Am. J. Hum. Genet. 45:381–387, 1989

Localization of the Genes for Histatins to Human Chromosome 4q13 and Tissue Distribution of the mRNAs

Johanna C. vanderSpek,* Herman E. Wyandt,† James C. Skare,*,† Aubrey Milunsky,† Frank G. Oppenheim,*,‡ and Robert F. Troxler*,‡

*Department of Biochemistry and †Center for Human Genetics, Boston University School of Medicine; and ‡Department of Oral Biology, Goldman School of Graduate Dentistry, Boston

Summary

A cDNA coding for histatin 1 was isolated from a human submandibular-gland library and sequenced. This cDNA was used to probe RNAs isolated from a variety of tissues to investigate tissue-specific regulation and to determine whether histatins might play a role other than in the oral cavity. The same probe was also used for Southern blot analysis of human genomic DNA restricted with various enzymes, and it showed that the genes coding for histatins are on the same chromosome. In situ hybridization of the cDNA probe to metaphase chromosome spreads was performed to determine chromosomal location of the genes for histatins. A genomic fragment isolated using the cDNA probe was also hybridized to chromosome spreads, and the same chromosome was identified. The genes for histatins are located on chromosome 4, band q13. We have shown that three histatin mRNAs are expressed in human parotid and submandibular glands but in none of the other tissues studied. These results suggest that histatins are specific to salivary secretions.

Introduction

The parotid and submandibular glands of humans secrete a family of small, mostly cationic, histidine-rich proteins termed *histatins*. The major histatins range in size from 24 to 38 amino acids and are similar in primary structure. Histatins 1, 3, and 5 comprise 85%–90% of the total histatin proteins and are termed the *major histatins*. Histatin 1 is a phosphoprotein thought to be active in the stabilization of mineral-solute interactions of the oral fluid. It adsorbs selectively to hydroxyapatite and enamel powders, implicating it as a precursor of the acquired enamel pellicle, a proteinaceous layer covering tooth surfaces that forms a barrier between tooth enamel and the oral environment (Hay 1973, 1975; Oppenheim et al. 1986). A cDNA coding for histatin 3 has previously been isolated by Dickinson et al. (1987). Histatins 1, 3, and 5 have been tested for candidicidal activity, and all have been shown to kill *Candida albicans* blastopores in a dose-dependent manner. The ability to kill *C. albicans* was related to the size of the histatins, with the smallest protein, histatin 5, being most effective and the largest, histatin 1, being least effective (Pollock et al. 1984; Oppenheim et al. 1988). Antibacterial properties of the histatins against three different strains of *Streptococcus mutans* have also been demonstrated (MacKay et al. 1984).

In the present study we show that the genes for histatins are at a single locus, within 15 kb of each other on chromosome 4, band q13, and that histatin mRNAs are found only in the parotid and submandibular gland.

Received February 22, 1989; revision received May 1, 1989.

Address for correspondence and reprints: Robert F. Troxler, Department of Biochemistry, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118.

0002-9297/89/4503-0007$02.00

Methods

A cDNA library was prepared with RNA extracted from a submandibular gland (Oppenheim et al. 1987) and poly A+ selected by affinity chromatography on oligo (dT) cellulose (Aviv and Leder 1972). First-strand cDNA synthesis was performed in a 50-μl reaction mixture containing 4 μg heat-denatured mRNA, 50 mM

Tris-HCl, pH 8.3, 75 mM potassium chloride, 10 mM dithiothreitol, 3 mM magnesium chloride, 0.5 mM of each dNTP, 1 μg oligo (dT), and 800 units Maloney murine leukemia reverse transcriptase. The reaction mixture was incubated at 37°C for 1 h, and the RNA:DNA hybrid was recovered. Second-strand synthesis was performed in a 100-μl reaction mixture containing heat-denatured first-strand product, 50 mM potassium phosphate buffer, pH 7.5, 3 mM magnesium chloride, 1 mM 2-mercaptoethanol, 0.5 mM each dNTP, and 30 units Klenow fragment. The reaction mixture was incubated at 16°C for 18 h, and the cDNA was precipitated. The cDNA was digested with S1 nuclease under conditions determined to be optimal and was recovered, methylated, polished, and ligated to *Eco*RI linkers according to a method described by Huynh et al. (1985). An *Eco*RI cut was performed, excess linkers were removed by gel filtration, and the linkered cDNAs were ligated to λGT10 arms and packaged. The library was screened using a 30-base-long, nondegenerate oligonucleotide probe, designed to hybridize the coding regions of both histatin 1 and histatin 3, based on the sequence obtained for histatin 3 by Dickinson et al. (1987). After being subcloned into M13, inerts were sequenced by the method of Sanger et al. (1977).

Human tissues for the tissue distribution studies were obtained at autopsy by the National Disease Research Institute and were snap-frozen in liquid nitrogen less than 8 h postmortem. They were shipped on dry ice and stored frozen at −70°C until needed. Human tissues tested included heart, kidney, liver, pancreas, psoas, testes, submandibular gland, and parotid gland. Total RNA was prepared by the method of Chomczynski and Sacchi (1987). The submandibular-gland mRNA was prepared by poly A+ selection as above. The RNA samples were electrophoresed on 1.5% formaldehyde denaturing gels and were transferred to nitrocellulose (Davis et al. 1986). RNA integrity was assessed by UV shadowing and by observing the appearance of the 28s and 18s ribosomal subunits of total RNAs from the various human tissues. The markers used were a 123-bp ladder. The Northern blot was hybridized with the radiolabeled 292-bp cDNA coding for histatin 1 (fig. 1). The probe was random primer-labeled (Feinberg and Vogelstein 1983), with ^{32}P dCTP as the radioactive nucleotide. The filters were prehybridized for 3 h at 42°C in 50% formamide, 4 × SSPE (1 × SSPE = 150 mM sodium chloride, 10 mM sodium phosphate monobasic, 1 mM disodium EDTA, pH 7.4), 5 × Denhardt's (1 × Denhardt's = 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone,, 0.2% BSA), 0.2% SDS, 50 μg denatured calf thymus DNA/ml, and 0.05% sodium pyrophosphate. The probe was hybridized to the filter for 16 h at 42°C in the same solution containing 0.5 ng denatured probe/ml. Washes were performed in 1 × SSC (1 × SSC = 150 mM sodium chloride, 15 mM sodium citrate, pH 7.5), 0.1% SDS twice for 1 h at room temperature and then once at 42°C for 1 h. The filters were exposed to Kodak XAR-5 film with an intensifying screen at −70°C, for 20 h for the mRNA lane and for 70 h for the total lanes (fig. 2).

```
                                    1
Ile Ser Met Ile Ser Ala Asp Ser His Glu
ATT TCC ATG ATT AGC GCT GAT TCA CAT GAA   30

                               10
Lys Arg His His Gly Tyr Arg Arg Lys Phe
AAG AGA CAT CAT GGG TAT AGA AGA AAA TTC   60

                               20
His Glu Lys His His Ser His Arg Glu Phe
CAT GAA AAG CAT CAT TCA CAT CGA GAA TTT   90

                               30
Pro Phe Tyr Gly Asp Tyr Gly Ser Asn Tyr
CCA TTT TAT GGG GAC TAT GGA TCA AAT TAT  120

            38
Leu Tyr Asp Asn       3' untranslated region
CTA TAT GAC AAT TGA   TATCCTTAGTAATCATG  152

GGGCATGATTATAGAGGTTTGACTGGCAAATTCACTTTT  191

TACTCATTTATTCTCATTCATCATACCGCATCACACTAC  230

CACTGCTTTTTGAAGAATTATCATAAGGCAATGGAGAAT  269

AAAAGAAAGACCATGATTTAGTG                  292
```

Figure 1 cDNA sequence of the clone for histatin 1. The amino acid sequence of the coding region is shown and is numbered separately.

Southern blot analysis was performed on human genomic DNA digested with seven different restriction enzymes (fig. 3). The DNA was electrophoresed on 0.8% agarose gels and was blotted onto nitrocellulose (Davis et al. 1986). The markers used were *Hin*dIII-cut lambda DNA. Prehybridization, hybridization, and washes were performed as for the Northern blot analysis. Exposure to film was for 20 h, as described above.

Peripheral blood lymphocytes from a normal individual were obtained and cultured using standard techniques (Yunis 1974). Spreads were prepared and treated with RNAse (100 μg/ml in 2 × SSC) for 1 h at 37°C, rinsed in 2 × SSC and a graded alcohol series, and air-dried.

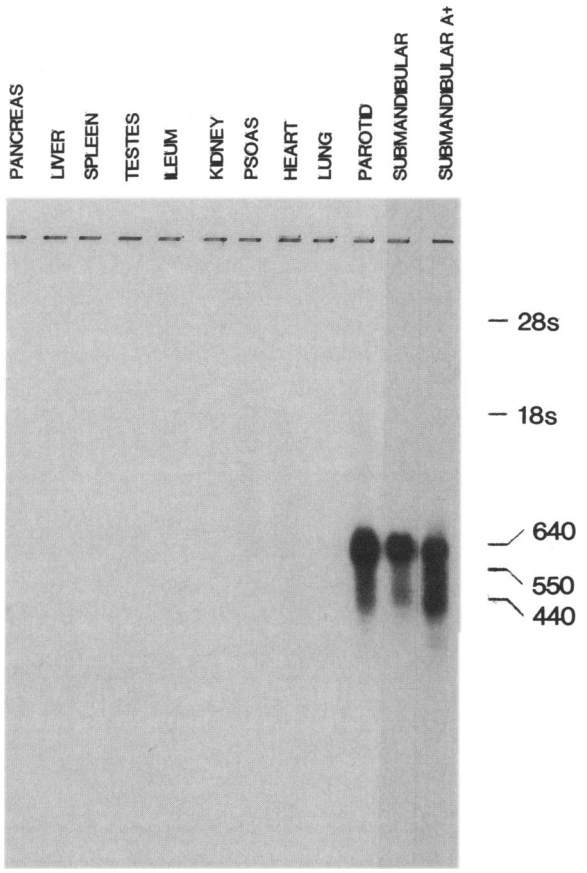

Figure 2 Tissue distribution of histatin mRNAs. The filter was prepared and hybridized to the histatin 1 cDNA probe as described. The lanes were all loaded with 15 μg total RNA from the indicated tissues, except for the submandibular A+ lane, which contains 2 μg of poly A+ selected mRNA. Exposure time for the total RNA samples was 70 h, and for the mRNA sample it was 20 h. The 28s and 18s ribosomal subunits were visible with ethidium bromide staining and are labeled.

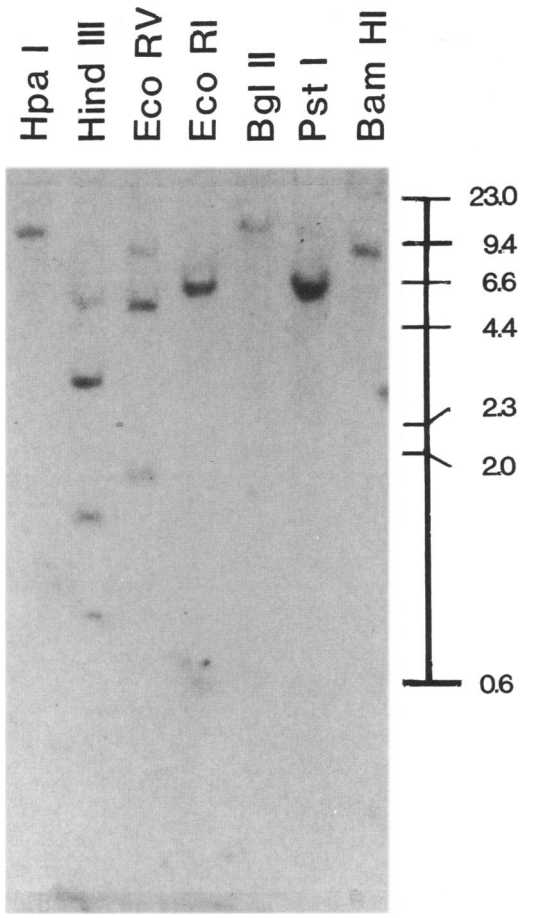

Figure 3 Southern blot analysis of human genomic DNA. The filter was prepared and hybridized to the histatin 1 cDNA probe as described. Each lane contains 15 μg genomic DNA restricted with the indicated enzyme. Lambda phage *Hin*dIII-cut markers were used to determine sizes of hybridizing bands. Genomic fragments hybridizing to the probe are indicated by solid squares.

The slides were denatured in 70% formamide in 2 × SSC, pH 7.5, at 70°C for 2 min, dehydrated in an ethanol series, and air-dried. The cDNA and genomic probes, as well as the control, DYZ2, a 2.1-kb-long repetitive probe to Yq12 (donated by Dr. Kirby Smith), were labeled (Feinberg and Vogelstein 1987) with all four ^{3}H nucleotides to a specific activity of 6×10^7 cpm/μg. The probes were hybridized to the smears for 16 h in a humid environment. The Y probe was hybridized at 37°C in a solution containing 10% dextran sulfate, 2 × SSCP (1 × SSCP = 120 mM sodium chloride, 20 mM sodium phosphate monobasic, and 15 mM trisodium citrate), 50% formamide, 100 μg sonicated salmon sperm/ml, and 200 ng denatured probe (3×10^5 cpm/smear)/ml. The cDNA and genomic probes were hybridized under similar conditions, except that two different concentrations and hybridization temperatures were tried. Smears were hybridized with either 200 ng probe/ml hybridization solution (3×10^5 cpm/smear) or 20 ng probe/ml hybridization solution, (3×10^4 cpm/smear). The smears were hybridized at 37°C or 42°C for 16 h.

The slides were washed three times in 50% formamide in 2 × SSC, pH 7.0, at 40°C for 2 min each wash and were dehydrated, air-dried, coated with nuclear track emulsion NTB-2, autoradiographed for 6–11 d at 4°C, developed, and stained with 0.005% quinacrine mustard (Sigma Q-2000) in McIlvaine's buffer

(0.1 M citric acid monohydrate, 0.2 M sodium phosphate dibasic, pH 5.4).

Location of silver grains over the chromosomes was determined by simultaneous observation of metaphases by epiluminescence and by transmitted light. The distribution of grains was mapped onto a 550-band karyotype ideogram (Caspersson et al. 1970; ICSN 1985), in the form of a histogram (fig. 4).

The cloning reagents used in the present study were purchased from Promega (Madison, WI), the enzymes and markers were from Bethesda Research Labs (Gaithersburg, MD), the random primer-labeling kit was from Boehringer Mannheim (Indianapolis), all radioactive nucleotides were from Amersham (Arlington Heights, IL), and the emulsion was from Kodak (Rochester).

Results

The sequence of the 292-bp-long cDNA coding for histatin 1 is shown in figure 1. The clone is not complete but does contain the entire coding region, six amino acids of the signal peptide, and 157 bases of the 3′-untranslated region. The cDNAs we isolated for histatin 1 and histatin 3 were very similar and possessed 93% homology. The sequence we obtained for histatin 3 was identical to the sequence Dickinson et al. (1987) reported, except for two base changes in the 3′-untranslated region. Owing to their high homology, the cDNA for histatin 1 or histatin 3 could be used as a probe to identify the genes for both. In addition, although we have not isolated a cDNA coding for histatin 5, it is likely that an mRNA coding for this protein would be identical—or at least highly homologous—to a portion of the mRNA coding for histatin 3. This assumption is based on the protein sequences for histatins 3 and 5. The amino acid sequence for histatin 5 is identical to that of the amino terminal 24 residues of histatin 3 (Oppenheim et al. 1988). A cDNA probe would therefore be expected to hybridize mRNAs to all three major histatins. We random primer labeled the cDNA coding for histatin 1 (fig. 1) and used this to perform a distribution study on RNAs isolated from various tissues.

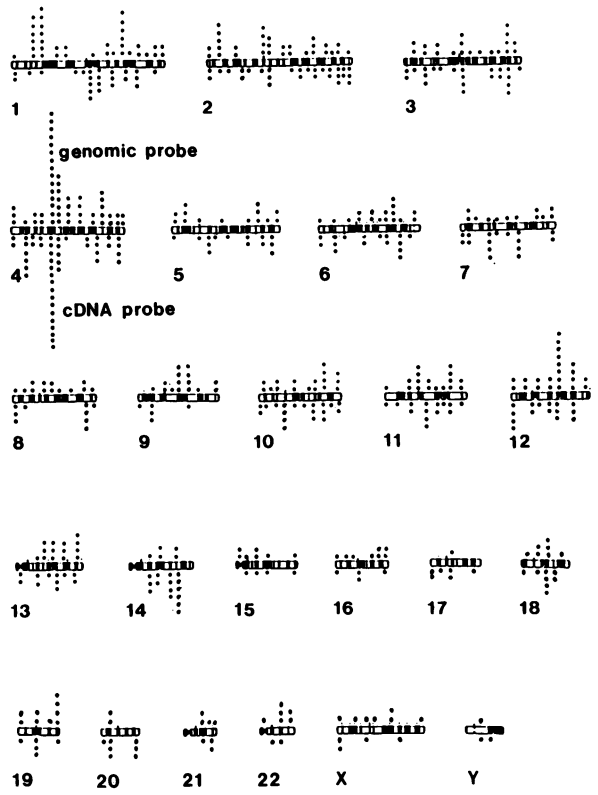

Figure 4 Silver-grain distribution in 200 cells hybridized to probes for histatins. The dots above the 550-band ideogram represent the genomic probe, and the dots below the ideogram represent the cDNA probe. Chromosome numbers are indicated beneath the ideogram.

The tissue distribution study showed that the histatin 1 cDNA probe hybridized to RNA from parotid and submandibular gland only. The probe hybridized three species approximately 640, 550, and 440 bases in length (fig. 2). The parotid-gland total RNA showed that the 640 species was the most abundant and that the 550 and 440 RNAs were present in much lesser amounts. The submandibular-gland total RNA contained less of the 640 RNA, and the 550 and 440 RNAs were barely visible; however, they were present, as they were clearly visible in the submandibular-gland mRNA lane.

Southern blot analysis using the same cDNA probe showed that three bands hybridized to the *Hin*dIII and *Eco*RV lanes and that two bands to the *Eco*RI, *Bam*HI, and *Pst*I lanes, and one band was observed in the *Hpa*I and *Bgl*II lanes (fig. 3). The *Hpa*I lane showed a single band of less than 15 kb that hybridized the probe.

The probe was next used to screen a human genomic library, and a clone for histatin 1 was isolated (authors' unpublished data). A 2.0-kb probe was generated from the isolate that contained 266 bases of the 297-base-long 3′-untranslated region, approximately 1,300 bases of intron within the 3′-untranslated region, and 430 bases 3′ to the end of the 3′-untranslated region. The cDNA and genomic probes were used to hybridize chromosome spreads and yielded the same results, although

the conditions for optimal hybridization varied between the probes.

The best results for the in situ chromosome hybridization studies were obtained with the cDNA probe when 3×10^4 cpm of the probe was hybridized to the smears at 42°C (fig. 4). A total of 200 cells were scored, with an average of fewer than 2 grains/cell. The grains were located on a 550-band histogram. The concentration of grains located on 4q13 was three times the next highest concentration of grains at 14q24. Of the 299 silver grains scored, approximately 5% were located on 4q13. Hybridization of 3×10^4 cpm at 37°C or of 3×10^5 cpm at either 37°C or 42°C resulted in nonspecific hybridization. Optimal results with the genomic probe were obtained when 3×10^4 cpm of the probe was hybridized at 37°C. The grains over 200 cells were once again located on the histogram. The concentration of grains localized over 4q13 was two times the next highest concentration of grains (fig. 4). Of the 337 silver grains scored, approximately 5% were located on 4q13. The genomic probe appeared to be less specific than the cDNA probe, with concentrations of grains occurring over 1p33, 1p34, 1q25, and 12q21. Hybridization of 3×10^4 cpm genomic probe to smears at 42°C or of 3×10^5 cpm probe at either 37°C or 42°C resulted in nonspecific binding. These results combined allow us to conclude that the genes coding for histatins are located on band 4q13. The Y probe control hybridized the Y chromosome, with very little nonspecific binding occurring (data not shown).

Discussion

Human salivary-gland secretions contain proteins that perform various roles in the oral cavity. In addition to digestive, antimicrobial, and immunological functions, these secretions are involved in regulating remineralization of tooth surfaces, and they play a role in taste acuity (Boackle and Suddick 1980). Whole saliva, as well as parotid and submandibular-gland secretions, have recently been shown to inhibit infectivity of human immunodeficiency virus 1 (Fox et al. 1988). Further study of proteins from glandular secretions will elucidate their individual functions as well as the functions of saliva as a whole.

The functions of histatins in the oral cavity include their role in the maintenance of the integrity of tooth surfaces and their anticandidal and antimicrobial activities. The tissue distribution study was performed to determine whether histatins mRNAs might exist in other tissues, thereby suggesting other possible functions. Recently Sabatini et al. (1988) showed that a probe for histatin hybridized RNAs from parotid and submandibular gland. Their results differed from ours in that their probe apparently only hybridized one mRNA, 610 bases in length. Dickinson et al. (1987) electrophoresed submandibular-gland total RNA on a 1.5% gel and hybridized it to their cDNA probe coding for histatin 3. One mRNA, approximately 600 bases in length, hybridized the probe. This is consistent with our results for the submandibular-gland total RNA sample. The three mRNA species hybridizing the probe were not readily apparent until the RNA was poly A+ selected. Our results for the histatin tissue distribution show that they are exclusive to the parotid and submandibular glands, limiting their functions to these secretions (unless they exist in tissues we did not examine). Three mRNA species coding for histatins were shown to exist in both parotid and submandibular gland. This is consistent with the fact that there are three major histatins, 1, 3, and 5. The results also showed that the amounts of these mRNAs varied in these two tissues. (The same amount of total RNA [15 μg] was electrophoresed for each sample.) The parotid gland contained more mRNAs coding for histatins than did the submandibular gland, and the 640 mRNA was the most abundant form. The cDNA probe hybridized mainly to the 640 mRNA species in the total RNA sample from submandibular gland, and the smaller species were barely visible. All three mRNAs appeared to be present in equal amounts in the submandibular gland poly A+ sample. This could be explained by the fact that the 640-base mRNA species had less affinity for oligo (dT) cellulose, possibly owing to the presence of either a shorter poly A tail or higher-order structural features.

The results for the Southern blot analysis indicate that the genes coding for histatins are on the same chromosome. The genomic DNA, when cut with the enzymes *Hpa*I and *Bgl*II, hybridized the cDNA probe at only one band. Generation of exactly the same-sized pieces of DNA from different chromosomes is unlikely to occur. The *Hpa*I lane showed a band of approximately 15.0 kb that hybridized the probe. This indicates that the genes coding for histatins are within 15.0 kb of one another on the same chromosome.

Both the cDNA and genomic probes localized the genes coding for histatins to chromosome 4, band q13. The cDNAs coding for histatins 1 and 3 are very homologous; however, the differences that do exist are spread throughout their length, ruling out the possibility of these genes arising by alternative splicing (fig. 1; compare the histatin 1 sequence to Dickinson et al.

[1987] sequence for histatin 3). The cDNA for histatin 5 has not been isolated, and it is possible that the mRNAs for histatin 3 and histatin 5 are both generated from the same gene by alternative splicing. Either way, these results show that the genes coding for the major histatins are all located on 4q13. Another salivary-gland protein, statherin, has been located to 4q11-q13, in studies using hybrid panels (Sabatini et al. 1987). Statherin is a phosphoprotein that maintains saliva supersaturated with respect to calcium phosphate salts (Hay et al. 1984). On the basis of the fact that some regions of nucleotide sequence homology exist, it has been suggested that statherin and histatin 3 arise from the same common ancestral gene (Dickinson et al. 1987). The finding that they are in the same chromosomal region is consistent with this hypothesis. (The cDNA probe used in this study did not hybridize to Statherin mRNA or cDNA [data not shown].)

Linkage analysis studies have revealed that three dental disorders—an autosomal dominant form of localized juvenile periodontitis and dentinogenesis imperfecta types II and III (DGI II and III)—are located near the vitamin-D-binding protein group–specific component, which is mapped to 4q12-q13 (Ball et al. 1982; Conneally et al. 1984; Boughman et al. 1986). Localized juvenile periodontitis (LJP) is characterized by bone loss around the first molars and incisors in otherwise healthy adolescents and young adults. A tendency for LJP to occur in familial patterns exists, and genetic predispositions to several factors has been suggested (Krill and Fry 1986; Boughman et al. 1987). DGI II and III are characterized by opalescent discoloration of the deciduous and permanent teeth, which undergo rapid attrition (Bixler 1976).

The specific biochemical defects causing these disorders are unknown; however, all involve bone loss of the teeth and possible defects of the calcifying matrix. Statherin and histatin 1 are both phosphoproteins and have been implicated as stabilizers of mineral-solute interactions of the oral fluid. In addition, histatins are known to possess antimicrobial activity. As the genes for histatins are on 4q13 and as the statherin gene is at 4q11-q13, they are linked to the loci for LJP and DGI II and III. The probes for these genes could be used to further map these diseases. The possibility also exists that they are involved in these disorders.

Acknowledgments

This research was supported in part by National Institutes of Health grants DE 05672 and DE 07652.

References

Aviv H, Leder P (1972) Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408–1412

Ball SP, Cook PJL, Pars M, Buckton KE (1982) Linkage between dentinogenesis imperfecta and Gc. Ann Hum Genet 46:35–40

Bixler D (1976) Heritable disorders affecting dentin. In: Stewart RE, Prescott GH (eds) Oral facial genetics. C. V. Mosby, St. Louis, pp 227–261

Boackle RJ, Suddick RB (1980) In: Menaker L, Morhart RF, Navia JM (eds) The biologic basis of dental caries. Harper & Row, Hagerstown, MD, pp 113–131

Bougham JA, Beaty TH, Yang P, Goodman SB, Wooten RK, Suzuki JB (1987) Problems of genetic model testing in early onset periodontitis. J Periodontol 59:332–337

Bougham JA, Halloran SL, Roulston D, Schwartz S, Suzuki JB, Weitkamp LR, Wenk RE, et al (1986) An autosomal-dominant form of juvenile periodontitis: its localization to chromosome 4 and linkage to dentinogenesis imperfecta and Gc. J Craniofacial Genet Dev Biol 6:341–350

Caspersson T, Zech L, Johansson C, Modest EJ (1970) Identification of human chromosomes by DNA-binding fluorescing agents. Chromosoma 30:215–227

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Conneally PM, Bixler D, Horton-Kelly S, Daugherty L (1984) Confirmation of linkage between dentinogenesis inperfecta and Gc. Cytogenet Cell Genet 37:438

Davis LG, Dibner MD, Battey JF (1986) In: Davis LG, Dibner MD, Battey JF (eds) Basic methods in molecular biology. Elsevier Science, New York, pp 62–65, 143–146

Dickinson DP, Ridall AL, Levine MJ (1987) Human submandibular gland statherin and basic histidine-rich peptide are encoded by highly abundant mRNA's derived from a common ancestral sequence. Biochem Biophys Res Commun 149:784–790

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Fox PC, Wolff A, Yeh CK, Atkinson JC, Baum B (1988) Saliva inhibits HIV-1 infectivity. J Am Dent Assoc 116:635–637

Hay DI (1973) The interaction of human parotid salivary proteins with hydroxyapatite. Arch Oral Biol 18:1517–1529

——— (1975) Fractionation of human parotid salivary proteins and the isolation of an histidine-rich acidic peptide which shows high affinity for hydroxyapatite surfaces. Arch Oral Biol 20:553–558

Hay DI, Smith DJ, Schluckebier SK, Moreno EC (1984) Relationship between concentration of human salivary statherin and inhibition of calcium phosphate precipitation in stimulated human parotid saliva. J Dent Res 63:857–863

Huynh TV, Young RA, Davis, RW (1985) In: Glover DM (ed) DNA cloning: a practical approach. Vol 1. IRL, Washington, DC, pp 49–78

ISCN (1985) An international system for human cytogenetic nomenclature (Published in collaboration with the March of Dimes–Birth Defects Foundation and Cytogenetics and Cell Genetics) Karger Medical and Scientific, Basel

Krill DA, Fry HR (1986) Treatment of localized juvenile periodontitis (periodontosis): a review. J Periodontol 58:1–8

MacKay BJ, Denepitiya L, Iacono VJ, Krost SB, Pollock JJ (1984) Growth inhibitory and bactericidal effects of human parotid salivary histidine-rich polypeptides on *Streptococcus mutans*. Infect Immun 44:695–701

Oppenheim FG, Hay DI, Smith DJ, Offner GD, Troxler R (1987) Molecular basis of salivary proline-rich protein and peptide synthesis: cell-free translations and processing of human and macaque statherin mRNAs and partial amino acid sequence of their signal peptides. J Dent Res 66: 462–466

Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offner GD, Troxler RF (1986) Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure and fungistatic effects on *Candida albicans*. J Biol Chem 263:7472–7477

Oppenheim FG, Yang YC, Diamond RD, Hyslop D, Offner GD, Troxler RF (1986) The primary structure and functional characterization of the neutral histidine-rich polypeptide from human parotid secretion. J Biol Chem 261: 1177–1182

Pollock JJ, Denepitiya L, MacKay BJ, Iacono VJ (1984) Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans. Infect Immun 44:702–707

Sabatini LM, Carlock LR, Johnson GW, Azen EA (1987) cDNA cloning and chromosomal localization (4q11–13) of a gene for statherin, a regulator of calcium in saliva. Am J Hum Genet 41:1048–1060

Sabatini LM, Warner TF, Saitoh E, Azen EA (1988) Tissue distribution of RNAs for salivary type proteins in humans and monkeys: proline-rich proteins, statherin, cystatins and histidine-rich proteins. Am J Hum Genet [Suppl] 43:A199

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Yunis JJ (1974) In: Yunis JJ (ed) Human chromosomal methodology, 2d ed. Academic Press, New York and London.