

ent close proximity to receptors for colony-stimulating factor 1 (*Csfmr*) and platelet-derived growth factor (*Pdgfr*) and to a caudal type homeobox gene (*Cdx-1*) (3).

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Sublocalization of the Gene Encoding Manganese Superoxide Dismutase (MnSOD/SOD2) to 6q25 by Fluorescence *in Situ* Hybridization and Somatic Cell Hybrid Mapping

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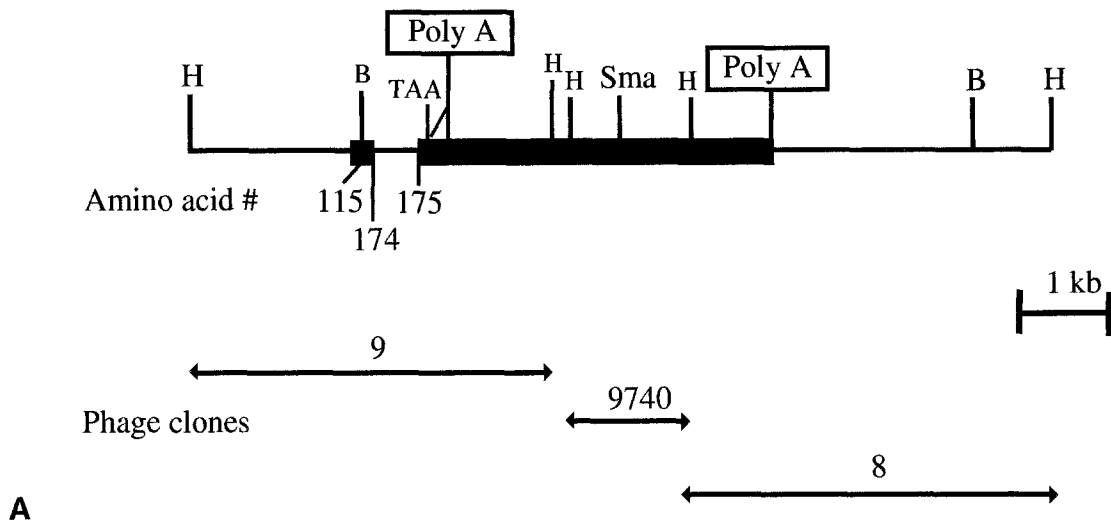
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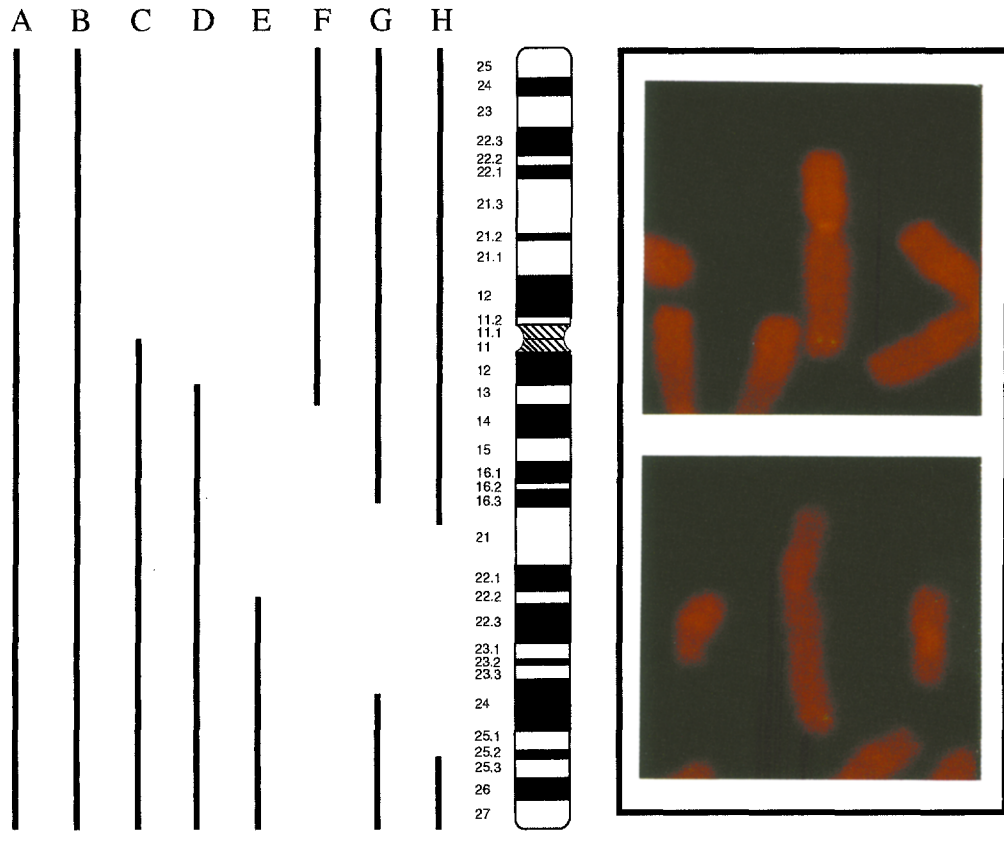
Manganese superoxide dismutase (MnSOD/SOD2) (E.C. 1.15.1.1) is one of a group of enzymes involved in the conversion of oxygen free radicals to hydrogen peroxide (6). MnSOD has been linked both to the removal of toxic oxygen radicals formed during hyperoxia and ischemic reperfusion (1) and to cellular differentiation (1, 3, 10). It is also interesting that cellular transformation and the malignant cell phenotype are both associated with a reduction in the enzyme activity of MnSOD (10).

MnSOD was initially localized to chromosome 6 using enzymatic analysis of mouse/human hybrids (4) and sublocalized to 6q21 following similar analyses to a hybrid with an apparent deletion of chromosome 6q21 (12). Further analysis has demonstrated multiple *TaqI* RFLPs using the MnSOD amino acid coding region cDNA as a probe (14). The long arm of human chromosome 6 is a frequent site of chromosome abnormalities in leukemia, neuroblastoma, and melanoma (5, 9, 13). Therefore, we sought to more closely define the regional localization of the MnSOD locus in humans by *in situ* hybridization and hybrid mapping.

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A



B

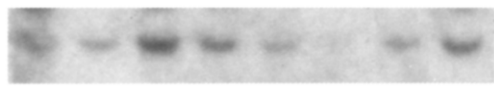


FIG. 1. (A) Partial restriction map of the 3'-portion of the human MnSOD gene used for *in situ* hybridization (see text). Phage clones depicted contain exons (represented by black boxes) encoding the COOH-terminal 118 amino acids and all but 114 nt of the 3'-untranslated region of the human MnSOD gene. Corresponding amino acid numbers are indicated. TAA signifies the MnSOD stop codon. Boxes enclosing Poly(A) denote alternative polyadenylation sites. B, *Bam*HI; H, *Hind*III; and SMA, *Sma*I, are restriction enzyme cleavage sites. (B) **Left:** Human chromosome 6 content of a hybrid mapping panel [after (7)]. Below each panel member is the autoradiographic documentation (following Southern analysis) indicating that only HAL26-12 fails to provide a hybridization signal [confirming that SOD2 lies within 6q25]. **Right:** Examples of FISH using the SOD2 probe shown in A localizing this gene to 6q25 (see idiogram).

The MnSOD probes utilized for the fluorescent *in situ* hybridization (FISH) study were generated using three *Hind*III genomic fragments isolated from a human chromosome 6 human/hamster cell line genomic library. The partial restriction map and the exon/intron organization of these clones are depicted in Fig. 1. The 4.3-kb phage clone 9 contains MnSOD exon 4 (amino acids 114–176) and exon 5 (amino acids 177–222 and 857 nt of the 3'-untranslated sequence). The 1.4-kb phage clone 9740 contains additional 3'-untranslated region sequence. The 6-kb phage clone 8 contains the terminal 984 nt MnSOD 3'-untranslated region, including the terminal polyadenylation signal (2). The phage clones were subcloned into pSp-72.² One microgram of DNA from the three clones was labeled with biotin and hybridized to human metaphase chromosomes as previously described (8). A total of 25 metaphase cells were examined; 23 of these had "double" fluorescent signals, one on each chromatid over the distal long arm of chromosome 6. Only chromosomes in which both chromatids had a signal were included for analyses, making the background hybridization extremely low. The same cells hybridized for FISH had been previously G-banded (using Wright's stain) and photographed to allow direct comparison of the results and confirmed that the hybridization signal was localized to 6q25 (Fig. 1B).

A recently published somatic cell hybrid panel composed of eight cell lines (7) was used to assist in regional localization of SOD2. The chromosome 6 content of this panel is shown in Fig. 1. DNA was digested with *Eco*RI and electrophoretically separated from each hybrid line and control DNA from human, mouse, and Chinese hamster cell lines. Southern blots were then probed with a SOD2 probe (pHMnSO₄) as previously described (9). As indicated in Fig. 1, SOD2 was localized to the most distal of these regions (6q25.3–qter) (Fig. 1). This confirms the FISH analysis and strongly suggests that SOD2 maps to the distal portion of 6q25.

Previous reports (4, 12), including the most recent report of the Committee on Chromosome 6 for the Human Gene Mapping Workshop (15), had indicated 6q21 as the reference map position for SOD2. Clarification of the localization of SOD2 to 6q25 should assist in the further detailed genetic mapping of the long arm of chromosome 6.

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Assignment of the Human CD30 (Ki-1) Gene to 1p36

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In Hodgkin's disease (HD) the lymphoid activation antigen CD30 (Ki-1) is expressed on the tumor cells (9). Furthermore,

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² The human MnSOD genomic sequence for these exons and partial intronic sequences have been deposited with the EMBL Data Library under Accession No. X65965.