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Genetics

Localization of the human *JUN* protooncogene to chromosome region 1p31–32

(genetics of human cancer/chromosomal mapping)

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ABSTRACT **The oncogene *jun* is the putative transforming gene of avian sarcoma virus 17; *jun* appears to be derived from a gene of the chicken genome and has homologues in several other vertebrate species. Recent genetic and immunological data indicate that *jun* codes for a protein that is closely related and probably identical to the transcription factor AP-1. We have isolated a genomic DNA clone encompassing the human cellular counterpart of the gene, *JUN*, and used this DNA to determine the chromosomal location of the gene. A panel of DNA preparations derived from rodent–human somatic cell hybrids with defined chromosome complements was first screened with the *JUN* probe. This Southern blot analysis indicated that *JUN* is situated on the short arm of chromosome 1. *In situ* hybridization then assigned *JUN* to chromosome region 1p31–32, a chromosomal region involved in both translocations and deletions of chromosomes seen in human malignancies.**

The avian sarcoma virus 17 (ASV17) is a replication-defective acutely transforming retrovirus that was initially isolated from a spontaneous fibrosarcoma in a chicken (1). ASV17 causes fibrosarcomas in chickens and also induces transformed foci in chicken embryo fibroblasts *in vitro*. Studies on the molecular structure of ASV17 have demonstrated that its genome comprises *gag* and *env* sequences flanking a cell-derived oncogene of 0.93 kilobases (kb), *jun* (2). The virus encodes a transformation-specific fusion protein with an apparent M_r of 65 kilodaltons (kDa), $p65^{gag\text{-}jun}$ (T. Bos, personal communication). Unlike the transforming proteins of all other avian sarcoma viruses, $p65^{gag\text{-}jun}$ lacks tyrosine-specific protein kinase activity. No homology was detected between *jun* and other oncogenes by nucleic acid hybridization (2). However, nucleotide sequence analysis detected similarity between the predicted amino acid sequence of *jun* and the GCN4 yeast protein (3). The GCN4 protein is a transcriptional activator of yeast, functioning to induce expression of 30–50 unlinked genes that code for amino acid-synthesizing enzymes. GCN4 binds specifically to well-characterized regulatory sequences (4, 5). Similarity between GCN4 and *jun* is restricted to the 66 carboxyl-terminal residues of GCN4, the region thought to constitute its DNA-binding domain (3). Recently this homology has been demonstrated to also possibly extend to function. A chimeric gene was constructed in which the DNA-binding region of GCN4 was replaced by the putative DNA-binding domain of *jun*, and this recombinant molecule was linked downstream to partial sequences of the *E. coli lexA* gene. The product of the entire construct retains transcriptional activator capacity in yeast (6). The significance of *jun* was further enhanced by the discovery that GCN4 and the human transcription factor AP-1 bind to the same DNA target sequence (7), an observation suggesting a relationship between AP-1 and *jun*. An immunologic comparison of AP-1 and the jun protein and functional studies of expressed cellular *jun* sequences have now demonstrated that *jun* codes for a protein that is very similar, if not identical, to AP-1 (ref. 8; M. Karin, personal communication). AP-1 is known to activate transcription from several genes that are induced following phorbol diester treatment (9, 10).

Transcription factors are key regulatory elements in the cell. The alteration of a transcription factor could change patterns of gene expression and could conceivably lead to oncogenesis. It is not known whether the human *jun* gene (designated *JUN*) is activated and plays an etiologic role in cancer. Because activation of oncogenes in human tumors is frequently the result of chromosomal rearrangement (11, 12), it becomes important to map *JUN* in the human genome. In this study we have determined the chromosomal location of *JUN* by Southern blot analysis of a mouse–human somatic cell hybrid panel and by *in situ* chromosomal hybridization.

MATERIALS AND METHODS

Human Genomic *JUN* Clone. The human *JUN* clone was isolated from a genomic library constructed from the T-cell line Jurkat in the λ phage EMBL3, a gift of Louise C. Showe (Wistar Institute, Philadelphia). The library was screened with a probe specific for the viral *jun* sequences as described previously (2). One clone was obtained that included the entire region of homology to the probe; this clone is illustrated in Fig. 1. From this phage clone we subcloned into the plasmid pIBI 25 (International Biotechnologies) a 4.9-kb *Eco*RI fragment after determining it to be free of repetitive sequence elements by Southern blot hybridization. This probe was designated pjun4.9E and was used for the Southern blot and *in situ* hybridization experiments described below.

Southern Blot Analysis. DNAs from human cells, mouse cells, and mouse–human somatic cell hybrids were extracted by cell lysis, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. DNAs were digested with appropriate restriction enzymes, separated through agarose gels, and transferred to nitrocellulose filters essentially according to Southern (13). Hybridization was done in 4× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 0.2 mg of sonicated salmon sperm DNA per ml, 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) at 68°C overnight. ^{32}P-

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Abbreviations: *jun*, the oncogene of avian sarcoma virus 17; *JUN*, the human homologue of *jun*; GCN4, a transcriptional regulator of yeast; N-*RAS*, human homologue of the N-*ras* oncogene; ASV17, avian sarcoma virus 17.

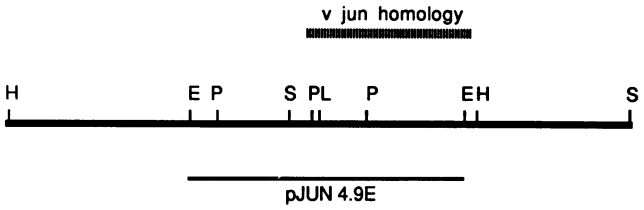

FIG. 1. Restriction map of the human *JUN* genomic clone in the phage EMBL3. Shaded bar denotes the region of homology to the v-*jun* probe. Extent of pjun4.9E is also indicated. H, *Hin*dIII; E, *Eco*RI; P, *Pst* I; S, *Sma* I; and L, *Sal* I.

labeled probes were prepared by nick-translation. After hybridization and washing, filters were exposed to Kodak XAR- 5 film at −70°C using calcium tungstate intensifying screens.

Cell Lines. The preparation and characterization of the mouse–human hybrid cell lines have been described (12, 14–16). Hybrids were studied for the expression of enzyme markers localized to each human chromosome (14). Some hybrid clones were karyotyped by trypsin/Giemsa or G-11 banding as described (12). In addition, previous Southern blotting experiments using probes for genes assigned to specific human chromosomes have confirmed the presence or absence of human chromosomes in many of the mouse–human hybrid (12, 14–16).

***In Situ* Hybridization.** Metaphase spreads were prepared from normal human male *in vitro* lymphocyte cultures stimulated for 72 hr with phytohemagglutinin. Total pjun4.9E plasmid DNA was nick-translated with [^{3}H]dATP (43.0 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) to a specific activity of 1.1×10^7 cpm/μg. The *in situ* hybridization was done essentially as described (16, 17). Chromosome spreads were treated with pancreatic RNase A (Sigma) and were then denatured in 70% formamide/2× SSC at 70°C for 2 min. Preparations were hybridized with the ^{3}H-labeled pjun4.9E at a concentration of 150–300 ng/ml in 50% formamide, 2× SSCP (1× SSCP is 0.15 M NaCl/0.015 M sodium citrate/0.02 M $NaPO_4$, pH 6.0), and 10% dextran sulfate at 37°C for 20 hr in the presence of a 300-fold excess of sonicated salmon sperm DNA. Slides were washed in 50% formamide/2× SSCP at 39°C, then washed in 2× SSC at 39°C, then dried and exposed to Kodak NTB2 nuclear tracking emulsion for 10 or 19 days at 4°C, and developed in Kodak Dektol developer at 15°C. G-banding was done essentially as described by Cannizzaro and Emanuel (18) with a mixture of six parts of borate buffer (50 mM Na_2SO_4/2.5 mM $Na_2B_4O_7$, pH 9.2) to one part of Wright's Giemsa stain solution (2.4 g of Wright's stain and 1.4 g of Giemsa stain per liter in methanol).

RESULTS

Chromosomal Localization of *JUN* by Southern Blot Analysis of Somatic Cell Hybrids. DNA from a panel of mouse–human somatic cell hybrids retaining overlapping subsets of human chromosomal regions (see Fig. 2) was digested with restriction enzyme *Hin*dIII, separated electrophoretically on agarose, transferred to nitrocellulose, and hybridized to the radiolabeled *JUN* probe, pjun4.9E. Results of hybridization of pjun4.9E to a Southern blot containing sixteen hybrid DNAs are shown in Fig. 3. The ≈7.0-kbp human *Hin*dIII *JUN* fragment seen in lane 2 (human control DNA) is also seen in three hybrid DNAs (Fig. 3, lanes 5, 7, and 15). The human *JUN Hin*dIII fragment is absent from all other hybrid DNAs (lanes 3, 4, 6, 8–14, and 16–18) and from mouse parental DNA (Fig. 3, lane 1).

The *JUN*-positive hybrids (Fig. 3, lanes 5, 7, and 15) retain human chromosome 1, whereas the *JUN*-negative hybrids (Fig. 3, lanes 3, 4, 6, 8–14, 16–18) have lost human chromosome 1 (see legend for Fig. 3). In addition to the major ≈7-kbp *Hin*dIII fragment detected by the *JUN* probe, human DNA (Fig. 3, lane 2) also exhibits two less prominent restriction fragments (4.6 and 3.4 kbp) detected by the *JUN* probe that do not segregate concordantly with chromosome 1. These less prominent fragments indicate the presence of additional *JUN*-like gene(s) on a different chromosome in the human genome. To confirm concordancy of human chromosome 1 and the major *JUN* 7.0-kbp fragment in the hybrid panel, filters were routinely stripped of probe and rehybridized to N-*RAS* and α-spectrin (SPTA) probes, which have been mapped to 1p and 1q, respectively (14, 19).

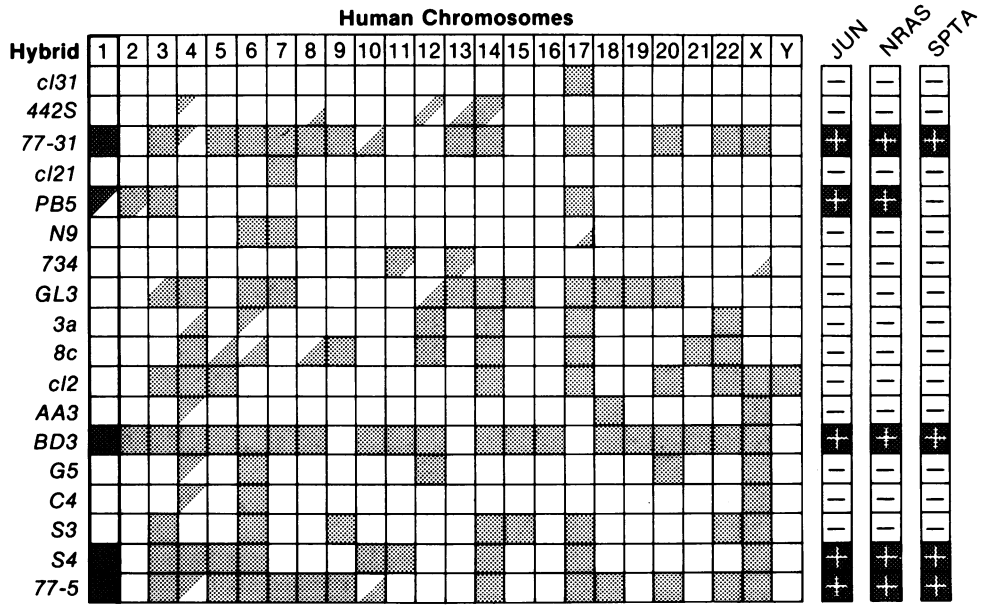

FIG. 2. Presence of human *JUN*, N-*RAS*, and α-spectrin (SPTA) genes in a panel of 18 mouse–human hybrids. ▩, Hybrid listed in left-hand column contains chromosome indicated above; ▨, presence of long arm (or in some cases a portion of long arm as indicated by less stippling) of the chromosome shown above; ▧, presence of short arm of chromosome named above; and □, absence of chromosome listed above. Column for chromosome 1 is outlined and stippled in boldface to highlight correlation of presence of chromosome 1 with presence of the genes for *JUN* and N-*RAS* shown in columns to right.

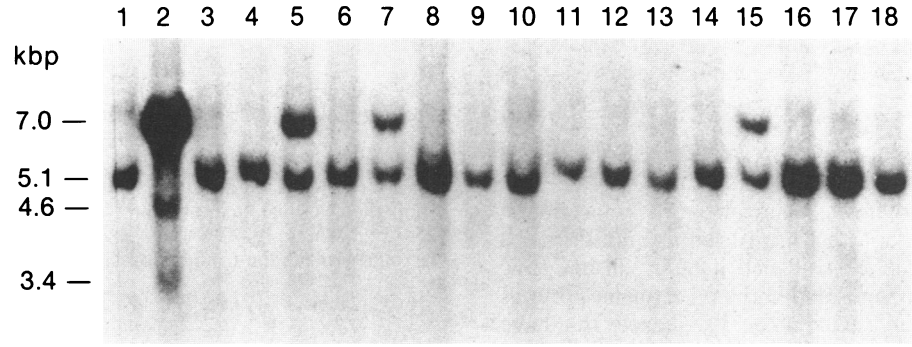

FIG. 3. The *JUN* gene segregates with chromosome 1 in mouse–human somatic cell hybrids. DNA (≈10 μg per lane) from mouse cell line (lane 1), human cell line (lane 2), mouse–human hybrid c131 retaining human chromosome 17 (lane 3); hybrid 442S retaining partial chromosome 4, 8, 12, 13, and 14 (lane 4); hybrid 77-31 retaining chromosomes 1, 3, partial 4, 5–9, partial 10, 13, 14, 17, 18, 20, 22, and X (lane 5); hybrid c121 retaining chromosome 7 (lane 6); hybrid PB5 retaining partial chromosomes 1, 2, 3, 5, and 17 (lane 7); hybrid N9 retaining chromosomes 6, 7, partial 17, and 21 (lane 8); hybrid p11 retaining chromosome region 11pter→11q23, partial 13, and Xq25→Xqter (lane 9); hybrid GL3 retaining chromosome 4, 6, 7, partial 12, 14, 15, and 17–20 (lane 10); hybrid 3a retaining partial chromosome 4 and 6 and chromosomes 12, 14, 17, and 22 (lane 11); hybrid 8c retaining chromosome 4, partial 5, partial 6, 7, 8, 12, 14, 17, and 22 (lane 12); hybrid c12 retaining 3–5, 14, 17, 20, 22, X, and Y (lane 13); hybrid AA3 retaining partial 4, 18, and X (lane 14); hybrid BD3 retaining chromosomes 1–3, 6–8, 10–12, 14–16, 18–22, and X (lane 15); hybrid G5 retaining partial chromosome 4 and chromosomes 6, 12, 20, and X (lane 16); hybrid C4 retaining partial 4 and chromosomes 6 and X (lane 17); hybrid S3 retaining 3, 4, 6, 9, 11, 14, 17, 22, and X (lane 18) was cleaved with an excess of restriction enzyme *Hin*dIII, fractionated on an agarose gel, transferred to nitrocellulose filter, and hybridized to radiolabeled pjun4.9E DNA. Approximate size of mouse and human *JUN*-specific fragments is shown at left.

A summary of all somatic cell hybrid data is presented in Fig. 2. As can be seen by inspection of Fig. 1, presence of the human *JUN* gene in the somatic cell hybrid panel correlates with presence of chromosome 1 (and the N-*RAS* gene) and not with any other human chromosome region.

Chromosomal Localization of *JUN* by *in Situ* Hybridization. The precise assignment of *JUN* was established by *in situ* chromosomal hybridization (16, 17). Grains were scored for their localization after autoradiography. The distribution of grains on 150 metaphases was assessed and is illustrated in Fig. 4 *Left*. Significant labeling occurs over the distal portion of the short arm of chromosome 1, with a strong peak evident at 1p31–32. Of 150 cells examined, 40 (27%) exhibited label on one or both chromosomes 1. About 13% of all grains were located on the short arm of chromosome 1, with most grains at 1p32. A typical metaphase is illustrated in Fig. 4*B*. The short arm of chromosome 1 includes ≈4.57% of the haploid human genome. Our finding that 13% of hybridi-

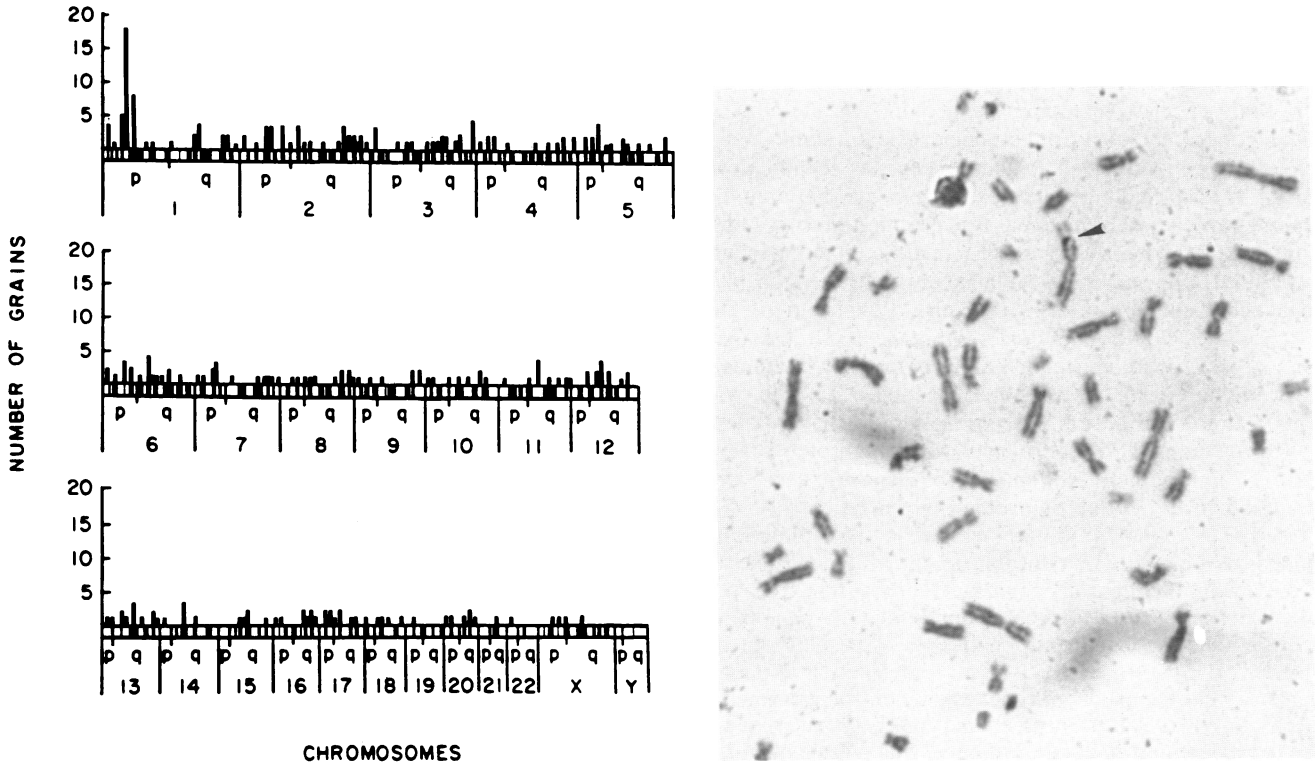

FIG. 4. Localization of the human *JUN* gene by *in situ* hybridization. (*Left*) Histogram illustrating the grain distribution in 150 metaphases hybridized with pjun4.9E. The abscissa is a schematic representation of the chromosomes in their relative size proportions; the ordinate indicates the number of grains counted at each band. Thirteen percent of grains localized to the short arm of chromosome 1, with most of these at chromosome region 1p31–32. (*Right*) Photograph of a G-banded lymphocyte metaphase showing a cluster of silver grains (arrow) over chromosome region 1p31–32.

zation with the pjun4.9E probe localizes to this region of chromosome 1 is highly significant ($\chi^2 = 44.27, P < 0.005$). In sum, we have localized the *JUN* gene to region p31–32 of human chromosome 1.

DISCUSSION

The human JUN protein is identical to the transcription factor AP-1 in all criteria that have been examined: in the DNA consensus sequence to which it binds, in antigenic determinants of the amino- and carboxyl-terminal portions of the molecule, and in the amino acid sequences that have been determined (ref. 8; M. Karin, personal communication). The product of the viral oncogene *jun* is also related structurally and functionally to the yeast transcriptional activator GCN4 and has affinity for the same DNA consensus sequence as AP-1 and GCN4 (refs. 3 and 6; T. Bos, personal communication). We therefore conclude that the cellular and viral jun proteins are specific regulators of transcription.

The *jun* sequence carried by ASV17 is altered as compared to cellular *jun* (ref. 8; T.N., unpublished work). V-*jun* is truncated at the 3′ end and has suffered deletions and base substitutions. It is also fused to part of the viral *gag* gene. The *gag–jun*-encoded fusion protein appears to be responsible for the oncogenic potential of this virus. This oncogenicity of an altered and transduced *jun* gene may be caused by a change in the normal transcriptional regulatory functions of the ASV17 *gag–jun*-encoded protein. Such a functional change could be quantitative or qualitative. The change may affect transcription in a positive or in a negative way. Thus, an altered *jun* could fail to interact with genes that it normally regulates, or it could regulate genes that do not belong to the target spectrum of cellular *jun*. Although GCN4, AP-1, and *jun* are primarily thought of as *activators* of transcription, a DNA-binding protein also has the potential of inhibiting transcription.

Cellular *jun* sequences have been identified in several vertebrate species, including the human. An altered *JUN* could conceivably play an etiologic role in some human cancers. "Activation" of cellular oncogenes in human tumors can result from genetic rearrangements that are often demonstrable by chromosomal translocation. For example, the mapping of the *MYC* gene (13) and the immunoglobulin loci (20–22) allowed a molecular analysis of the translocations occurring in Burkitt lymphoma, translocations that juxtapose *MYC* and immunoglobulin genes (23). The mapping of *JUN* within the human genome now permits a determination of its chromosomal location relative to cytogenetic alterations specific to various tumors.

We have assigned the *JUN* gene to chromosome 1 bands p31 to 32 by Southern blot analysis of a somatic cell hybrid panel and by *in situ* chromosomal hybridization. Chromosome 1p32 is known to be involved in the cytogenetic changes associated with neuroblastoma, which consistently shows a deletion of chromosome 1 extending from 1p32–pter (24). Deletion of the *JUN* gene would doubtless have pleiotropic effects; an oncogenic change could result from the failure to activate genetic elements that exert a dominant regulatory influence on oncogenes or control growth and differentiation (25, 26). Chromosome 1p32 is also involved in rare translocations in human leukemias and lymphomas (27). Thus, it is possible that translocation may activate the *jun* protooncogene, allowing augmented transcription of its substrates.

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