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# The enamelin (tuftelin) gene

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**ABSTRACT This paper reviews the primary structure, characteristics and possible function of tuftelin/enamelin protein. It describes the distribution of tuftelin in the ameloblast cell and in the extracellular enamel matrix, employing high resolution protein-A gold immunocytochemistry. The chromosomal localization of the human tuftelin gene and its possible involvement in autosomally linked Amelogenesis Imperfecta, the most common hereditary disease of enamel, is also discussed.**

KEY WORDS: *enamel, tuftelin, gene, chromosomal localization, mineralization*

## Introduction

Enamel, a unique and highly mineralized ectodermal tissue covering vertebrate teeth, is synthesized and secreted by specialized cells of the enamel organ called ameloblasts. During the process of development and mineralization, the extracellular enamel matrix is originally rich in protein and water, and relatively poor in mineral. The maturing matrix then loses most of its protein and acquires mineral ions, calcium and phosphorus, finally becoming highly mineralized. The removal of most of the extracellular matrix proteins as the enamel mineralizes and matures is a unique feature of enamel in comparison with the mesenchymal mineralizing tissues, bone dentine and cementum, the major matrix constituents of which remain embedded within the mineralized mature tissue (for review see Deutsch, 1989).

Autoradiography, light microscopy, transmission electron microscopy, ultrastructural immunolocalization, amino acid sequencing, biochemical studies and molecular biology studies (Weinstock and LeBlond, 1971; Frank, 1979; LeBlond and Warshawsky, 1979; Warshawsky, 1985; Nanci *et al.*, 1987; Smith *et al.*, 1989; Fincham *et al.*, 1992; Deutsch *et al.*, 1995) have indicated that the biosynthetic events in the secretory ameloblast are typical of other extracellular protein secretory cells. The enamel proteins to be secreted follow the merocrine secretory pathways (Smith, 1979; Simmerlink, 1982; Warshawsky, 1985) through the rough endoplasmic reticulum, the Golgi apparatus, the secretory granules, and are finally released at the cell surface.

The ameloblast cells and the secreted extracellular organic matrix (which are thought to be in constant communication) provide the ideal environment for the commencement of enamel mineralization and the growth of the elongated thin enamel mineral crystallites.

The extracellular organic matrix is composed of two major classes of proteins (Termine *et al.*, 1980): the abundant hydrophobic amelogenins (over 90% of the secreted extracellular matrix proteins), rich in proline, histidine and glutamine, and the acidic enamelins (less than 10%), rich in glutamic acid, aspartic acid, serine and glycine. These have been postulated to play major roles in the mineralization and structural organization of forming enamel.

Indeed, there is accumulating evidence that the hydrophobic amelogenins are involved in the control of crystallite size, morphology and orientation (Robinson and Kirkham, 1985; Aoba and Moreno, 1989; Fincham *et al.*, 1992; Diekwisch *et al.*, 1993; Moradian-Oldak *et al.*, 1995), while the acidic enamelins have been suggested by many laboratories to act as a nucleator (or template) and regulator in the initiation of enamel mineralization (Termine *et al.*, 1980; Deutsch *et al.*, 1984; Robinson and Kirkham, 1985; Traub *et al.*, 1985; Slavkin *et al.*, 1988; Deutsch *et al.*, 1989, 1991; Arsenault and Robinson, 1989).

## Characterization of enamelins

In the late 70's, evidence accumulated from detailed studies on the changes in amino acid composition of enamel during development (Glimcher *et al.*, 1977; Robinson *et al.*, 1977; Eastoe, 1979) that, in addition to the bulk amelogenin fraction, a second class of proteins, which was acidic and rich in aspartic acid, glutamic acid, serine and glycine, also existed during the secretory stage of development. In contrast to the amelogenins, these proteins remained, in part, in a degraded form in the mature tissue.

In 1980, employing their newly developed sequential extraction method, Termine *et al.* (1980) succeeded in separating these non-amelogenin acidic proteins, which they termed enamelin proteins, from the hydrophobic amelogenin fraction. This was a turning point in the isolation, purification and characterization of these two classes of proteins.

The enamelins extracted from developing extracellular matrix of enamel of a number of species were shown to have a molecular weight of 70 kDa and below (Termine *et al.*, 1980; Belcourt *et al.*, 1982; Deutsch *et al.*, 1984, 1986, 1987, 1989; Shimokawa *et al.*,

*Abbreviations used in this paper:* AI, Amelogenesis Imperfecta.

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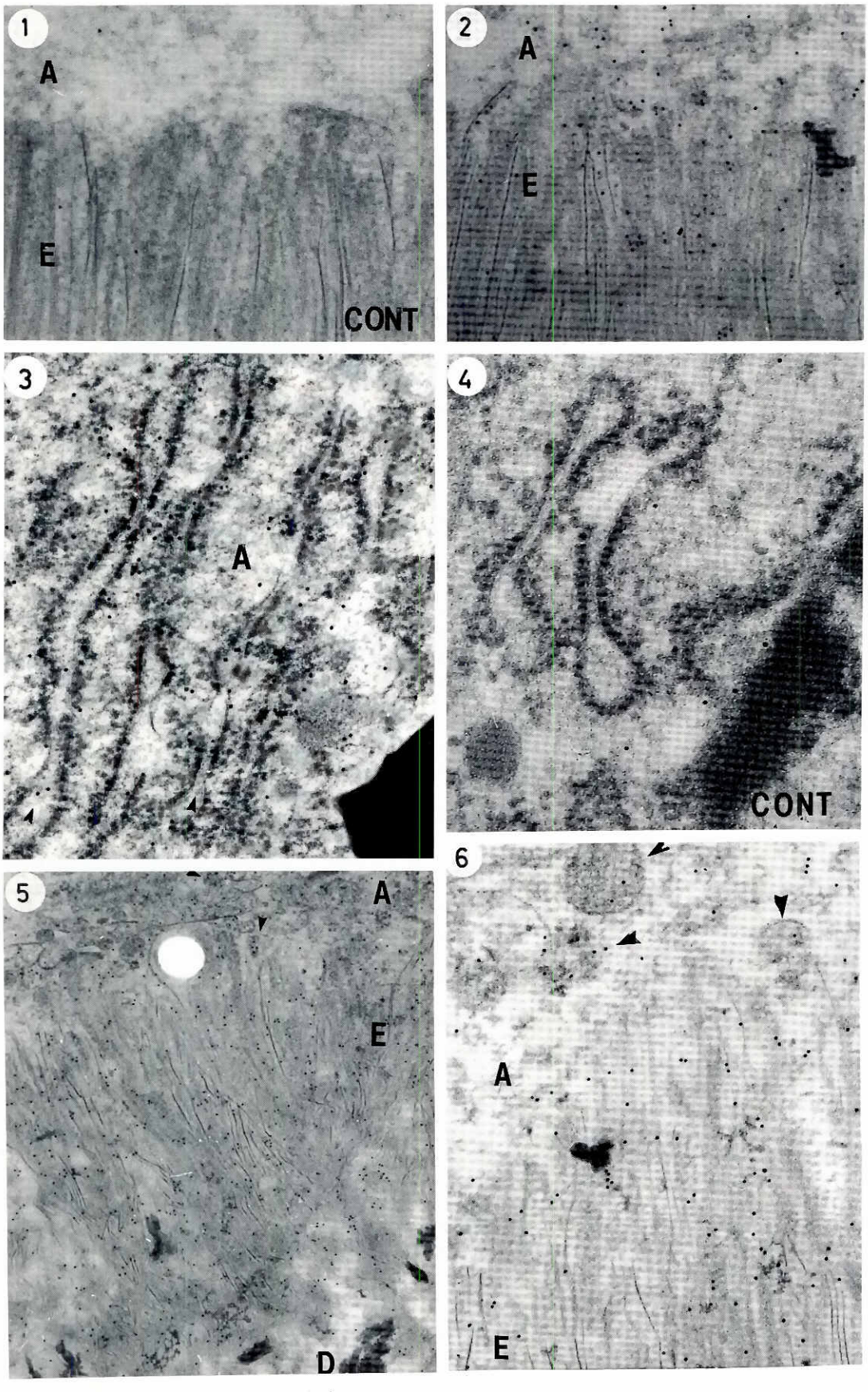


**Fig. 1. High resolution immunochemistry using the protein-A gold method.** *Reaction of tuftelin synthetic peptide antibodies (see Deutsch et al., 1991) with developing enamel* **(2,3,5,6,7,10)**. *Reaction with pre-immune sera served as control* **(1,4,11)**. *Double labeling of developing enamel with amelogenin monoclonal antibodies (large, 18 nm gold particles) and tuftelin synthetic peptide polyclonal antibodies (small, 12 nm gold particles)* **(8,9)**. *A, ameloblast; E, enamel; D, dentin; ODONT, odontoblast; CONT, control.* **(1)** *Control E+A x55,500;* **(2)** *Experimental E+A x55,500;* **(3)** *Experimental A. Rough endoplasmic reticulum (RER) x55,500;* **(4)** *Control A. Rough endoplasmic reticulum x55,500;* **(5)** *Experimental A+E+D. Young enamel. Arrows point to small granules on enamel surface x27,500;* **(6)** *Experimental E+A x55,500. Arrows point to vesicles containing tuftelin protein.*

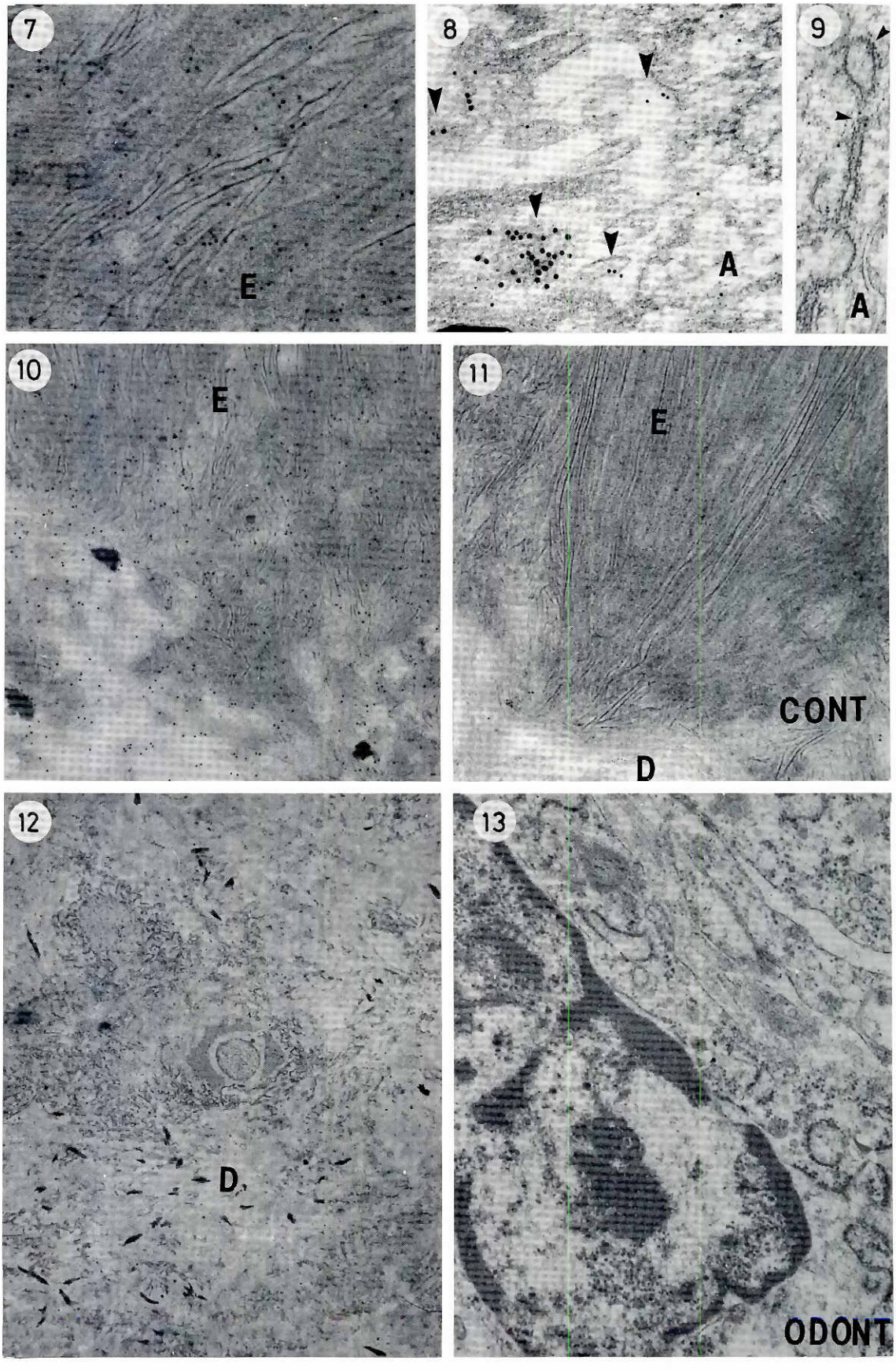


**Fig. 1. High resolution immunochemistry using the protein-A gold method (cont.). (7)** *Experimental E x55,500.* **(8)** *Double labeling (ameloblast= A). Arrows on vesicles containing amelogenin (large particles) and vesicles containing tuftelin (small particles) x55,500* **(9)** *Double labeling, experimental (ameloblast= A). Rough endoplasmic reticulum (RER). Arrows pointing to amelogenin (large particles) and to tuftelins (small particles) x27,500* **(10)** *Experimental E+D enamel-dentin junction x27,500* **(11)** *Control E+D, enamel-dentin junction x27,500* **(12)** *Experimental (dentin= D) x27,500* **(13)** *Experimental (odontoblast= O) x27,500.*

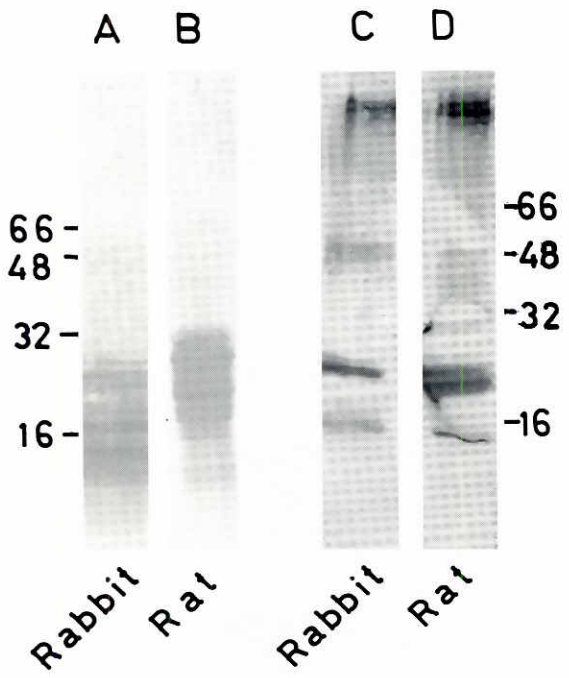


**Fig. 2. Western blot analysis of rat and rabbit developing enamel (mixture of amelogenin and enamelin) using synthetic peptide antisera to the deduced tuftelin predicted sequences (Deutsch *et al.*, 1991). (A)** *Rabbit developing enamel proteins separated on SDS PAGE electroblotted onto nitrocellulose and stained with amido-black.* **(B)** *Rat developing enamel proteins separated on SDS PAGE and stained with amido-black.* **(C)** *Rabbit developing enamel proteins reacted with tuftelin synthetic peptide antisera.* **(D)** *Rat developing enamel proteins reacted with tuftelin synthetic peptide antisera. The antisera was diluted 1:300.*

1984). More recently, even higher enamelin protein species were reported to be present in porcine and, it seems also, in bovine developing enamel (Tanabe *et al.*, 1990; Fukae *et al.*, 1993). It was suggested that at least some of the lower molecular weight enamelin species were a result of the degradation of a higher molecular weight enamelin species. The existence of a number of enamelin polypeptides with different molecular weights could also be due to mRNA alternative splicing (one gene giving rise to a number of mRNA, which in turn code for a number of proteins) or to the existence of a number of genes coding for different acidic proteins. In fact, studies on the biosynthesis of enamel matrix proteins *in vivo* and *in vitro* revealed the existence of 4 enamelin species of 70, 45, 30 and 28 kDa in the cow, and 2 in the rabbit (Ogata *et al.*, 1988; Zeichner-David *et al.*, 1988).

Cell-free translation studies employing human, mouse, rat, bovine, porcine and rabbit enamel organ mRNA (Zeichner-David *et al.*, 1985; Farge *et al.*, 1987) indicated the presence of one or two nascent enamelin proteins.

Two-dimensional studies (Deutsch *et al.*, 1986; Slavkin *et al.*, 1988) revealed the enamelin proteins to be acidic (as was already predicted from the amino acid composition of the different enamelin species) with an isoelectric point of 5.5 and below.

The acidic nature of the enamelin proteins was in line with their ability to bind to the surface of the growing, thin, elongated enamel mineral crystallites. Since the early studies of Termine *et al.* (1980) many studies coupling the biochemical extraction procedure for amelogenins and enamelins with freeze-fracture and electron microscope procedures (Warshawsky *et al.*, 1984; Bai and Warshawsky, 1985; Yanagisawa and Takuma, 1982; Daculsi *et al.*, 1984) as well as ultrastructure localization of amelogenin and enamelin in the extracellular matrix using high resolution immunocytochemistry (Hayashi *et al.*, 1986) and studies on isolated developing enamel crystallites (Daculsi *et al.*, 1984) (for review see Deutsch *et al.*, 1989) have revealed the acidic enamelins to be bound mainly to the mineral surface, while the hydrophobic amelogenins were localized to the intercrystalline spaces.

A number of reports have suggested the enamelin proteins may be highly phosphorylated. This characteristic was established indirectly on the basis of their electrophoretic staining properties with "Stains-All" (Termine *et al.*, 1980). However, direct methods such as those used by Takagaki *et al.* (1984) and very recently by Fincham (1994) (employing Edman degradation sequencing, electro-spray and FAB mass spectroscopy, respectively), are also required to establish the precise nature of this phosphorylation. Phosphorylation of of the enamelin proteins, could provide sites for specific chelation of calcium ions, and thus could be important in understanding the role of these proteins in enamel mineralization.

X-ray studies (Traub *et al.*, 1985; Jodaikin *et al.*, 1986) suggested that enamelin, which is rich in aspartic acid and serine, could be arranged in a beta-sheet conformation such that functional amino acid groups (e.g. aspartic acid) could nucleate the mineral component. They further showed that some of the beta-sheet proteins are preferentially oriented perpendicular to the crystallographic c axis of the mineral, implying a specific role of these proteins in controlling crystal nucleation and growth.

Detailed amino-acid composition studies of enamel along and across the different stages of development indicated that initially, in the very early stages of enamel formation, proteins relatively rich in amino acids characteristic of enamelin, are present in the extracellular matrix (Robinson *et al.*, 1977; Deutsch *et al.*, 1984; Deutsch and Alayoff, 1987). Further evidence that enamelin is the first protein to be secreted by the ameloblasts in the very early stages of formation came from the studies of Slavkin *et al.* (1988). Employing high resolution 2-D gel electrophoresis coupled with immunoblotting and immunocytochemistry, Slavkin *et al.* found that an acidic (pI= 5.5), 46 kDa enamel protein (enamelin) was first secreted by the ameloblast cells prior to the mineralization process and prior to the secretion of the less acidic (pI= 6.5-6.7) 26 kDa protein (amelogenin). Farge *et al.* (1987), using cell-free translation, have also indicated that the high molecular weight enamelin protein is the major gene product at the early stage of human enamel development.

The secreted acidic enamelin proteins persist throughout the forming and maturing stages of development and are present in the mature tissue (some in a partially degraded form) as part of the mature enamel proteins often referred to as tuft-proteins (Weatherell *et al.*, 1968; Robinson *et al.*, 1975; Paulson, 1981). The origin of some components of tuft-proteins has been shown to be the acidic enamelin proteins secreted at the early stages of amelogenesis (Robinson *et al.*, 1989). Antibodies against tuft-proteins, extracted from mature enamel, reacted with secretory organelles of early ameloblasts and with 50-70 KDa enamelin components in developing extracellular matrix enamel proteins. In addition, antibodies against the acidic enamelin protein, extracted from forming enamel, reacted with the tuft-proteins, found in the mature tissue (Amizuka and Ozawa, 1989; Deutsch *et al.*, 1991).

Immunological studies have indicated that the acidic enamelin proteins have common antigenic determinants across a wide

range of vertebrates, suggesting that at least certain portions of the enamelin protein structures have been highly conserved throughout 450 million years of vertebrate evolution (for review see Slavkin *et al.*, 1984), and that these acidic extracellular matrix proteins have an important biological role in the process of enamel mineralization. These immunological studies also indicated that the acidic enamelins are predominant in certain aquatic vertebrates such as fishes and sharks, whereas in terrestrial verterbrates, the enamelin proteins are detected in low proportion relative to the predominant amelogenins. This is supported by biochemical studies and high resolution protein-A gold immunocytochemistry studies, which showed that shark teeth enameloid contains enamelin-like proteins with no evidence of amelogenin proteins (Levine *et al.*, 1966; Kawasaki *et al.*, 1980; Clement, 1984; Graham, 1984, 1985; Herold *et al.*, 1989).

In recent years it has become clear that some of the proteins found in what was originally described as the enamelin fraction are serum albumin proteins (Limeback and Simic, 1989; Strawich and Glimcher, 1990; Strawich *et al.*, 1993). These proteins may have adsorbed to developing enamel during the development of the extracellular enamel matrix; not directly from the ameloblast cells themselves but from the other enamel organ cells, embedded blood vessels, or, as has recently been raised as a possibility, from the underlying dentin (Robinson*et al.*, 1994). Alternatively, these proteins may have become associated with the enamel during tooth extraction procedures. In any case, employing northern and southern hybridization techniques, Couwenhoven*et al.* (1989) reported that ameloblast cells do not synthesize or secret serum albumin protein.

The finding that the enamelin fraction might also contain proteins which are not synthesized by ameloblasts does not exclude the likelihood that in the enamelin protein fraction there indeed do exist specific acidic proteins which are secreted by the ameloblast cells, are specific to the developing enamel tissue, and play a major role in enamel mineralization. This has been the major underlying stimulus in recent years to try and isolate, purify, characterize and sequence such specific acidic enamel proteins employing contemporary molecular biology techniques. Elucidating the primary structure (from Edman degradation and from the cDNA and genomic structure) would establish the identity and specificity of the protein and could, in turn, help to establish the gene expression (and its control) and role of these proteins in amelogenesis. Establishing the gene structure and chromosomal localization will assist in determining whether mutation of the genes coding for the specific acidic enamel proteins are involved in Amelogenesis Imperfecta, the most common heredity disease of enamel.

## Tuftelin/enamelin primary structure and localization

Until recently, virtually nothing was known about the primary structure of the enamelin proteins. No amino acid sequence typical of enamelin had been reported. Neither had any gene coding for these proteins been identified. The deciphering of the enamelin primary structure seemed to be of utmost importance because many researchers have considered the acidic enamelin protein to be a potential nucleator and regulator of enamel crystal growth.

Recently (Deutsch *et al.*, 1989, 1990, 1991, 1994), we have cloned, sequenced and characterized a novel acidic enamel protein (which we called tuftelin) belonging to the family of proteins originally classified as enamelins. The identity and localization of the deduced protein was confirmed by amino acid composition,

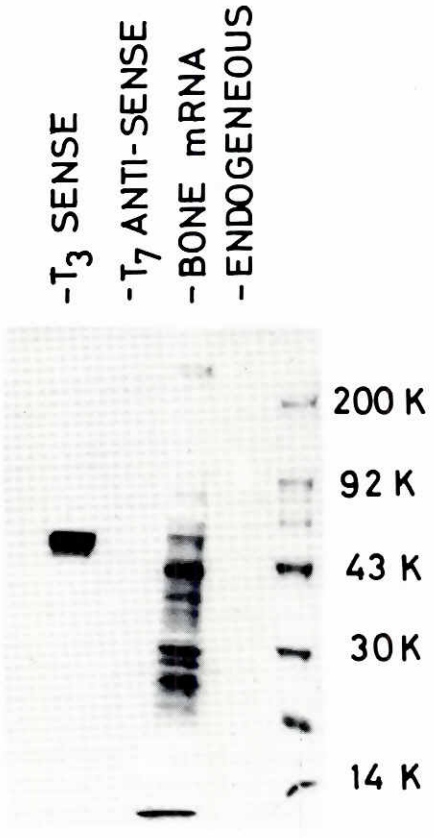


**Fig. 3. Approximately 1.0 ug of DNA from clone 2b was linearized with *Hind III* and sense RNA, prepared using T3 RNA polymerase, using cell free transcription reagents from Promega.** *RNA was capped by addition of $m^7G(5')ppp(5')G$ (Pharmacia Cat # 776-785) using a final concentration of 5 mM and transcription carried out using the conditions recommended by the manufacturers. Transcription products were treated with RNase-free DNase (Boehringer Mannheim). The integrity of the transcription product was analyzed using a 1.2% agarose gel containing formaldehyde and shown to be a single species of approximately 2.4 kb (not shown). For control, antisense RNA was prepared as above except that DNA was linearized with Xbal and then transcribed using T7 RNA polymerase. Approximately 1 μg of sense and antisense RNA were incubated in a rabbit reticulocyte cell-free translation system (NEN Dupont) using [3H] leucine as tracer. Cell free translation products were then separated on a denaturing gradient (4-20%) acrylamide gel (Novex). The radioactive gel was treated for fluorography and exposed to XAR-2 film for 16 h prior to development. For control of the translation reaction RNA containing a wide range of mRNA species was isolated from cultured human bone cells and used in parallel with the sense and antisense tuftelin RNA.*

enzyme-linked immunosorbant assay, Western blots, indirect immunohistochemistry, and high resolution protein-A gold immunocytochemistry. The results revealed the deduced protein to be a novel, conserved (Deutsch *et al.*, 1990) acidic enamel protein, rich in glutamic and aspartic acid. It contains 389 amino acids and has a calculated molecular weight of 43,814 (pre-posttranslation). However, posttranslation modification of the nascent tuftelin protein, such as glycosylation, would increase the actual molecular weight of the protein from that predicted from its amino acid sequence. One potential N-glycosylation site was determined in the present 44 kDa (nascent) deduced protein.

Enamelins have, indeed, been reported to be glycosylated (Menanteau *et al.*, 1988). In this respect, the molecular weight of the deduced tuftelin protein fits well with the range of molecular weights reported for the enamelin proteins (Termine *et al.*, 1980; Ogata *et al.*, 1988; Deutsch *et al.*, 1991).

The amino acid composition of the deduced tuftelin protein is also similar to tuft proteins (Robinson *et al.*, 1975) (enamel matrix proteins remaining in the mature enamel, the origin of which has been shown to be the acidic enamelins secreted in the early stages of development; Robinson *et al.*, 1989). This suggested that at least some of the tuftelin which was secreted during the early stages of enamel formation remains throughout the development of enamel and is found (probably in a partially degraded form) in the mature tissue.

This conclusion is supported by indirect immunohystochemical studies (light level), employing the synthetic tuftelin peptides antisera (Deutsch *et al.*, 1991). The antibodies reacted with the ameloblast cells and particularly with the secretory region (Tomes Process) and with the underlying developing extracellular enamel matrix. No definite reaction was observed with either the odontoblast (dentin-secreting cells) or with the extracellular dentin matrix. The antisera also reacted with bovine and human mature enamel tuft proteins radiating from the dentin-enamel junction towards the enamel surface. The reaction with forming extracellular enamel matrix on a light microscope level was localized mainly interprismatically (between the enamel prisms). In the human mature enamel, the typical horseshoe pattern was seen. This agrees well with high resolution protein-A gold studies of Amizuka and Ozawa (1989), and is similar to recent indirect immunochemical results of Tanabe *et al.* (1990), who employed synthetic peptide antisera to the first 18 N-terminal amino acid sequence (the only sequence available) of a 32 kDa enamel protein fragment. This protein fragment, it was suggested, was part of a high molecular weight non-amelogenin enamelin protein (different from tuftelin), which was originally isolated from porcine enamel (Tanabe *et al.*, 1990; Fukae *et al.*, 1993).

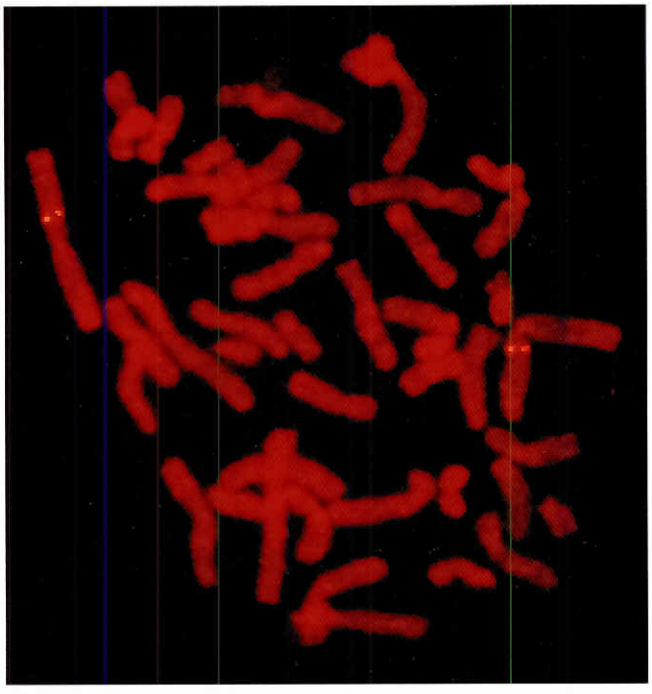

**Fig. 4. Localization of the TUFT 1 locus by fluorescent *in situ* hybridization.** *Metaphase chromosomes are counterstained with propidium iodide and the TUFT 1 gene localization is by FITC fluorescent signals. Reprinted from Deutsch et al. (1994).*

Employing high resolution protein-A gold, and the tuftelin specific antibodies, revealed (Fig. 1) that the tuftelin acidic enamel protein was localized in the ameloblast cells and in the extracellular enamel matrix. Virtually no tuftelin was found in the odontoblast cells or in the extracellular dentin matrix. In the ameloblast, tuftelin was localized at the RER and in vesicles and secretory granules concentrated at the Tomes Processes. In the extracellular matrix, tuftelin was present mainly at the immediate crystallite regions, confirming earlier results on the ultrastructural localization of enamelin. The distribution of tuftelin throughout the thickness of enamel was not homogeneous; tuftelin, it seems, is more concentrated at the DEJ (see also biochemical results of Termine *et al.*, 1980). Preliminary studies on the simultaneously ultrastructural localization of amelogenin and tuftelin proteins employing double-labeling high resolution protein-A gold immunocytochemistry (Bendayan, 1984) revealed that tuftelin and amelogenin proteins do not seem to co-localize in the cell, and are often found in different vesicles and secretory granules.

Our immunological studies (indirect immunohystochemistry and Western blotting) (see Deutsch *et al.*, 1990, 1991) revealed cross reactivity between tuftelin (enamelins) of different species such as bovine, human and shark, suggesting high conservation of tuftelin structure between species. Immunodetection on Western blots of enamelin-enriched fractions from bovine developing enamel employing tuftelin synthetic peptide antisera recognized 66-58, 48, and 28 kDa enamelins (Deutsch *et al.*, 1991). Similar protein bands were also recognized when rat and rabbit enamel proteins were detected on Western blots using the tuftelin synthetic peptide antisera (see Fig. 2). A number of high molecular weight and low molecular weight bands were also observed.

Cell-free translation of tuftelin riboprobe (mRNA) made from the cloned tuftelin cDNA (see Fig. 3) resulted in the single protein band. The Mr on the gel of the synthesized acidic tuftelin protein was more than predicted by cDNA. In this context, it is interesting that the acidic fractions of osteopontin and bone, sialoprotein (BSP) are always higher in Mr on gels than predicted (Fisher, unpublished). It seems that the acid-rich domains cause an increase in Mr over Mw. Control antisense RNA produced no protein bands.

In addition to the consensus sequence for one N-glycosylation site, the cDNA sequence of tuftelin also contains one phosphorylation site, a tripeptide (Glu-Ser-Leu), the phosphorylated form of which appears in all mineralizing tissues (Glimcher, 1979) and, with one mismatch, a consensus sequence of EF-hand calcium-binding domain. In view of the possible role of tuftelin in the mineralization of enamel, these findings are intriguing.

An N-terminal decapeptide sequence of a 22 kDa unidentified protein band isolated from enamelin extract has been published (Strawich and Glimcher, 1990). It contains a -Pro-Ser-Ser-X-X-Ala-Gln- sequence. A homologous sequence can be found in the tuftelin predicted sequence -Pro-Ser-Pro-Pro-Ala-Gln- (the former sequence has an extra serine). This may indicate that the published peptide sequence is a tuftelin derivative or fragment.

To further characterize the tuftelin protein and its structure and function, attempts are currently being made to highly express tuftelin in *E. coli* (Deutsch *et al.*, 1995).

Using our bovine tuftelin cDNA data (Deutsch *et al.*, 1991) Zeichner-David *et al.* (1993) more recently produced specific tuftelin oligonucleotide probes, and employing PCR and Northern blot hybridization, showed that tuftelin was also expressed in mouse ameloblasts from early enamel formation to maturation. It was not expressed in the mesenchymal odontoblast cells. Partial sequencing of mouse tuftelin cDNA showed 85% homology with the corresponding sequences in bovine. Very recently, employing synthetic peptide antisera made to our predicted bovine tuftelin sequence (Deutsch *et al.*, 1991) and indirect immunohistochemistry, Zeichner-David *et al.* (1994) confirmed (see Deutsch *et al.*, 1991) that tuftelin was first secreted at the very early stage of enamel development before the bulk amelogenin was secreted (see also Robinson *et al.*, 1977; Termine *et al.*, 1980; Slavkin *et al.*, 1988; Deutsch, 1989). Tuftelin as well as amelogenin were used by MacDougall *et al.* (1993) as markers for amelogenesis when successfully preparing for the first time primary cultures of mouse ameloblast.

## The role of tuftelin/enamelin

The acidic enamelin has been considered by many researchers to be a potential nucleator and regulator of enamel crystal growth. This was based on its acidic nature (Termine *et al.*, 1980; Belcourt *et al.*, 1982; Deutsch *et al.*, 1986), some aspects of its 2-D structure (Traub *et al.*, 1985), its localization on the crystal surface (Termine *et al.*, 1980; Yanagisawa and Takuma, 1982; Daculsi *et al.*, 1984; Warshawsky *et al.*, 1984; Hayashi *et al.*, 1986; Deutsch *et al.*, 1991) and on the time of its secretion (which commences prior to enamel mineralization). The presence of a relatively high concentration of the acidic enamelin protein at the dentino-enamel junction area, already at very early stages of development (Termine *et al.*, 1980; Deutsch *et al.*, 1984; Robinson and Kirkham, 1985; Slavkin *et al.*, 1988; Deutsch, 1989; Deutsch *et al.*, 1991; Zeichner-David *et al.*, 1994) has suggested to some that the enamelin proteins might be associated with the mineralization of the hypermineralized enamel region adjacent to the dentino-enamel junction, present in the very early forming enamel of all species (Fearnhead, 1979; Suga, 1982) thus creating a mineralization front in enamel (Deutsch *et al.*, 1984, 1991). Based on EM studies, Arsenault and Robinson (1989) have suggested that the acidic enamelins might bind to the collagen fibers of the underlying dentin and such chemical interaction between the two matrices may serve to promote enamel crystal nucleation. Recently it has been reported that ameloblast cells *in vitro* can synthesize and secrete an enamel ECM which is capable of *de novo* biomineralization in the absence of any pre-existing dentin crystals (MacDougall *et al.*, 1994).

The finding that enameloid mineralization, in sharks for example, occurs in the presence of enamelins but does not require the presence of amelogenin is possibly another indicator of the biological role of the acidic enamelins. Perhaps the acidic enamelin/tuftelin (Deutsch *et al.*, 1990) participates in the nucleation of the mineral crystal, whereas the hydrophobic amelogenins found in more advanced vertebrates regulate the size, shape and growth of the crystal.

In the search for the basic mechanism associated with mineral nucleation and growth, the model (Veis, 1985) of matrix-mediated mineralization (Lowenstam, 1981; Lowenstam and Weiner, 1983; Weiner *et al.*, 1983) is an attractive hypothesis. According to this, the organic matrix comprises two component systems. There is the structural bulk phase, which defines the architecture of the system, and the ultimate orientation of the crystal axis. The second phase is an interactive protein that has the dual role of interacting specifically with the structural component and nucleating crystal. Perhaps the role of structural bulk protein is taken up by the abundant hydrophobic amelogenin and the role of interacting protein is accomplished by the acidic enamelins. Enamelins have been shown to bind strongly both to the nucleating and growing crystals and to the bulk amelogenin (Deutsch *et al.*, 1989).

## Human tuftelin gene, chromosomal localization and Amelogenesis Imperfecta — the most common enamel hereditary disease

We have recently cloned (from a human genomic library contained in Stratagene lambda-fix) and partially sequenced (4 exons) the human tuftelin gene (Deutsch, unpublished results), employing the bovine tuftelin cDNA probe (Deutsch *et al.*, 1991). These sequences include exon 1 and over one thousand bases upstream which might contain the promoter region. Southern hybridization and sequencing indicated the clone to contain the entire gene.

Employing fluorescent *in situ* hybridization, we (Deutsch *et al.*, 1994) have mapped the human tuftelin gene to chromosome 1 q 21-31 (Fig. 4). The localization of the human tuftelin gene to a well-defined cytogenetic region may be important in understanding the etiology of the autosomally inherited Amelogenesis Imperfecta (AI), the most common hereditary disease of enamel.

Amelogenesis Imperfecta is a heterogeneous group of hereditary disorders, which affects primarily the enamel of teeth. The different types of AI have been subclassified according to their clinical appearance, enamel characteristics and mode of Mendelian inheritance. The enamel abnormalities have been categorized as hypoplastic, hypocalcified, hypomaturation, or a combination of these. The inheritance patterns include autosomal dominant, autosomal recessive, sex-linked dominant or sex-linked recessive modes of transmission (Witkop and Sauk, 1976). The combined prevalence of all forms has been reported as 1:14,000 in the United States (Witkop and Sauk, 1976), 1:8,000 in Israel (Chosack *et al.*, 1979), and 1:4,000 in Sweden (Sundell and Kock, 1985). Despite the numerous studies describing the clinical, histological and anatomical features and mode of Mendelian inheritance, virtually no genetic or molecular biology information was available until very recently on the involvement of enamel genes in this disease. Having mapped the amelogenin gene to the X and Y chromosomes, it was suggested (Lau *et al.*, 1989; Nakahori *et al.*, 1991; Fincham *et al.*, 1992) and indeed recently confirmed (Lagerstrom *et al.*, 1990, 1991, 1994) that gene mutation of this most abundant enamel protein is associated with the X-linked Amelogenesis Imperfecta. However, inherited X-linked Amelogenesis Imperfecta comprises only a small number of all AI cases. Over 90% of AI cases are autosomally linked and could therefore only be explained by mutation of genes residing on autosomal chromosomes. Since the acidic tuftelin is specifically expressed in the ameloblast, is secreted in the early stages of amelogenesis, persists throughout the development and mineralization of enamel, is conserved throughout vertebrate evolution (Deutsch *et al.*, 1990), and its gene is localized to an autosomal chromosome (chromosome 1), it is a good candidate (the mutated gene) for the cause of at least one form of autosomal Amelogenesis Imperfecta. Attempts are currently being made to study the involvement of the human tuftelin gene in more than 12 autosomally-linked AI families

(one or more families for each of the available sub-classes of this hereditary disease have been identified and characterized and DNA samples from affected and non affected members will be available for this study). This information will provide a baseline for understanding the molecular mechanisms involved in these hereditary diseases.

The opportunities provided by contemporary molecular biology to carry out transgenic and gene "knock-out" experiments together with high expression of the protein in *E. coli* and Baculovirus is an exciting challenge for future understanding of the function, expression and regulation of the tuftelin gene and protein in developing enamel.

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