6, derived from the original cloned UR2 cDNA clone. The UR2 proviral genome was excised from the pBR322 vector using *Eco*RI, recircularized, and reinserted into pBR322 such that the v-*ros* gene was intact. A 2.1-kb *Sma* I–*Hin*dIII fragment containing all of the v-*ros* gene plus some flanking *gag* and *env* sequences was radiolabeled by calf thymus primer extension (32) and used to screen a human genomic library under conditions of hybridization (6× SSC/2× Denhardt's/10 μg of salmon sperm DNA per ml, 68°C), which allowed cross-hybridization of the v-*ros* *Sma* I–*Hin*dIII fragment with human insulin receptor cDNA sequences. Upon screening 330,000 recombinant phages, a single 17-kbp clone, λhrosc16 (Fig. 1), hybridized to the v-*ros* probe.

A unique (i.e., repeat free) 5.5-kbp *Hin*dIII fragment, phrosHi6 (Fig. 1), which spans a large part of the region of homology to v-*ros*, was subcloned into pUC19 and used as the probe in mapping studies.

Human c-*ros* Gene, *ROS*, Is on Chromosome 6 Between *PIM* and *MYB*. We have generated and characterized a panel of rodent–human somatic-cell hybrid clones (33, 38–41) in which the human chromosomes are retained in different, overlapping subsets such that each chromosome is contained in some hybrid cell clone. Restriction endonuclease-cleaved hybrid cell DNAs were transferred to nitrocellulose membranes by the method described by Southern (44) and hybridized to the radiolabeled human c-*ros* subclone, phrosHi6.

A total of 21 mouse–human hybrid cell DNAs were analyzed for presence of the human c-*ros* gene by Southern blot analysis after cleavage with *Eco*RI or *Hin*dIII endonuclease. An example of results obtained with 15 *Hin*dIII-cut hybrid DNAs and controls is shown in Fig. 2. Mouse sequences homologous to human c-*ros* are not detected under conditions of hybridization used (lane 1). Human DNAs (lanes 2 and 3) exhibit a *Hin*dIII restriction fragment length polymorphism with the more frequent allele of 5.5 kb in lane 2 and the less frequent allele at 6.5 kbp (lane 3). *ROS* positive hybrids in lanes 4, 8, and 9 (which are clearly positive on long exposure), 10–13, 15, and 18 all exhibit the 5.5-kbp c-*ros* fragment. Other lanes contain DNA from hybrids that do not retain the c-*ros* gene. These results give an immediate indication of the chromosomal location of the human c-*ros* gene, since lane 18, which is *ROS* positive, contains DNA from a hybrid, MCP-6, that retains only human chromosome region 6p21→6qter (see Fig. 2 legend). Table 1 summarizes the results of Southern blot analyses of 21 hybrid DNAs and demonstrates the correlation between presence of human chromosome 6 and the human c-*ros* sequences. Somatic cell hybrids tested for the presence of c-*ros* have also been tested for three other chromosome 6-linked genes by Southern analysis using DNA probes for human *PIM* (33), vasoactive intestinal polypeptide gene (*VIP*) (I. Gozes, R. Avidor, Y. Yahov, D. Katznelson, C.M.C., and K.H., unpublished data), and *MYB* (34–36); since seven of these hybrids retain only a partial chromosome 6, it has been possible to regionally localize the c-*ros* gene relative to the other six-linked genes. Table 2 summarizes these results and demonstrates that the *ROS* gene resides between the *PIM* (at 6p21) and *MYB* (at 6q22) loci.

Human c-*ros* Gene Is at Chromosome Region 6q16→6q22. To determine more precisely the location of the c-*ros* gene on chromosome 6, chromosomal *in situ* hybridization was performed. The ^{3}H-labeled phrosHi6 plasmid was hybridized to metaphase preparations from a normal male. On examination of 130 metaphases, 42 of 245 chromosomally localized grains, or 17%, were found over chromosome region 6q16→6q22; see Fig. 3 for a graphical representation of these data. No other chromosome region shows significant clustering of grains. Thus, the human c-*ros* gene is located at chromosome region 6q16→6q22.

DISCUSSION

The recently completed sequence of the avian sarcoma virus UR2 oncogene, v-*ros* (31), shows a significantly higher degree of homology with the insulin receptor tyrosine kinase domain (21) than all the presently known *src* gene family members but, nevertheless, is not extensive enough to allow the interpretation that v-*ros* represents a truncated avian

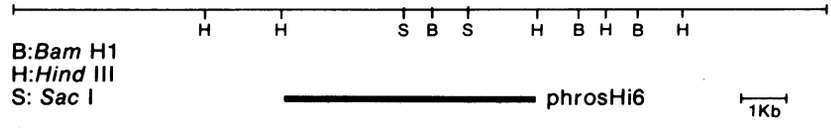

FIG. 1. Human c-*ros* molecular clone. The restriction map represents the 17-kbp λhrosc16, and the closed bar is the v-*ros* homologous 5.5-kbp *Hin*dIII subclone used for mapping studies.

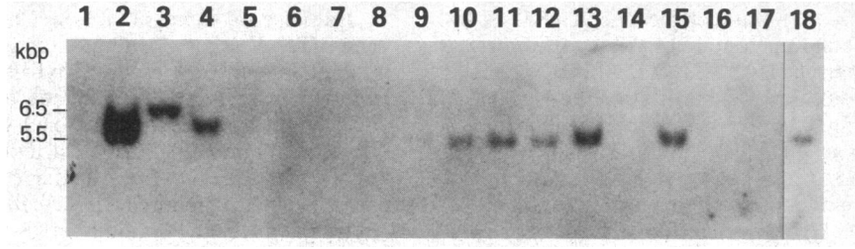

FIG. 2. Human c-*ros* homolog maps to chromosome region 6p21→6qter. Southern blot analysis of DNA (≈10 μg/lane) from a mouse cell line (lane 1); human peripheral blood lymphocyte (PBL) from a normal female (lane 2); human PBL from a normal male (lane 3); and mouse–human hybrids containing the following human chromosomes: chromosomes 6, 7, 17q, and 21 (lane 4); chromosome 7 (lane 5); human translocation chromosome t(6;17)(6pter→6p21::17p13→17qter) (lane 6); chromosome 17 (lane 7); partial chromosome 6, chromosomes 9, 12–14, 17, and 22 (lane 8); partial chromosome 6, chromosomes 9, 12–14, 17, 21, and 22 (lane 9); chromosomes 1, 3–10, 13, 14, 17, 18, 20, 22, and X (lane 10); partial chromosome 4, chromosomes 6, 12, 20, and X (lane 11); partial chromosome 4, chromosomes 6 and X (lane 12); chromosomes 4, 6, 7, 13, 14, 17, 18, and 20 (lane 13); chromosomes 4, 7, 8, 13, 15, 17, 18, 20, and X (lane 14); chromosomes 3–5, partial chromosome 6, and chromosomes 7, 11, 13–15, 17, 18, and X (lane 15); chromosomes 17 and 18 (lane 16); chromosome 4, partial chromosome 6 and chromosome 17 (lane 17); human translocation chromosome t(6;X)(6p21→6qter::Xq13→Xqter) (lane 18). DNA was digested with an excess of restriction enzyme *Hin*dIII, fractionated in agarose, transferred to nitrocellulose, and hybridized to nick-translated ^{32}P-labeled phrosHi6 probe. Approximate size of the human specific c-*ros* fragment in lane 3 is 6.5 kbp; the human c-*ros* band seen in lane 2 and in all positive hybrid DNAs is 5.5 kbp.

insulin receptor (31). The fact that the cellular homolog of v-*ros* is likely to code for a receptor and the extreme tissue specificity of expression of the gene (24) prompted us to isolate a human *ros* homolog. A preliminary restriction map of the human *ROS* locus has been prepared, and a subclone of this region has been used to map the human *ROS* locus to chromosome region 6q16→6q22 proximal to the human c-*myb* locus. Rabin *et al.* (48) have recently mapped *mcf3*, a transforming gene from a mammary carcinoma, to the same region of chromosome 6; the *mcf3*-deduced amino acid sequence shows homology to v-*ros* (48). Thus *mcf3* and our c-*ros* clone probably represent the same genomic locus. This region of chromosome 6 has been shown to be involved in translocations in ovarian carcinoma (49) and deletions in various lymphoproliferative neoplasias (50) and in melanomas (51). In screening more than 40 DNA samples from normal human donors, donors with various malignancies, or human cell lines established from normal or malignant cells, we have not detected rearrangement of the human *ROS* locus (K.H., L.N., J Finan, P. C. Nowell, and C.M.C., unpublished results). We cannot yet rule out deletion of a *ROS* allele in all of our samples since detection of 2-fold differences in intensity of hybridizing bands depends upon very careful quantitation of amount of DNA loaded or upon presence of polymorphic alleles. We have thus far detected only two different *ROS* alleles with the DNAs (>40) and enzymes (*Hin*dIII, *Pst* I) tested. These alleles are the *Hin*dIII 5.5- and 6.5-kbp fragments seen in Fig. 2 (lanes 2 and 3). One heterozygote containing both *Hin*dIII alleles was also seen (data not shown). If *ROS* restriction fragment length polymorphisms that occur at a higher frequency are found using other regions of the *ROS* locus and other enzymes, the occurrence of deletions of the *ROS* region in melanomas and lymphoproliferative malignancies could be assessed.

In previous studies the pattern of expression of several members of the *src* gene family in normal chicken tissues was compared. Expression of the c-*ros* gene was not detectable (i.e., less than 0.3 RNA copies per cell) in spleen, thymus,

Table 1. Correlation of presence of c-*ros* gene and specific human chromosomes in 21 mouse–human hybrids

Human chromosome	No. of hybrid clones with c-*ros*/chromosome retention +/+	−/−	+/−	−/+	Discordant
1	3	9	9	0	9
2	0	9	12	0	12
3	4	8	8	1	9
4	7	7	5	2	7
5	4	8	8	1	9
6	12	9	0	0	0
7	5	7	7	2	9
8	2	7	10	2	12
9	4	9	8	0	8
10	3	9	9	0	9
11	2	9	10	0	10
12	3	9	9	0	9
13	6	8	6	1	7
14	7	7	5	2	7
15	1	7	11	2	13
16	0	9	12	0	12
17	9	4	3	5	8
18	6	7	6	2	8
19	0	9	12	0	12
20	4	7	8	2	10
21	2	9	10	0	10
22	4	8	8	1	9
X	6	7	6	2	8

DNA from a panel of hybrid cells characterized for the presence of specific human chromosomes (by isozyme analysis and, in some cases, karyotypic analysis and DNA·DNA hybridization using DNA probes for genes assigned to specific chromosomes) was analyzed for the presence of the human *ROS* gene.

Table 2. Regional localization of *ROS* gene on chromosome 6

Hybrid	*PIM*	*ROS*	*VIP*	*MYB*	Chromosome region
56–47 c122	+	−	−	−	6pter → p21
3a	+	+	+	−	6pter → q22
3c	+	+	+	−	6pter → q22
D2 c16S5	+	+	+	−	6pter → q22
8c	−	+	+	+	6q16 → qter
GB-13	−	−	NT	+	6q22 → qter
MCP-6	−	+	+	+	6p21 → qter

The regions of chromosome 6 retained in 56–47 c122 and MCP-6 have been determined by Giemsa-band staining of hybrid metaphases (42, 43). The regions of chromosome 6 retained by the other hybrids are deduced by the Southern blot analysis of 6-linked markers retained. Since the *ROS* locus is not in the 56–47 c122 hybrid but is in the MCP-6 hybrid, it must be in region 6p21 → 6qter. Since it is present in hybrids that retain *PIM* but not *MYB* (3a, 3c, and D2 c16S5), it must be between *PIM* and *MYB*. The *VIP* gene, previously mapped to 6p21 → 6qter (I. Gozes, R. Avidor, Y. Yahav, D. Katznelson, C.M.C., and K.H., unpublished data), segregates concordantly with the *ROS* genes and is thus also proximal to the *MYB* locus.

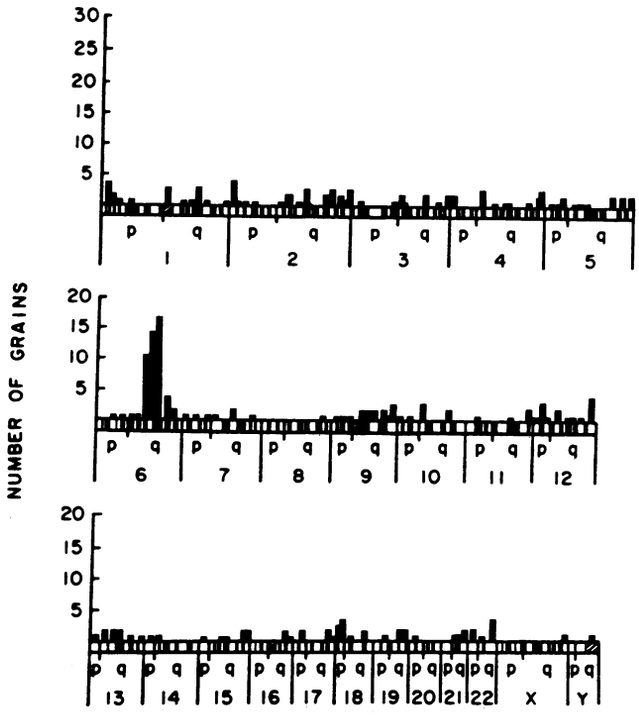

FIG. 3. Human *ROS* gene maps to chromosome region 6q16→6q22. The histogram shows the distribution of silver grains in 130 metaphases from a normal male using ^{3}H-labeled phrosHi6 as probe. The abcissa represents the G-banded chromosomes and their relative sizes; the ordinate represents the number of grains present on the bands of the chromosomes. On 130 metaphases, 42 out of 245 grains (17%) were found over chromosome region 6q16→6q22, with most grains at 6q22.

muscle, heart, liver, and brain; bursa and bone marrow contained less than 1 copy per cell, while kidney was the highest expresser of the tissues tested with only 2.5 copies per cell (24). In agreement with the previous studies, RNA blots of total cellular RNA (20 μg) from many human cell lines (lymphoblastoid, Burkitt lymphoma, various carcinomas, melanoma, myeloid, and others), we have not detected c-*ros* expression, indicating that human c-*ros* has a very restricted tissue specificity, or that it is expressed at low steady-state levels in the cell lines we have tested (A. ar-Rushdi, K. H., L. N., and C.M.C., unpublished data).

In summary, the human c-*ros* homolog that is likely to code for a cell surface receptor of particular tissue specificity has been isolated and mapped to human chromosome region 6q16→6q22. This region of chromosome 6 is involved in nonrandom chromosomal rearrangement in specific neoplasias—including acute lymphoblastic leukemias, malignant melanoma, and ovarian carcinomas.

We thank Dr. Axel Ullrich for the human insulin receptor cDNA and Dr. Peter N. Goodfellow for the MCP-6 DNA. The skillful technical assistance of Felicia Watson and Esther Angert is gratefully acknowledged. We also thank Siu-Long Yao, an undergraduate student who was extremely helpful in cloning and subcloning of the *ROS* locus. This work was supported by National Institutes of Health Grants CA-10815, CA21124, CA39860, and CA25875.

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