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cDNA Cloning and Chromosomal Localization (4q11-13) of a Gene for Statherin, a Regulator of Calcium in Saliva

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SUMMARY

On the basis of the known amino acid sequence of statherin, a human salivary protein, mixed synthetic oligonucleotides were synthesized and used to screen a cDNA library constructed from human parotid-gland mRNA. A cDNA clone coding for statherin was isolated from this library and has been completely sequenced. The cDNA represents a full-length (or nearly full-length) copy of an ~640-bp statherin mRNA. Statherin appears to be coded by a single-copy gene that maps to chromosome 4q11-4q13 when somatic-cell hybrids are used.

INTRODUCTION

Statherin may play an important role in the maintenance of oral health, since, together with a group of anionic proline-rich proteins (PRPs), it functions in calcium binding and inhibition of crystal growth and exhibits high affinities for hydroxyapatite surfaces (Bennick 1982). Statherin is a highly stable, small-molecular-weight (5,380), acidic (pI 4.2) human salivary protein that is rich in the amino acids tyrosine, proline, and glutamine (16.3% each). Its complete amino acid sequence is known (Schlesinger and Hay 1977). The PRPs are a major constituent of human saliva, comprising ~70% of parotid salivary proteins. They can be subdivided into acidic, basic, and glycosylated proteins. Statherin and the acidic PRPs maintain a supersaturated environment with respect to calcium phosphate salts while preventing the build-up of harmful

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deposits in salivary glands and on tooth surfaces. Although functionally related to the acidic PRPs, statherin possesses the additional property of inhibiting spontaneous precipitation of calcium phosphate salts (Hay et al. 1984). This provides an environment important in the protection of teeth by preventing dissolution of dental enamel and promoting the recalcification of early carious lesions, thereby counteracting the destructive action of bacterial acids. The concentration of statherin in saliva varies 10-fold between individuals, ranging from 3.0 to >27.3 μM (Hay et al. 1984). These concentrations are nonetheless consistent with its proposed biological function.

On the basis of the known amino acid sequence of statherin (Schlesinger and Hay 1977), we constructed mixed synthetic oligonucleotide probes that were used to isolate a statherin cDNA clone. This cDNA clone was then completely sequenced. Statherin appears to be encoded by a single gene that maps to chromosome 4q11-4q13 when somatic-cell hybrids are used.

MATERIAL AND METHODS

Materials

Restriction endonucleases were purchased from New England Biolabs, Bethesda Research Laboratories, or Promega Biotec. Calf intestinal alkaline phosphatase was purchased from Boehringer Mannheim, T4 polynucleotide kinase from Pharmacia, and the Klenow fragment of DNA polymerase I from New England Biolabs or Promega Biotec. All enzymes were used according to the suppliers' recommendations. [^{35}S]dATP and ^{32}P-labeled nucleotide triphosphates were purchased from New England Nuclear. Unlabeled nucleotide triphosphates were purchased from P-L Biochemicals. Synthetic oligonucleotides were synthesized by the University of Wisconsin Protein Sequence–DNA Synthesis facility. The cDNA library in pGFY279 from human parotid-gland mRNA was a gift from N. Maeda; its construction has been described elsewhere (Maeda et al. 1985). Genomic DNA was isolated (Poncz et al. 1982) from white blood cells (WBCs) or a human embryonic fibroblast cell line, 563 (courtesy of R. DeMars, University of Wisconsin). The preparation and characterization of hamster × human hybrid cell lines have been described elsewhere (Carlock et al. 1986; Wasmuth et al. 1986). The hamster parent cell line used in generating these hybrids was UCW 56. Several independent fusions were made using leukocytes isolated from individuals with either karyotypically normal chromosomes or known rearrangements. The human chromosome content of the hybrid cell lines was determined by means of karyotype and isozyme analysis and verified by means of hybridization to a panel of previously mapped chromosome 4 markers.

Isolation of Statherin cDNA

Mixed synthetic oligonucleotides were designed as hybridization probes for statherin cDNA on the basis of the known amino acid sequence (Schlesinger and Hay 1977). Two mixed probes, 14 and 17 bases long, corresponding to the 5′ and 3′ ends of the coding region, respectively, were synthesized. Their

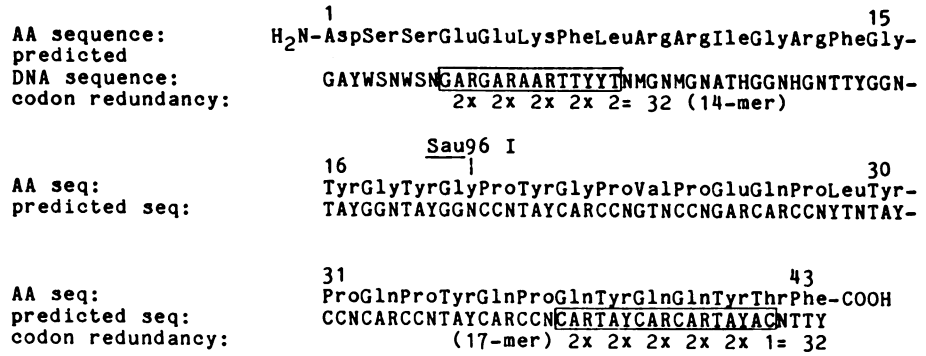

FIG. 1.—Design of synthetic oligonucleotides. The amino acid sequence for statherin was taken from Schlesinger and Hay (1977). In the predicted DNA sequence, N = any base; Y = pyrimidine; R = purine; W = A or T; M = A or C; S = C or G; and H = A, C or T. Sequences used for the synthesis of mixed oligonucleotides are shown in the boxes.

sequences are described below (fig. 1). ^{32}P-labeled oligonucleotides were used to screen a cDNA library constructed from human parotid-gland mRNA (Maeda et al. 1985). Colonies were transferred in an ordered array to nitrocellulose filters placed on NZY agar plates supplemented with 40 μg ampicillin/ml and grown for 16–20 h. Filters were then transferred to NZY agar plates supplemented with 40 μg ampicillin/ml and 10 μg chloramphenicol/ml and amplified for 12 h. Cells were lysed, and the DNA was fixed to the filters (Grunstein and Hogness 1975). Filters were prehybridized in 6 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 100 μg heparin/ml at 37 C for 1 h. Hybridizations were done in 6 × SSC, 50 μg heparin/ml with 8 ng labeled oligonucleotides/ml (Singh and Jones 1984). The mixture was heated briefly to 68 C and hybridized at 22–24 C for 15–18 h. The filters were washed for 3 h with several changes of buffer. The filters hybridized with the 17-base probe were washed with 2 × SSC at 37 C; the filters hybridized with the 14-base probe were washed with 6 × SSC at 30 C. Optimal hybridization and wash conditions were determined empirically. The estimated melting temperature (T_d) for oligonucleotides (4 C/GC base pair + 2 C/AT base pair), −15 C, was used as a starting point (Suggs et al. 1981; Singer-Sam et al. 1983). Those candidates that hybridized to both oligonucleotide probes and had the predicted restriction-endonuclease recognition sites were selected for further characterization.

Nucleotide Sequence Analysis

Appropriate restriction fragments from the cDNA insert were cloned into M13mp19 (Yanisch-Perron et al. 1985) for sequencing by dideoxy-chain termination (Sanger et al. 1977). Sequencing was carried out with [^{35}S]dATP. Alternatively, restriction fragments were end-labeled with ^{32}P by using polynucleotide kinase and sequenced by means of chemical degradation (Maxam and Gilbert 1977). All of the reported sequences were determined from both strands. Sequence analysis of overlapping fragments was performed to read across restriction sites used in subcloning. Sequence data were analyzed using

software provided by the University of Wisconsin Genetic Computer Group (Devereux et al. 1984).

Molecular-Weight Determination for Statherin mRNA

Total parotid-gland RNA (5 μg; provided by N. Maeda) was glyoxylated and separated electrophoretically in 1.5% agarose gels in 10 mM sodium phosphate, pH 7.0 (McMaster and Carmichael 1977). RNA was transferred to GeneScreen Plus (DuPont) and hybridized with a ^{32}P-labeled cDNA probe by using protocols recommended by the manufacturer (Cat. no. NEF-976). Filters were prehybridized in 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin (BSA), 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate (SDS), 1 M NaCl, 50 mM Tris-HCl, pH 7.5. Filters were hybridized in the same buffer with 100 μg sonicated salmon-sperm DNA/ml and 2 × 10^6-cpm ^{32}P-labeled statherin cDNA/ml. Whole plasmid was used as probe in these experiments, such that both plasmid-DNA size markers and statherin mRNA were labeled. The filters were washed twice in 2 × SSC at room temperature for 30 min each, twice in 2 × SSC, 1% SDS at 68 C for 60 min each, and twice in 0.1 × SSC at room temperature for 60 min each. XAR-5 X-ray film (Kodak) was exposed at −70 C for 4–12 h.

Analysis of Genomic DNA

Total genomic DNA (6–10 μg) was digested with restriction endonucleases. DNA was separated electrophoretically in 0.8% agarose gels and transferred to nitrocellulose (Southern 1975). Filters were prehybridized in 3 × SSC, 20 mM sodium phosphate, pH 6.8, 2 mg polyvinylpyrrolidone/ml, 2 mg Ficoll/ml, 250 μg BSA/ml, 50 μg poly r(A)/ml, 0.1% SDS, and 100 μg sonicated salmon-sperm DNA/ml at 68 C and hybridized in the same buffer for 12–18 h at 68 C (Vanin et al. 1983). ^{32}P-labeled statherin cDNA or plasmid pRD5 was used as a hybridization probe. The characterization of the cDNA used as a probe in these experiments will be described below. Plasmid pRD5 contains a 4.8-kb *Eco*RI restriction fragment of human genomic DNA cloned into plasmid pUC119 (pUC119 was a gift from J. Vieira, Rutgers University). This fragment represents the 3′ region of the statherin gene (the identity of the 4.8-kb insert has been confirmed by sequence analysis [L. Sabatini, unpublished data]). Filters were washed in 3 × SSC, 0.5% SDS at 68 C with three changes of buffer, 45 min each change. XAR-5 X-ray film (Kodak) was exposed at −70 C with a single intensifying screen (DuPont) for 1–3 days.

RESULTS

Isolation and Analysis of Statherin cDNA

The design of the oligonucleotide probes is shown in figure 1. The target sites for the 14- and 17-base oligonucleotides were selected to be near the NH_2-terminus and COOH-terminus, respectively. Each probe is composed of a mixture of 32 sequences. The oligonucleotides were end-labeled to a specific activity of ~10^8 cpm/μg and used to screen a human cDNA library from

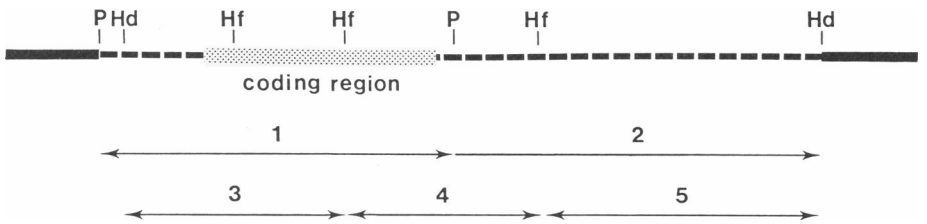

FIG. 2.—Strategy used for the sequence analysis of a statherin cDNA, plasmid H772b. Fragments 1 and 2 were subcloned into M13mp19 and sequenced according to the method described by Sanger et al. (1977). Fragment 1 was sequenced in both orientations. Both strands of fragments 3–5 were sequenced according to the method described by Maxam and Gilbert (1977). Fragment 4 overlaps the *Pst*I restriction site used for subcloning into M13mp19. The solid black line represents vector DNA; the dashed line represents insert DNA; and the stipled region represents the protein-coding region of the cDNA. P = *Pst*I; Hd = *Hin*dIII; and Hf = *Hin*fI.

parotid-gland mRNA (Maeda et al. 1985). Three candidates were isolated that hybridized to both oligonucleotide probes and had a predicted *Sau*96I restriction site (as based on the decoded amino acid sequence). The cDNA inserts ranged in size from ~620 to ~670 bp. The plasmid containing the largest insert, H772b, was chosen for sequence analysis. The fragments used for sequence determination are shown in figure 2. Fragments 1 and 2 were subcloned into M13mp19 and sequenced according to the method described in Sanger et al. (1977). Fragment 1 was sequenced in both orientations. Both strands of fragments 3–5 were sequenced according to the method described by Maxam and Gilbert (1977). The resulting nucleotide sequence (671 bp) and the decoded amino acid sequence are shown in figure 3. The nucleotide sequence of the coding region (189 bp including a UAA stop codon) is compatible with that of the known amino acid sequence (Schlesinger and Hay 1977). The transcript extends 71 nucleotides upstream from the first AUG codon, 291 nucleotides 3′ to the UAA termination codon, and contains a presumptive polyadenylation signal, AATAAA (fig. 3, underlined portion), 10 nucleotides upstream from the ~120 nucleotide poly(A) tail (Fitzgerald and Shenk 1981). Analysis of parotid-gland RNA reveals a major hybridizing RNA species ~640 nucleotides in length (fig. 4). Plasmid H772b therefore contains a full-length (or nearly full-length) cDNA copy of statherin mRNA. Two minor RNA bands, ~490 and ~440 nucleotides, respectively, also hybridize to statherin cDNA. It is not known whether these minor bands represent degradation products.

Estimation of Statherin Gene Copy Number

The number of copies of statherin DNA present in the human genome was estimated as follows: 7 μg human genomic DNA was digested with *Eco*RI endonuclease and separated in 0.8% agarose gels. The presence of an internal *Eco*RI restriction site results in two unique hybridizing fragments, a 7.6-kb fragment and a 4.8-kb fragment. Adjacent lanes contained varying amounts of *Eco*RI-digested plasmid pRD5 DNA containing the 4.8-kb *Eco*RI fragment, equivalent to 0.5, 1.0, 2.0, 5.0, and 10.0 copies of statherin DNA/haploid genome (if a genome size of 3×10^9 bp is assumed). DNA was transferred to

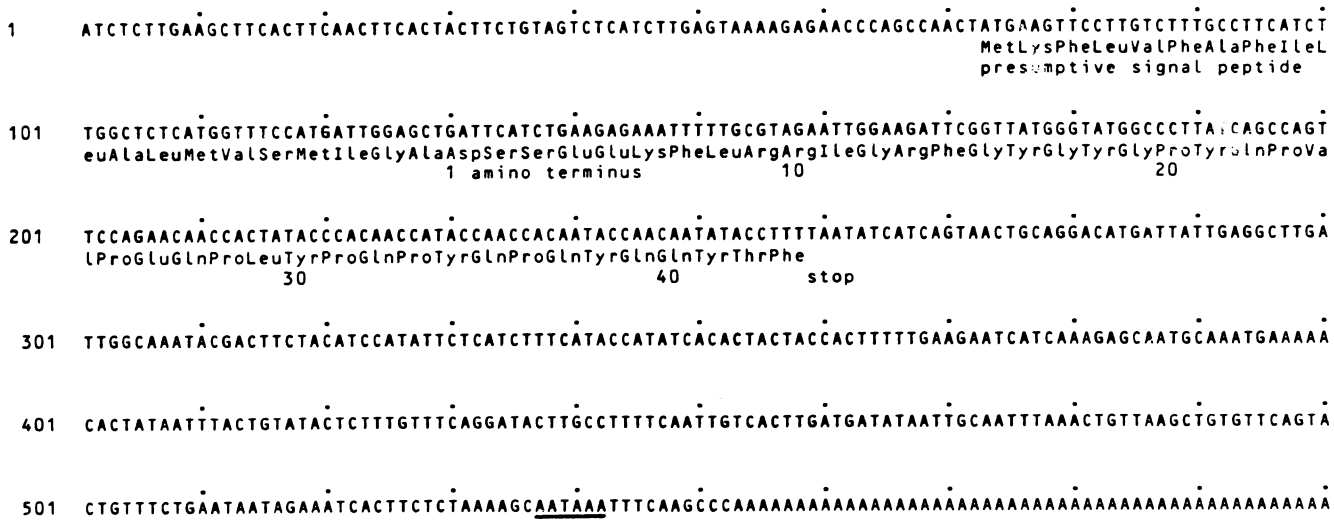

FIG. 3.—Nucleotide sequence of statherin cDNA and the decoded amino acid sequence. Sequence analysis indicates that the plasmid H772b contains a 671-bp cDNA insert. The cDNA includes a 71-nucleotide 5′ leader sequence, a 189-nucleotide coding region, a 291-bp 3′-untranslated region, and an ~120-bp poly(A) tail (truncated above). The decoded amino acid sequence is shown below the nucleotide sequence beginning at the first ATG codon. The decoded sequence includes a 19–amino acid secretory signal peptide preceding the statherin sequence. Numbering for the amino acid sequence begins at the amino terminal residue of statherin. The statherin sequence matches that determined by Schlesinger and Hay (1977; see fig. 1). These data have been submitted to the National Institutes of Health–GenBank nucleotide sequence libraries.

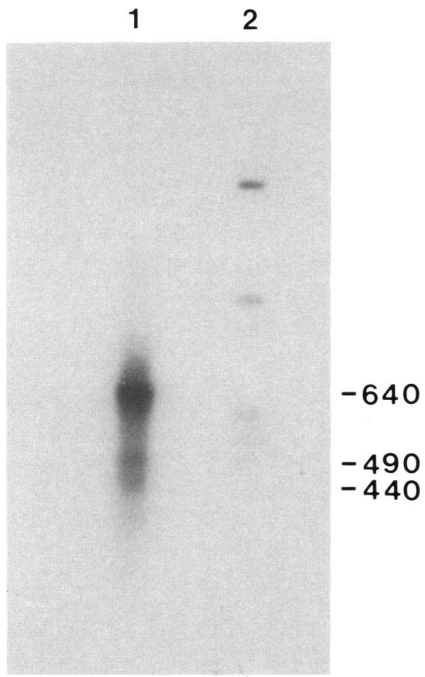

FIG. 4.—Analysis of statherin mRNA. Total parotid-gland RNA (5 μg) was glyoxylated and separated electrophoretically in 1.5% agarose in 10 mM sodium phosphate, pH 7.0 (McMaster and Carmichael 1977). RNA was transferred to GeneScreen Plus (DuPont) and hybridized with ^{32}P-labeled plasmid H772b containing a statherin cDNA insert. Lane 1, Glyoxylated parotid-gland RNA; lane 2, glyoxylated plasmid-DNA size markers. A major hybridizing RNA band migrates at ~640 nucleotides; two minor RNA bands migrate at ~490 and ~440 nucleotides, respectively.

nitrocellulose and hybridized to ^{32}P-labeled pRD5 (see Material and Methods). Exposures were kept within the linear range of the film, and the intensity of the hybridizing bands in genomic samples was compared with that in the plasmid lanes by using a Hoefer GS300 scanning densitometer. The intensity of hybridization to the 4.8-kb *Eco*RI fragment in genomic DNA from two different sources (fig. 5, lanes 3 and 4) was comparable to that of the plasmid sample representing 1 copy/haploid genome (fig. 5, lane 2).

Chromosomal Localization of Statherin DNA

DNA from hamster × human hybrid cell lines was digested with *Xba*I restriction endonuclease and separated in 0.8% agarose gels. The DNA was transferred to nitrocellulose and hybridized with the ^{32}P-labeled pRD5 probe under conditions decribed above. Plasmid pRD5 (containing the 4.8-kb *Eco*RI statherin-gene fragment) hybridizes to 3.1-, 1.8-, and 1.0-kb *Xba*I restriction fragments in human genomic DNA (fig. 6, lanes 1 and 10). Hamster DNA does not hybridize with human statherin sequences (fig. 6, lanes 9 and 18). The presence or absence of the expected *Xba*I restriction fragments was correlated

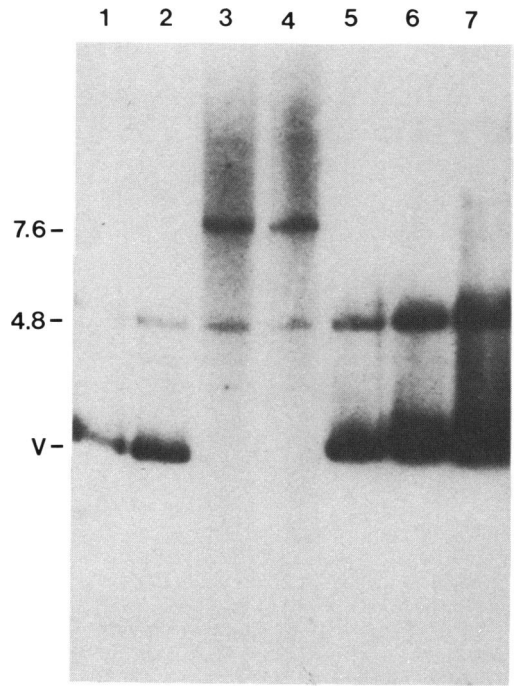

FIG. 5.—Estimation of gene copy number. Human DNA from two different sources (embryonic fibroblasts and WBC) was digested with *Eco*RI restriction endonuclease and hybridized to ^{32}P-labeled plasmid pRD5 containing the 4.8-kb *Eco*RI statherin-gene fragment. *Eco*RI-digested pRD5 DNA was loaded in adjacent lanes as standards in amounts equivalent to 0.5, 1.0, 2.0, 5.0, and 10 copies/haploid genome, respectively (a genome size of 3×10^9 bp being assumed). Lane 1, 9.7 pg pRD5; lane 2, 19 pg pRD5; lane 3, 7 μg embryonic fibroblast DNA; lane 4, 7 μg WBC DNA; lane 5, 39 pg pRD5; lane 6, 96 pg pRD5; and lane 7, 193 pg pRD5. The positions of the 7.6 and 4.8 *Eco*RI statherin-gene fragments are labeled. The position of the vector DNA is marked with a V.

with the human chromosome content of the individual hybrid cell lines as determined by karyotype and isozyme analysis.

Five independent cell hybrids, containing only human chromosome 4 in common (Carlock et al. 1986), are all positive for hybridization to pRD5 (fig. 6, lanes 3–7). In addition, hybrid KO1, which contains only human chromosome 4 in a Chinese hamster background (Carlock et al. 1986), confirms our localization of the statherin gene to chromosome 4 (fig. 6, lane 6). Hybrids HHW661 (Wasmuth et al. 1986), KO2, and KO7 do not hybridize with pRD5 (fig. 6, lanes 11, 16, and 17). Hybrids KO2 and HHW661, which contain an intact 4p segment or the telomere-proximal region, and hybrid KO7, containing the 4q25-4qter fragment, can be used to regionally assign pRD5 to the 4q centromere-proximal area. Hybrids KO4 and KO5, which hybridize to pRD5 (fig. 6, lanes 14 and 15), both contain, in addition to the short arm, a segment of the long arm extending from the centromere to band 4q13. These results localize the statherin gene on chromosome 4 in the centromere-to-4q13 region. Table 1 details

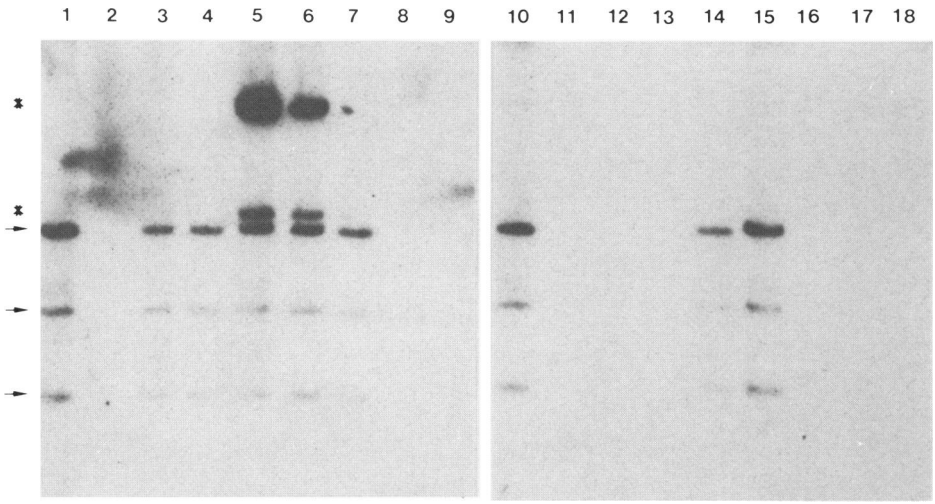

FIG. 6.—Chromosomal localization of statherin DNA. Human, hamster, and human × hamster somatic-cell hybrid DNA was digested with *Xba*I restriction endonuclease and hybridized with ^{32}P-labeled plasmid pRD5 containing the 4.8-kb *Eco*RI statherin-gene fragment. Hybridizing bands in human DNA are marked with arrows (lanes 1 and 10). Hamster DNA does not hybridize with pRD5 DNA (lanes 9 and 18). Lanes 2–8 and 11–16 are somatic-cell hybrid DNA samples. Hybrid samples in lanes 3–7, 14, and 15 are positive for human DNA fragments hybridizing to plasmid pRD5; hybrid samples in lanes 2, 8, 11–13, 16, and 17 are negative. The bands marked with the * in lanes 5 and 6 have been shown to be due to plasmid-DNA contamination in these samples (data not shown). Lanes 1 and 10, 563 (human fibroblast DNA); lane 2, HHW432 DNA; lane 3, KO18; lane 4, KO19; lane 5, HHW658; lane 6, KO1; lane 7, KO20; lane 8, HHW105; lanes 9 and 18, UCW56 (Chinese hamster ovary DNA); lane 11, HHW661; lane 12, HHW108; lane 13, HHW693; lane 14, KO4; lane 15, KO5; lane 16, KO2; and lane 17, KO7.

the results for pRD5 hybridization and the chromosomal content of each somatic-cell hybrid as determined by isozyme and karyotype analysis. One additional cell line (HHW323), established from primary orangutan fibroblasts, hybridized strongly with pRD5 (data not shown). The hybridization pattern is the same as that of human DNA except that the orangutan DNA fragment corresponding to the 1.8-kb *Xba*I fragment in human DNA migrates at ~1.6 kb.

DISCUSSION

A full-length (or nearly full-length) cDNA copy of statherin mRNA has been isolated by using synthetic oligonucleotides to probe a human parotid-gland cDNA library. The nucleotide sequence of the cDNA is compatible with the 43–amino acid sequence of statherin (Schlesinger and Hay 1977). Translation most likely initiates at the first AUG codon 57 nucleotides upstream from the NH_2-terminal GAU (Asp) codon (Kozak 1978). The primary translation product would therefore include a 19–amino acid secretory signal peptide composed predominantly (16/19) of hydrophobic amino acids, a composition similar to those of other signal peptides (Watson 1984).

To estimate gene copy number, the intensity of hybridization of the radiola-

TABLE 1

HUMAN CHROMOSOMAL CONTENT OF SOMATIC-CELL HYBRIDS AS ESTABLISHED BY CYTOGENETIC AND ISOZYME ANALYSIS

CELL LINE	CHROMOSOME CONTENT																							HYBRIDIZATION WITH pRD5
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
HHW423	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KO18	−	−	+	+	+	+	−	−	+	−	−	+	−	+	−	−	−	+	−	−	−	+	−	+	+
KO19	−	+	−	+	+	+	−	−	+	−	−	−	+	−	−	+	−	−	+	+	+	+	+	−	+
HHW658	−	−	+[a]	+	+	+	−	−	−	+	−	+	+	−	−	−	−	+[a]	−	+	−	−	−	−	+
KO1	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
KO20	−	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+
HHW105	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HHW661	−	−	−	+[b]	+[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HHW108	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
HHW693	−	−	−	+[c]	+[c]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KO4	−	−	−	+[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
KO5	−	−	−	+[e]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
KO2	−	−	−	+[f]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KO7	−	−	−	+[g]	−	−	−	−	+	−	−	+[g]	−	−	−	−	−	−	−	−	−	−	−	−	−
UC56	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
563	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
% DISC	70	60	50	0	30	40	60	70	60	60	70	60	50	60	70	60	70	50	50	50	50	50	60	60	

Karyotypes of derivative chromosomes are as follows:

[a] der 3,5/18,5.

[b] 4pter:4p15.2::5p15:5qter.

[c] 4pter:4p15.2::5p15:5q11.

[d] 4pter:4q13::CHO.

[e] 4pter:4q21::4q33:4qter.

[f] isochromosome 4p.

[g] 4qter:4q25::12p13:12qter.

beled 4.8-kb *Eco*RI statherin-gene fragment to the same fragment in genomic DNA was compared with that of a control plasmid, pRD5, containing this fragment. Plasmid DNA was loaded in adjacent lanes in amounts equivalent to 0.5–10.0 copies/haploid genome. These results suggest that statherin is coded by a single-copy gene.

Since statherin is functionally related to the acidic salivary PRPs, it might be expected that statherin is also contained within the salivary-protein gene complex (SPC) on human chromosome 12 (Azen et al. 1985; Mamula et al. 1985). However, our mapping data indicate that statherin is located on chromosome 4 and is therefore not linked to the PRP genes. Regional localization of statherin has established that several markers are in the same region of chromosome 4. Those markers include peptidase S (PEPS), metallothionein 2–processed pseudogene (MT2P1), alpha-fetoprotein (AFP), albumin (ALB), group-specific component (GC), and dentinogenesis imperfecta (DGI1). Hereditary persistence of AFP (HPAFP) and juvenile periodontitis (JP) have also been provisionally localized to this region of chromosome 4 (Kidd and Gusella 1985; McKusick 1986).

The fact that statherin maps in the same region as two dental disorders was especially interesting. DGI1 is an autosomal dominant mutation affecting the dental tissue protein matrix, dentin. Enamel formation may be clinically normal or hypoplastic. It is a common autosomal dominant mutation, occurring with a frequency of 1/8,000 and exhibiting nearly 100% penetrance (Witkop and Rao 1977). Tooth defects are apparent prior to tooth eruption; thus, salivary protein may not play a significant role in this process. However, the possibility that statherin is expressed in tissues other than salivary glands (e.g., in the tooth bud) has not yet been investigated. JP is characterized by an increased susceptibility to periodontitis—a disease resulting in the destruction of the alveolar bone and the fibers of the periodontal ligament—in otherwise healthy adolescents. JP differs from adult periodontitis in its familial pattern of inheritance, and the symptoms appear to be more of a degenerative than of an inflammatory type (Clark et al. 1977; Beube 1978). We are currently attempting more detailed genetic and cytogenetic mapping of the statherin gene to investigate a possible relationship and/or linkage with these disorders.

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