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Nucleotide sequence and expression of human chromosome 21-encoded superoxide dismutase mRNA

(Down syndrome/*SOD-1* mRNA species/gene dosage)

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ABSTRACT **Cytoplasmic superoxide dismutase (SOD-1; EC 1.15.1.1) is encoded by human chromosome 21. The *SOD-1* gene locus is located at chromosomal region 21q22, which is involved in Down syndrome. cDNA clones containing sequences of human *SOD-1* were previously isolated. In the present study the nucleotide sequence of one clone, designated pS61-10, was determined. It contains 459 nucleotides representing the entire coding region and 95 nucleotides of the 3′ untranslated region. In human cells two poly(A)-containing SOD-1 RNAs of 0.7 and 0.5 kilobases were detected. These two species are also present in monkey cells, whereas mouse cells contain only a 0.5-kilobase RNA. In a mouse/human hybrid line that contains chromosome 21 as the only human chromosome, the two human SOD-1 RNAs were detected, indicating that both are encoded by this chromosome. These RNAs were found in poly(A)-containing polysomal RNA and were translated *in vitro* to SOD-1 polypeptide; they are therefore functional mRNAs. In normal human fibroblasts 0.002–0.006% of the poly(A)-containing RNA was SOD-1 RNA. The level in monosomic 21 cells was 70% of this value and the level in fibroblasts from Down syndrome patients was about 2 times higher than normal.**

Superoxide radicals, which are normally produced within the cell, are toxic to biological systems (1). Eukaryotic cells possess the enzyme superoxide dismutase (SOD; EC 1.15.1.1), which catalyzes the oxidation–reduction reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ (reviewed in refs. 1–3) and thus provides a defense against oxygen toxicity (3, 4). SOD activity was discovered more than a decade ago (5) and has been extensively studied since. Eukaryotic cells contain two distinct forms of SOD (6)—a mitochondrial manganese-containing enzyme and a cytoplasmic copper/zinc-containing enzyme. The human Cu/Zn SOD-1 is a dimeric protein composed of identical noncovalently linked subunits (7). The complete amino acid sequence of the human erythrocyte SOD-1 has been published (8, 9). It is composed of 153 residues and shares about 78% homology with the bovine and horse enzymes. More recently, an additional Cu-SOD of high molecular weight was isolated from human lung tissue (10). The gene locus for human *SOD-1* was assigned to chromosome 21 (11). This chromosome is involved in the most frequent aneuploid genetic abnormality, known as trisomy 21 or Down syndrome (12, 13). About one in 600 newborn babies carries an extra chromosome 21. This chromosome imbalance is the cause of mental retardation, premature aging, and various other disease conditions, of which acute leukemia is a common example (14). In most cases, the patients with Down syndrome have a karyotype with 47 chromosomes (46 plus one additional 21). However, cases of Down syndrome in which a portion of chromosome 21 is translocated to another chromosome and is therefore present in triplicate have enabled the localization of the "responsible" region to segment 21q22, the distal portion of the long arm of chromosome 21 (15–18). Six genes have been assigned, so far, to human chromosome 21: ribosomal RNA (19), interferon receptor (11), cytoplasmic SOD (*SOD-1*) (11, 20), glycineamide phosphoribonucleotide synthetase (*GARS*) (21), aminoimidazole ribonucleotide synthetase (*AIRS*) (22), and liver-type 6-phosphofructokinase (*PFKL*) (23). From these, both the interferon receptor gene and *SOD-1* have been localized to 21q22, the chromosomal region involved in Down syndrome. Although trisomy 21 was identified as a human genetic disease over 20 years ago (12), there is no effective treatment and very little is known about the biochemical mechanisms by which the extra chromosomal segment (21q22) results in reduced viability and abnormalities of morphogenesis and mental function. It is generally assumed that the extra chromosomal segment codes for normal products and that the abnormalities found in Down syndrome are produced by imbalance due to changes in gene dosage (24); namely, the presence of additional genetic material in the cell will result in the production of commensurately increased amounts of the gene products coded by the extra chromosomal segment. Indeed, Down syndrome patients show an increase of about 50% in SOD-1 activity (25–27) due to higher levels of SOD-1 protein (28). However, it is not known whether this "gene dosage" phenomenon is a result of quantitative changes in the amount of SOD-1 mRNA. We believe that identifying the genes residing in the 21q22 chromosomal segment and analyzing their organization and expression is important for understanding how differential gene expression, as in the case of aneuploidy, may influence cellular differentiation. The *SOD-1* gene is located in the 21q22 band and it can therefore serve as a handle for the chromosomal segment involved in the Down syndrome phenotype. To this end we constructed a cDNA clone of human *SOD-1* (29) and report here the nucleotide sequence and expression of the SOD-1 mRNAs.

MATERIALS AND METHODS

DNA Sequence Analysis. The nucleotide sequence was determined by using both the chemical method of Maxam and Gilbert (30) and the M13 phage dideoxynucleotide technique (31, 32). The resulting fragments were resolved on 0.4 mm thin 6%, 8%, and 20% acrylamide/8 M urea gels.

Cells. The SV80 cells are a continuous line of simian virus 40-transformed human fibroblasts (33). Human foreskin diploid fibroblasts FS11 (34) were established in our department by D. Gurari-Rotman. COS cells are CV-1 cells (African green monkey kidney cells in culture) that have been transformed by simian virus 40 DNA lacking a functional origin of replication (35). The monosomy 21 skin fibroblast culture (culture no. GM-137)

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Abbreviations: SOD, superoxide dismutase; bp, base pair(s); kb, kilobase(s).

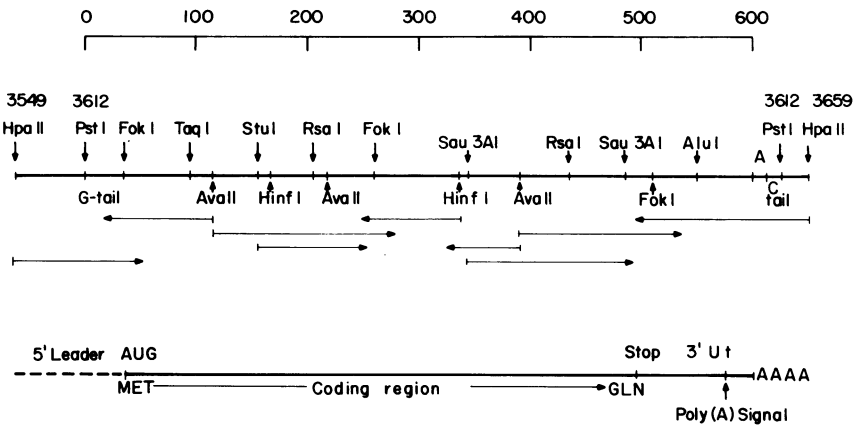

FIG. 1. Restriction map and sequencing strategy for the cDNA insert of pS61-10. Numbers above the *Hpa* II and *Pst* I sites correspond to sites on pBR322. The scale on the top is in base pairs (bp) and corresponds to the numbers used in Fig. 2. Lines below the map indicate the restriction fragments used in chemical sequence determination. 3' Ut, 3' untranslated region.

was obtained from the Human Genetic Cell Repository (Camden, NJ). K-193 human fibroblasts trisomic in chromosome 21 were obtained from the Department of Human Genetics, Hadassah Hospital, Jerusalem. The mouse/human hybrid cell line WAVR4dF9-4a (36) originating from the fusion of a mouse A9 cell and a human diploid fibroblast was obtained from F. Ruddle; it contains a full mouse genome plus human chromosome 21. The two Abelson murine leukemia virus-transformed cell lines—2M3, a nonproducer derived from BALB/c, and L1-2, of C57L/J origin—were obtained from V. Rotter (37). Friend erythroleukemic cells (745) (38) were obtained from H. Aviv, and the human histiocytic lymphoma cell line U937 (39) and the human myeloid leukemic cells HL-60 (40) were from A. Kimchi in our department.

RNA Extraction and Blot Hybridization. RNA was prepared and passed twice through an oligo(dT)-cellulose column, as described (41). Hybridization of DNA with filter-bound RNA was carried out as before (29). Polysomal RNA was prepared by using a modification of the procedure described by Palacios *et al.* (42). Cell-free translation in a rabbit reticulocyte lysate, immunoprecipitation, and $NaDodSO_4$/polyacrylamide gel electrophoresis were carried out as described (43). Quantitative dot-blot hybridization was modified from Kafatos *et al.* (44). Serial 1:2 dilutions of poly(A)-containing RNA (1–0.03 μg in 1 μl) were spotted, in duplicates, on nitrocellulose filters, soaked 20 min in 1.5 M NaCl/0.15 M sodium citrate, air dried, and baked 2 hr in a vacuum oven at 80°C. The amount of RNA per spot was kept constant (1 μg) by diluting the poly(A)-containing RNA with a 1 mg/ml solution of *Escherichia coli* tRNA. Filters were hybridized for 24 hr with an excess of ^{32}P-labeled SOD-1 or HLA-B antigen (45) cDNA (specific activity 1–3 × 10^8 cpm/μg) and autoradiographed to detect the spots, which were then cut out and their radioactivities were measured in scintillation fluid. Series of spots containing 1–0.03 ng of single-stranded SOD-1

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                1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26
                ALA THR LYS ALA VAL CYS VAL LEU LYS GLY ASP GLY PRO VAL GLN GLY ILE ILE ASN PHE GLU GLN LYS GLU SER ASN
G32 ATG,GCG,ACG,AAG,GCC,GTG,TGC,GTG,CTG,AAG,GGC,GAC,GGC,CCA,GTG,CAG,GGC,ATC,ATC,AAT,TTC,GAG,CAG,AAG,GAA,AGT,AAT,
          40          50          60          70          80          90          100         110

 27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48  49  50  51  52  53
 GLY PRO VAL LYS VAL TRP GLY SER ILE LYS GLY LEU THR GLU GLY LEU HIS GLY PHE HIS VAL HIS GLU PHE GLY ASP ASN
GGA,CCA,GTG,AAG,GTG,TGG,GGA,AGC,ATT,AAA,GGA,CTG,ACT,GAA,GGC,CTG,CAT,GGA,TTC,CAT,GTT,CAT,GAG,TTT,GGA,GAT,AAT,
      120         130         140         150         160         170         180         190

 54  55  56  57  58  59  60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80
 THR ALA GLY CYS THR SER ALA GLY PRO HIS PHE ASN PRO LEU SER ARG LYS HIS GLY GLY PRO LYS ASP GLU GLU ARG HIS
ACG,GCA,GGC,TGT,ACC,AGT,GCA,GGT,CCT,CAC,TTT,AAT,CCT,CTA,TCC,AGA,AAA,CAC,GGT,GGG,CCA,AAG,GAT,GAA,GAG,AGG,CAT,
  200         210         220         230         240         250         260         270

 81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99 100 101 102 103 104 105 106 107
 VAL GLY ASP LEU GLY ASN VAL THR ALA ASP LYS ASP GLY VAL ALA ASP VAL SER ILE GLU ASP SER VAL ILE SER LEU SER
GTT,GGA,GAC,TTG,GGC,AAT,GTG,ACT,GCT,GAC,AAA,GAT,GGT,GTG,GCC,GAT,GTG,TCT,ATT,GAA,GAT,TCT,GTG,ATC,TCA,CTC,TCA,
  280         290         300         310         320         330         340         350

108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134
GLY ASP HIS CYS ILE ILE GLY ARG THR LEU VAL VAL HIS GLU LYS ALA ASP ASP LEU GLY LYS GLY GLY ASN GLU GLU SER
GGA,GAC,CAT,TGC,ATC,ATT,GGC,CGC,ACA,CTG,GTG,GTC,CAT,GAA,AAA,GCA,GAT,GAC,TTG,GGC,AAA,GGT,GGA,AAT,GAA,GAA,AGT,
  360         370         380         390         400         410         420         430

135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153
THR LYS THR GLY ASN ALA GLY SER ARG LEU ALA CYS GLY VAL ILE GLY ILE ALA GLN STOP
ACA,AAG,ACA,GGA,AAC,GCT,GGA,AGT,CGT,TTG,GCT,TGT,GGT,GTA,ATT,GGG,ATC,GCC,CAA, TAAACATTCCCTTGGATGTAGTCTGAGG
  440         450         460         470         480         490         500         510         520

CCCCTTAACTCATCTGTTATCCTGCTAGCTGTAGAAATGTATCCTGATAAACATTAAACACTGTAATCTTAAAAAAAAAAAG17
      530         540         550         560         570         580         590         600
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FIG. 2. DNA sequence of pS61-10 insert. The sequence and the predicted amino acids for SOD-1 are given. The amino acid sequence corresponding to the entire mature protein is numbered 1–153.

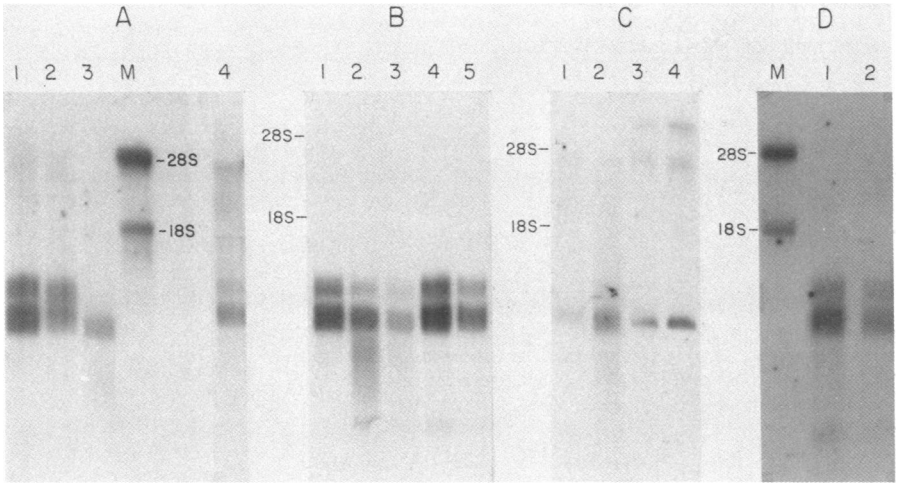

FIG. 3. Detection of SOD-1 RNA in human and mouse cells. Poly(A)-containing RNA was prepared and electrophoresed through a 1.5% agarose/formaldehyde gel, transferred to nitrocellulose filters, and hybridized to ^{32}P-labeled pS61-10 plasmid DNA. Lanes M, ^{32}P-labeled 28S and 18S rRNA. (*A*) RNA extracted from the following sources: lane 1, SV80 human cells (5 μg); lane 2, COS monkey cells (6.3 μg); lane 3, A9 mouse cells (5 μg); lane 4, poly(A)-containing nuclear RNA from SV80 cells (10 μg). (*B*) Five μg of RNA extracted from various human cell lines: lane 1, GM-137; lane 2, HL-60; lane 3, U937; lane 4, SV80; lane 5, FS11. (*C*) Five μg of RNA extracted from various mouse cell lines: lane 1, Friend cells; lane 2, A9; lane 3, 2M3; lane 4, L1-2. (*D*) RNA extracted from the mouse–human hybrid cell line: lane 1, WAVR4dF9-4a (10 μg); lane 2, FS11 (10 μg).

cDNA cloned in M13 phage were used as positive controls and references.

RESULTS AND DISCUSSION

Nucleotide Sequence of SOD-1 cDNA. Recombinant plasmids containing sequences complementary to human SOD-1 mRNA were recently obtained (29). The insert from the clone designated pS61-10 was mapped by restriction endonuclease digestion and used for sequence analysis (Fig. 1). Sites for cleavage and end labeling were chosen from the detailed restriction map and overlapping fragments were used for sequence analysis by the chemical methods of Maxam and Gilbert (30). The sequence was then confirmed by sequencing the pS61-10 DNA insert, using the dideoxy technique (31) after subcloning in M13 phage (32) (Fig. 2). The regions flanking the insert at the 5′ and 3′ sides have the expected nucleotide sequences surrounding the *Pst* I site of pBR322. The insert is composed of 620 bp. The lengths of the oligo(dG) and oligo(dC) tails were found to be 32 bp at the 5′ end and 17 bp at the 3′ end of the mRNA. The human SOD-1 is coded for by 459 bp followed by a single stop codon (TAA) at nucleotide positions 495–497. The noncoding sequence following the stop codon is 95 nucleotides in length. The A-A-U-A-A-A hexanucleotide found approximately 20 nucleotides upstream from the 3′ poly(A) tract in eukaryotic cellular mRNAs (46) is absent; it is replaced by A-U-U-A-A-A. This uncommon sequence has been reported for mouse pancreatic α-amylase (47), human leukocyte interferon (48), chicken lysozyme (49), and human α_1-anti-trypsin (50). It is also possible that the adjacent hexanucleotide G-A-T-A-A-A (positions 570–575) is the actual signal for the putative processing nuclease and poly(A) polymerase. The amino acid sequence of human SOD-1 has been reported (8, 9). The predicted amino acids obtained by translation of the pS61-10 insert (Fig. 2) agree with the published data with few exceptions. The codons beginning with nucleotides 84 and 328 corresponding to amino acid residues 17 and 98 of Fig. 2 specify isoleucine and serine in agreement with Barra *et al.* (9) but in contrast to Jabusch *et al.* (8). The other discrepancies are aspartate vs. asparagine or vice versa in residues 11, 26, 52, 53, and 92 and glutamate vs. glutamine in residue 49. It is likely that in part these discrepancies are the result of deamination during protein sequence determination. The coding sequence contained a nonrandom distribution of codon choices, the significance of which is unknown. The molecular weight of the SOD-1 polypeptide was calculated as 18,538 from the amino acid sequence of Fig. 2, in good agreement with our previous estimate of 19,000 (43).

Two Species of SOD-1 mRNA in Human Cells. Two SOD-1 mRNA species were previously detected in human cells, whereas only one band was seen in mouse RNA (29). To further examine this phenomenon total poly(A)-containing cytoplasmic RNA was prepared from human, monkey, and mouse cells, fractionated by electrophoresis on 1.5% agarose/formaldehyde gels, and transferred to nitrocellulose filters. The immobilized RNA was then hybridized with ^{32}P-labeled cloned SOD-1 cDNA probe (pS61-10) (Fig. 3). Two distinct size classes [0.5 and 0.7 kilobases (kb)] of SOD-1 mRNA were clearly identified in human and monkey cells. The two RNAs were present at the same ratio in nuclear RNA and cytoplasmic RNA (Fig. 3*A*), indicating that the larger is not a nuclear precursor of the smaller. Mouse cells contain only a 0.5-kb species. SOD-1 RNA was also examined in human cells monosomic for chromosome 21 as well as in human transformed cell lines (Fig. 3*B*). In all cases tested the 0.5- and 0.7-kb SOD-1 RNAs were detected. Although the total amount of SOD-1 RNA varies from one cell to another and was higher in trisomy 21 cells, the ratio between the two RNA species was almost constant. In

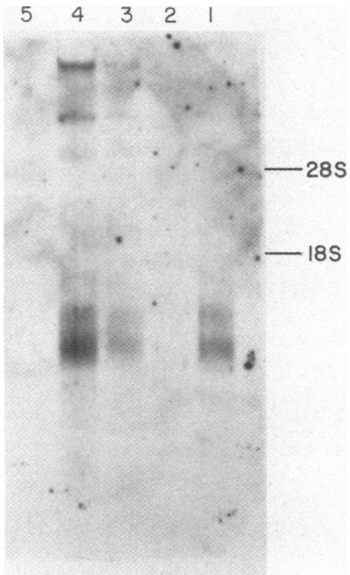

FIG. 4. Detection of SOD-1 RNA in polysomal RNA. Polysomes were isolated, and RNA was selected in oligo(dT)-cellulose and analyzed as described for Fig. 3. Lane 1, poly(A)-containing cytoplasmic RNA (5.5 μg); lane 2, polysomal RNA lacking poly(A) (50 μg); lane 3, poly(A)-containing polysomal RNA (4 μg); lane 4, poly(A)-containing polysomal RNA (11.5 μg); lane 5, cytoplasmic RNA lacking poly(A) (35 μg).

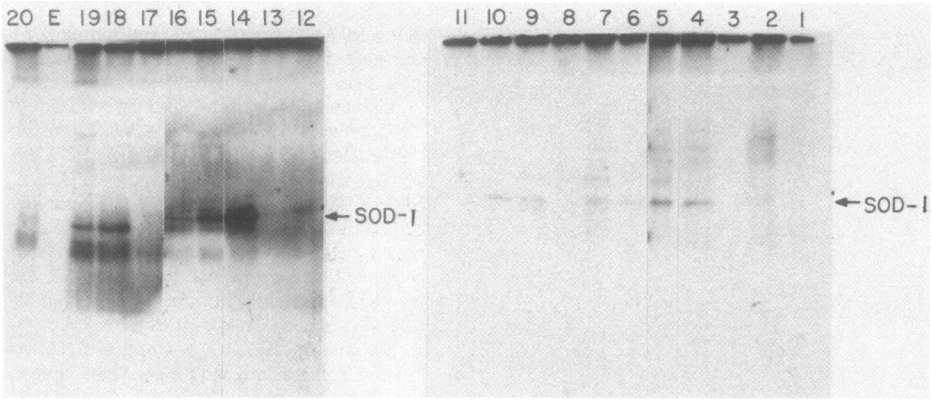

FIG. 5. *In vitro* translation products of fractionated poly(A)-containing RNA. The RNA (10 μg) was fractionated on a CH_3-HgOH/agarose gel. The relevant region of the gel was sliced and RNA was eluted. RNA recovered from the various gel fractions was then translated in rabbit reticulocyte lysates containing [^{35}S]cysteine. The products were immunoprecipitated with anti-SOD-1 serum and analyzed on NaDodSO$_4$/15% polyacrylamide gels. Lanes 1–20, gel slice numbers of region 6.5–8.5 cm from origin; lane E, control with no exogenously added RNA. SOD-1 arrows indicate authentic SOD-1 polypeptide stained with Coomassie brilliant blue.

a few cases, a third RNA band of about 0.1 kb was detected, but it was not further characterized. When RNA from different mouse cells was tested for SOD-1 sequences, only a 0.5-kb RNA species was present (Fig. 3*C*). On the other hand, the mouse/human hybrid cell line WAVR4dF9-4a contained both RNAs (Fig. 3*D*), indicating that the 0.7-kb species was also encoded by chromosome 21. These results and those documented below signified that both SOD-1 mRNAs were encoded by chromosome 21. It is, however, not yet known whether they resulted from transcription of the same gene and whether the difference in length was at their 3′ or 5′ ends.

The Two SOD-1 RNAs Are Functional *in Vivo* and Can Be Translated *in Vitro*. To determine whether the two SOD-1 RNAs were functional *in vivo*, polysomes were isolated by centrifugation onto a sucrose cushion, and RNA was extracted, fractionated on oligo(dT)-cellulose, and analyzed for SOD-1 sequences, as described above. The two SOD-1 species were located in the poly(A)-containing polysomal RNA, indicating that both were active mRNAs (Fig. 4).* The translational ability of the two SOD-1 mRNAs was assayed by *in vitro* translation in rabbit reticulocyte lysates. Poly(A)-containing RNA from SV80 cells was fractionated on CH_3HgOH/agarose gels in two parallel lanes. One lane was used for blotting to diazobenzyloxymethyl (DBM)-paper followed by hybridization to ^{32}P-labeled pS61-10 DNA to determine the position of the two SOD-1 mRNA species. The rest of the gel lanes were then sliced and RNA was eluted by freeze-thawing as described (43) and purified through oligo(dT)-cellulose. RNA eluted from the gel fractions was translated in the reticulocyte lysate. The products synthesized *in vitro* were immunoprecipitated with anti-SOD-1 and analyzed by NaDodSO$_4$ gel electrophoresis (Fig. 5). SOD-1 polypeptides migrating with purified SOD-1 were detected in two regions of the gel (fractions 4 and 5 and fractions 14 and 15) that contained the 0.5- and 0.7-kb RNA species detected by hybridization. These results show that both RNAs can encode *in vitro* the same 19,000-dalton SOD-1 polypeptide. Similar results were obtained with poly(A)-containing RNA extracted from the mouse/human hybrid cell line WAVR4dF9-4a (Fig. 6). Mouse SOD-1 mRNA is similar in size to the human 0.5-kb RNA (Fig. 3); nevertheless, the mouse and human SOD-1 polypeptides can be separated on NaDodSO$_4$/polyacrylamide gels (43). When RNA from the 0.7- and 0.5-kb regions of the CH_3HgOH/agarose gel was eluted and translated *in vitro* and the products were analyzed on NaDodSO$_4$ gels, both the mouse and human SOD-1 polypeptides were detected in translation products of the 0.5-kb RNA, whereas only the human SOD-1 was translated from the 0.7-kb RNA (Fig. 6). This shows that RNA from the hybrid cell line that migrated in the 0.5-kb region contained a mixture of mouse and human SOD-1 RNAs.

Quantitation of SOD-1 mRNA in Human Cells Monosomic, Disomic, and Trisomic for Chromosome 21. Kurnit has previously shown gene dosage at the transcriptional level for the bulk of poly(A)-containing RNA encoded by chromosome 21

* Additional high molecular weight RNAs were detected in the polysomal RNA (Fig. 4, lanes 3 and 4). These RNAs, which may code for other species of SOD (10), were not further characterized.

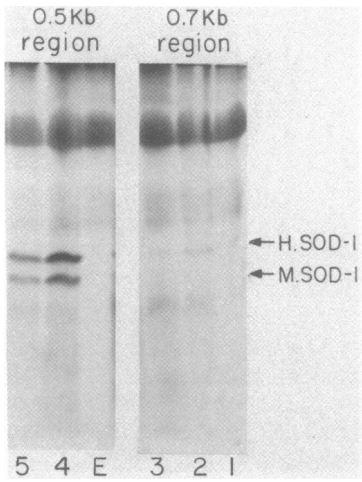

FIG. 6. *In vitro* translation products of poly(A)-containing RNA from mouse/human hybrid line. RNA (10 μg) extracted from the WAVR4dF9-4a cell line was fractionated on a CH_3HgOH/agarose gel and the relevant regions were identified by hybridization with ^{32}P-labeled pS61-10 DNA. The 0.5- and 0.7-kb regions were sliced and RNA was eluted and translated *in vitro*, and products were analyzed as in Fig. 5. Lanes 1–3 and 4–5, gel slices from the 0.7- and 0.5-kb regions, respectively; lane E, control with no exogenously added RNA. H. and M. SOD-1 arrows indicate positions of *in vivo* labeled human and mouse SOD-1 polypeptides.

Table 1. Quantitation of SOD-1 mRNA

Exp.	Cell line	cDNA hybridized, cpm/μg RNA*	SOD-1 RNA, %†
1	SV80	1,520	0.0020
	FS11	1,400	0.0017
2	SV80	2,660	0.003
	FS11	4,600	0.005
3	SV80	1,700	0.006
4	FS11	560	0.006

* The cpm/μg RNA value was derived by counting dot-blot hybridization signals obtained by hybridization of ^{32}P-labeled SOD-1 cDNA to serial dilutions of poly(A)-containing RNA.

† Percent SOD-1 RNA was calculated by comparing the values in the preceding column with dot-blot signals obtained by hybridization of ^{32}P-labeled SOD-1 cDNA to single-stranded SOD-1 DNA.

Table 2. Relative quantity of SOD-1 RNA in human cells with different numbers of chromosome 21

Cell line	cDNA hybridized, cpm/μg RNA* SOD-1	HLA	SOD/HLA		SOD-1, %†
SV80	1,520	4,400	0.34	0.35	100
FS11	1,400	3,680	0.38		
FS11	2,057	6,083	0.34		
GM-137	1,820	7,280	0.25		71
K-193	2,437	2,290	1.06		302
K-193	800	1,380	0.58		166

* Values obtained as described for Table 1.
† Percent SOD-1 relative to mean value of SV80 and FS11 disomic for chromosome 21.

(24). For SOD-1, a gene dosage effect at the enzyme activity level was reported (25–28). It was therefore interesting to determine the amount of SOD-1 mRNA in cells with different numbers of chromosome 21. The SOD-1 mRNA content was quantitated by a modification of the dot-blot hybridization procedure described by Kafatos *et al.* (44). The single-stranded DNA insert of pS61-10 subcloned in bacteriophage M13 was used as a reference. Quantitative analysis by this technique showed that both normal fibroblasts (FS11) and transformed cells (SV80) contain between 0.002 and 0.006% SOD-1 mRNA (Table 1). Because the proportion of mRNA varied between different poly(A)-containing RNA preparations, it was essential to use an internal standard for comparison. HLA mRNA, which is encoded by chromosome 6 (51), was used for this purpose. RNA samples were hybridized to both SOD-1 cDNA (pS61-10) and to HLA-B cDNA (45). The highest amount of SOD-1 mRNA was found in cells from a Down syndrome patient (K193), which contained 1.6–3 times more SOD-1 RNA than normal fibroblasts (FS11) or SV80 transformed cells (Table 2). This increase is probably related to the presence of three chromosomes 21 in K193 cells; accordingly, GM-137 cells, which are monosomic for chromosome 21, contained the least amount of SOD-1 mRNA (70% of FS11), indicating a gene dosage response of SOD-1 transcription.

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