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Review

Proteomic analysis of dental tissues

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Abstract

Teeth are highly refined structures formed by several types of specialised cell. Tooth formation embraces many areas of biomedical interest, including cellular mechanisms for calcium handling, protein secretion and mineralised tissue production. Proteomics offers great potential to elucidate these cellular roles, and to establish their relevance to general cell types. Here we review our proteomic investigations of dental enamel formation, covering both the approaches taken and some findings of general biomedical relevance. © 2002 Published by Elsevier Science B.V.

Keywords: Reviews; Proteomics; Dental tissues

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1. Introduction

Specialised cells offer valuable correlations between biological structure and function. By defining

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the molecular and anatomical departures from general cell types, biologists can infer which machinery and systems are central to the specialist role. However, a concern is whether such specialised mechanisms apply to cells in general. Global protein analysis (proteomics) is a powerful means to address this issue since specific differences in protein machinery can be identified and evaluated in a whole-cell context. Consequently, higher physiological relevance can be expected for the ensuing proteome profiles than with classical single-protein approaches.

Our teeth are highly refined structures formed by several types of specialised cell. Tooth formation embraces many areas of scientific interest, particularly in the calcium, development and dental fields. It follows that a variety of biomedical research areas will profit from improved understanding of tooth development, including cellular mechanisms for calcium handling, protein secretion, and mineralised tissue production. Traditionally, biochemical investigations have focussed on unique features of dental tissues, particularly the individual constituents of mineralised extracellular matrices. Although a reasonable understanding of tooth formation has accrued, much remains to be learned before this information can be applied to biomedical problems more generally. Most pressing is the need to elucidate roles that cells play in tooth formation.

We have initiated proteomic investigations of dental enamel, the consummate mineralised tissue. Our principal focus was on the formative cells, and the safety mechanisms they use to handle calcium in bulk. This article outlines the proteomic approaches we developed to investigate enamel formation, and reviews some findings that hold general biomedical relevance. It is hoped that this information will highlight the scientific value of enamel-forming cells as a specialist calcium-handling system, and illustrate the utility of proteomics for investigating enamel and other dental tissues.

2. Overview of dental tissues

Our teeth are composed of three distinct hard tissues (enamel, dentine, cementum) surrounding a soft tissue core named the dental pulp (Fig. 1A). Enamel is about 97% mineral and harder than iron,

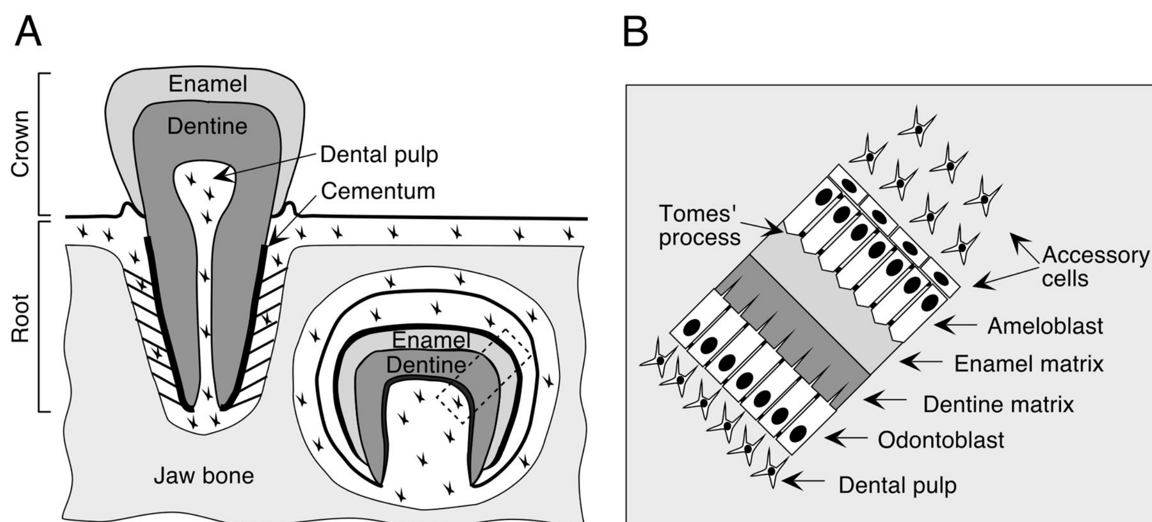


Fig. 1. Scheme depicting the structure and formation of teeth. (A) The mature tooth (left) is formed of a dentine core, with a resilient cap of enamel over the crown. Cementum coats the root, anchoring the fibrous ligament (diagonal lines) that links tooth to jaw bone. The dental pulp is a loose connective tissue in the centre of the tooth, that sustains and innervates the dentine-forming cells (odontoblasts). Formation of the tooth crown occurs in the jaw bone (right), before tooth eruption and associated formation of the root. (B) Detail of enamel and dentine formation (from dashed box in (A)). The enamel epithelium is comprised of elongate cells named ameloblasts that secrete enamel matrix through their apical ''Tomes' process'', plus a variety of accessory cell types (cuboidal ''stratum intermedium'' and ''stellate reticulum'' cells are depicted). Odontoblasts produce dentine matrix through apical membrane processes, that protrude into mature dentine.

providing the exposed ''crown'' with durable cutting and grinding surfaces. Dentine forms the main structure of the tooth and has some flexibility, being compositionally similar to bone. Cementum covers the dental ''root'' and anchors the fibrous ligament that links tooth to jawbone.

The dental hard tissues are first formed as soft extracellular matrices, and only harden after extensive deposition of calcium-based minerals. Enamel is produced entirely before eruption of the tooth, by an epithelium comprising specialised secretory cells (ameloblasts) and accessory cell types (Fig. 1B). Two principal stages of enamel formation (secretion and maturation) are recognised, each associated with anatomically distinct classes of ameloblast. A protein-rich ''enamel matrix'' is secreted first, and then subsequently hypermineralised and deproteinated to yield mature enamel that comprises ~40% calcium by mass. Unlike enamel, dentine maintains a lifelong association with its formative ''odontoblasts'', providing capability for repair (e.g., to reinstate a barrier over the dental pulp after removal of decayed dentine). Odontoblasts have long membrane processes that extend into dentine (Fig. 1B), rendering teeth sensitive to physical stimuli such as cold food and dentist's drills. Cementoblasts also retain their cementum-forming capacity throughout life. This enables the fibrous attachment to be rearranged, for example during orthodontic tooth movement [1–4].

3. Biomedical interests in dental tissues

Tooth formation holds major importance in calcium biology since it embraces specialist calcium-handling roles and involves large amounts of calcium. To remain viable and function properly, all cells in our bodies must regulate their internal calcium concentrations within strictly defined limits. Usually this means that only small amounts of calcium are admitted to a cell at any one time, for example as required to initiate a cell-signalling event. Prolonged exposure to elevated calcium concentrations is cytotoxic and can eventually lead to cell death [5]. Tooth-forming cells face the additional burden of biomineralisation, which requires calcium to be supplied in bulk, at a particular location, and in a well-controlled manner. These requirements are greatest in the case of enamel, and are met by calcium taking a regulated transcellular route across the ''tight'' enamel epithelium. Consequently, ameloblasts are thought to have specialised mechanisms for controlling the entry, transcellular transport and vectorial extrusion of bulk calcium, that successfully avoid calcium-induced cytotoxicity [6]. It seems likely that the calcium-oriented activities of dental tissues will hold broader biomedical relevance. For example, transcellular calcium transport in kidney and intestine has critical importance for nutrition, and calcium toxicity is central to the cell-damaging effects of stroke and neurodegenerative disorders [7,8]. Whether or not these diverse situations share common calcium-regulatory mechanisms remains an important, open question.

The oral biology and dentistry fields have more specific interests in tooth formation, as to be expected. An improved understanding of normal tooth development and its disorders is desirable, both from prevention and treatment viewpoints. Such knowledge depends on more detailed information about the formative cells and their molecular functions. For example, enamel formed in the presence of fluoride is relatively resistant to dental decay but, for poorly understood reasons, excess fluoride can cause undesirable defects that commonly manifest as white spots in enamel [9]. Better fundamental understanding of enamel formation could lead to more acceptable use of fluoride and to the development of alternative nutritional strategies that promote resistance to tooth decay. The ability to regulate odontoblast and cementoblast activities might also be translated into clinical benefits, such as improved repair processes after reimplantation of traumatically displaced teeth. Definition of dental tissue proteomes will provide stringent diagnostic markers for dental histopathology, and establish a comprehensive ''fingerprint'' for assessing authenticity of dental cell lines [10–13].

4. Proteomic challenges of dental tissues

To undertake proteome analysis of dental hard tissues, investigators face the obvious problem of accessing proteins buried in a calcified matrix. After

removal of mineral, usually with calcium chelators or acid, the next task is to overcome the poor solubility of many matrix proteins [14,15]. A further difficulty is substantial protein heterogeneity associated with alternative splicing and a variety of post-translational modifications (phosphorylation, glycosylation, sulfation). Proteolytic maturation of enamel proteins gives even more complexity [2–4,16]. While much can be learned from studies of hard tissues in isolation, a complete understanding will depend on parallel examination of their formative cells.

Obtaining tooth-forming cells is a major challenge. Developing human teeth are not readily available since tooth formation occurs during early childhood. Tooth development in rat and mouse has been investigated extensively and found to exhibit many similarities to human [1]. The small size of murine teeth imposes a major constraint on biochemical procedures, but they remain a sensible starting point for proteomic investigations given their general knowledgebase. Suitable isolation procedures are established for rat odontoblasts, cementoblasts [17,18] and enamel epithelium (Section 5.1).

5. Proteomic analysis of enamel development

We initiated proteome analysis of rat enamel epithelium with the primary intention of learning about the protein machinery used for bulk calcium handling [19,20]. Procedures were established to identify the major protein constituents of enamel epithelial cells (''enamel cells'') and freshly secreted enamel matrix. Subsequently, protein expression patterns were compared at the secretion and maturation stages to provide correlations with enamel protein production and calcium transport, respectively. Several discoveries arising from this comparative proteomic analysis have been investigated in more detail, using a variety of approaches.

5.1. Tissue isolation and sample preparation

5.1.1. Analysis of enamel-forming cells

Our initial endeavour was to obtain rat enamel cells in sufficient bulk for proteome analysis, but within a suitably short timeframe to avoid post-mortem degradation of proteins. For this reason, epithelia were rapidly microdissected from developing mandibular first molars, then stored frozen and subsequently pooled. Maxillary molars were not sampled, to save post-mortem time. This approach was successful since proteome analysis revealed only minor proteolytic nicking of calreticulin and actin, proteins that are particularly prone to degradation [20,21]. The secretion and maturation stages were sampled by using animals of different ages (5- and 10-day-old, respectively). Immunolocalisation of interest proteins was used to address some shortcomings of this approach [22–24]. First, the use of whole enamel epithelium meant that findings could not be specifically ascribed to ameloblasts, our primary focus. Second, not all cells were of the desired developmental stage at each sampling period due to the complex morphology of molars. Third, some connective tissue was included with microdissected epithelia, as evidenced by variable contamination with serum albumin. In future studies, individual cell types might be examined by use of split-epithelium preparations or dispersed cells. Developmental stages could be sampled at higher resolution by subsectioning epithelia, particularly in incisors where all stages are represented on discrete regions of a single tooth [19,25]. The integrity of ameloblasts during isolation of enamel epithelium is another area requiring consideration. During secretion, each ameloblast has an apical membrane process that extends into the developing enamel matrix (Tomes' process; Fig. 1B). Histology revealed apical blebbing of isolated ameloblasts, suggesting that Tomes' process is ruptured and resealed during microdissection [19] (and our unpublished data). It remains to determine whether such localised disruptions are accompanied by significant loss of soluble proteins.

We have used subcellular fractionation routinely, both to produce simplified proteome maps and to localise proteins. Initial two-dimensional gel electrophoresis (2-DE) of whole enamel epithelium revealed a hyperabundance of cytoskeletal proteins, many of which predominated in regions where we expected to find calcium-handling proteins. To avoid masking such lower-abundance proteins, we prepared soluble and particulate extracts using microscale homogenisation and ultracentrifugation procedures [20]. As anticipated, much of the cytoskeletal content partitioned to the particulate fraction, leaving the soluble proteins more clearly resolved. Two

practical matters were found to benefit this approach. Homogenisation in low-osmotic-strength buffer promoted disruption of major organelles including endoplasmic reticulum (ER) and mitochondria. As a consequence, soluble protein residents of these organelles (e.g., ER chaperones) partitioned more cleanly to the soluble fraction. Second, with ultracentrifugation of the particulate fraction into a compact pellet, it was possible to use the powerful detergent, sodium dodecyl sulfate (SDS), to effect complete solubilisation of cytoskeletal proteins. Only a small amount of SDS was required and so this step did not pose a problem for subsequent 2-DE analysis (Section 5.2). Further into our proteomic investigations, we became interested in preserving the integrity of organelles to help address questions about protein localisation. A freeze–thawing procedure was instigated to gently disrupt whole enamel epithelium, and we successfully obtained distinct 2-DE distributions for known cytosolic, ER and mitochondrial proteins. Strong support for the localisation of a novel ER protein eventuated from this microscale proteomic strategy [26]. An unexpected bonus was significant improvements in protein recovery, probably due to reduced sample handling compared with the earlier homogenisation procedure.

5.1.2. Analysis of enamel matrix

To characterise secreted enamel proteins, we sampled enamel matrix from the early secretion stage in rat incisors. Later stages of matrix development were excluded in an attempt to avoid proteolytically processed variants. Enamel matrix was grossly freed of cells by dissection, and then lyophilised to facilitate removal of residual cellular debris. Trifluoroacetic acid, an excellent protein solvent, was used to simultaneously demineralise and solubilise the isolated matrix, followed by exchange to a neutral-buffered SDS–chelator mixture in preparation for 2-DE analysis [20]. Minimal insoluble residue remained after this procedure, suggesting that most if not all protein constituents of enamel were represented in the samples examined.

5.2. Two-dimensional gel electrophoresis

For several years, our standard approach to 2-DE involved classical carrier ampholyte isoelectric focussing (IEF) in tube-gels followed by mini-format SDS–polyacrylamide gel electrophoresis (PAGE). More recently, we have adopted immobilised pH gradient (IPG) strips for the IEF dimension, in some but not all situations. In our experience, IPG separations exhibited superior stability and reproducibility in the basic range (p*I* 6–11), as expected. Higher protein-spot intensities were also achieved in the basic range with IPG, as compared to nonequilibrium ampholyte-based separations where sample precipitation problems were encountered. However, in the acidic range (p*I* 3.5–7), the reverse applied. As shown in Fig. 2, the carrier ampholyte separation yielded more protein spots and generally stronger staining than for IPG, even with a doubled loading in the latter case. Moreover, our classical 2-DE analysis of human liver revealed three major proteins absent from published IPG-based maps [27]. The carrier ampholyte procedure appears more tolerant of SDS and salts, and to retain less protein than occurs with IPG strips. With these experiences, we consider it beneficial to have both IPG and carrier ampholyte procedures available in the laboratory. For acidic-range separations, carrier ampholytes remain our first choice in the case of scarce samples and particulate fractions requiring solubilisation with SDS.

The sample limitation with enamel cells forced us into an early departure from the standard practice of using large-format gels. We found that considerably lower sample loads were required on mini-gels to achieve the same protein-spot intensity obtained with large gels. Given the difficulty of obtaining enamel cell samples, the attendant loss of resolving power on mini-gels was considered a lesser evil. In a similar vein, we modified a commercial mini-format 2-DE apparatus to accept thinner (0.75 mm) gels in the second dimension. This modification improved spot intensity with low sample loads, and provided higher yields from tryptic in-gel digests [20,23].

5.3. Protein identification

We routinely use sequence-based procedures (direct sequencing, peptide-mass fingerprinting) to identify proteins, but have also exploited ligand-overlay assays and immunoblotting to address specific questions [23]. For example, screening with the ^{45}Ca-overlay procedure led to identification of the F_1-ATPase β-subunit as a calcium-binding protein, and immunoblotting of the ER calcium pump was

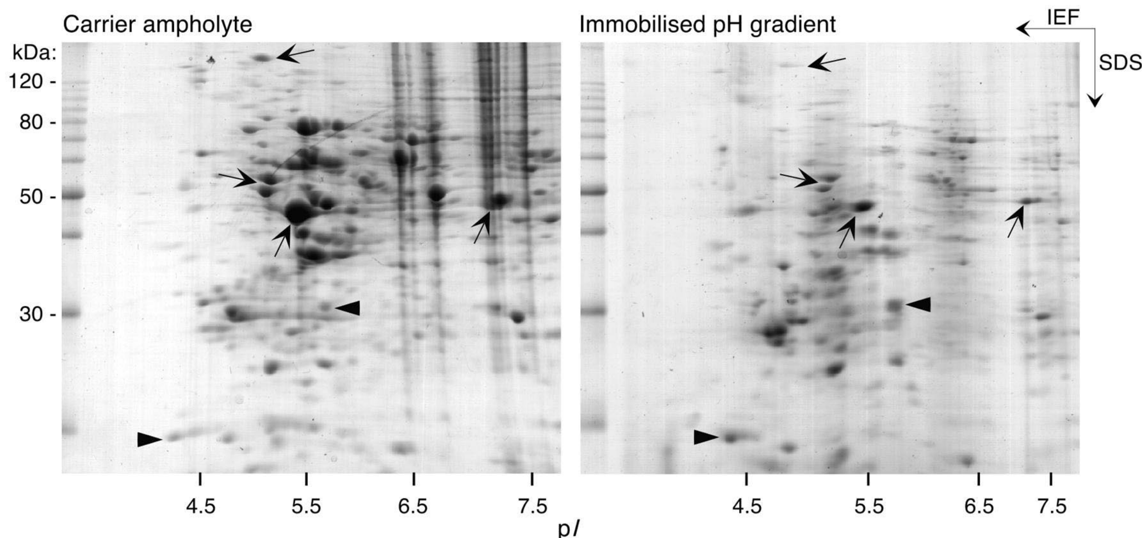


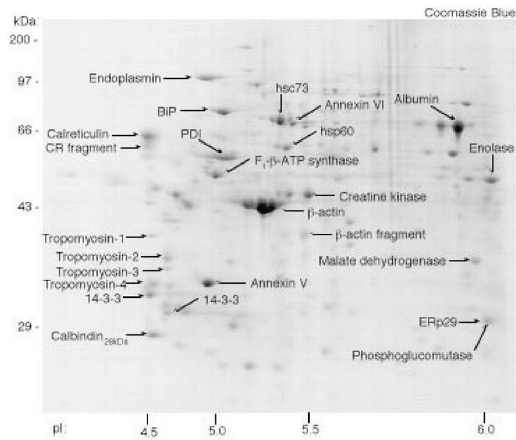
Fig. 2. Comparative analysis of carrier ampholyte and IPG separations. Mouse brain extract was subjected to parallel 2-DE analysis using mini-format, carrier ampholyte tube gels (150 μg protein loaded) and IPG strips (300 μg protein) as indicated, and stained with Coomassie Blue. Despite the doubled sample load for IPG, the carrier ampholyte procedure produced more and generally stronger protein spots particularly in the higher mass regions (e.g., arrows). The arrowheads indicate two of several spots that were more strongly stained in the IPG separation, as expected. For the carrier ampholyte procedure, ethanol-precipitated proteins were dissolved in 3 μl 10% SDS–10 *M* dithiothreitol then diluted with 20 μl sample-loading solvent (9 *M* urea, 4% CHAPS, 50 m*M* dithiothreitol, 2% carrier ampholytes, pH 3–10) before loading. For IPG, the protein precipitate was dissolved directly in 135 μl IPG sample buffer (5 *M* urea, 2 *M* thiourea, 2% CHAPS, 2% SB3-10, 100 m*M* dithiothreitol, 40 m*M* Tris–HCl, pH 9.5, 0.5% carrier ampholytes, pH 3.5–10) and loaded by rehydration at 50 V for 12 h (JCK and MJH, unpublished results).

used to sidestep its inaccessibility to 2-DE [28,29]. In the early stages, we used Edman microsequencing of HPLC-purified tryptic peptides to identify 2-DE-separated enamel cell proteins [19,20]. Our aim then was to get extensive sequence coverage, since rat proteins were poorly represented in the databases and we did not know to what extent enamel cells expressed unique proteins and isoforms. Although time consuming, this thorough approach generated de novo sequence reliably and we were subsequently rewarded with the identification of a novel protein, ERp29 [26,30]. When matrix-assisted laser desorption ionisation (MALDI)-MS became available, the peptide-mass fingerprinting approach provided better sensitivity and efficiency to protein identification [28]. However, it was interesting to find that many peptides invisible to MALDI-MS were readily characterised by the Edman approach, and vice versa. When it became necessary to obtain near-complete sequence coverage, as in the ERp29 studies, the combination of Edman analysis and MALDI-MS was used to good effect. Moreover, proteins could be identified unequivocally from only one or two peptides when this combination was used to generate sequence tags [26,27]. Our more recent capability to generate sequence tags by LC–MS–MS has provided better sensitivity and so made many more proteins accessible to primary structural characterisation.

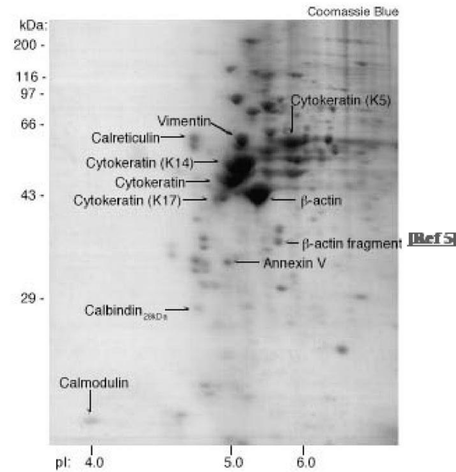
5.4. An initial view of the enamel cell proteome

Proteome analysis of enamel cells produced a molecular fingerprint that was complementary to the existing picture of enamel cell biology built largely from morphological data. It also revealed for the first time that, despite their specialised functional roles, enamel cells are largely composed of common protein machinery [21,23]. Distinct 2-DE maps were obtained for the soluble and particulate fractions of enamel cells, and for secretion-phase enamel (Fig.

A



B



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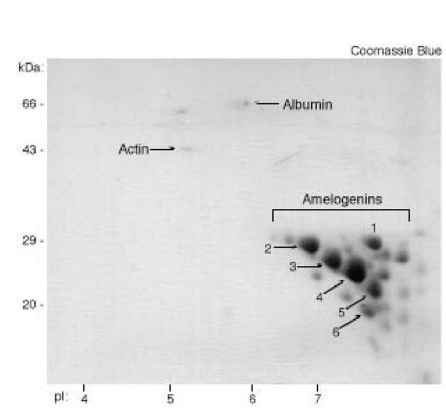

Fig. 3. Two-dimensional gel maps of rat enamel tissues, showing distinct protein patterns for: (A) soluble fraction from enamel epithelium homogenate; (B) particulate fraction from enamel epithelium homogenate; and (C) enamel matrix extract at secretion phase. Images were taken from the *ToothPrint* website [36].

3), attesting to the success of our sample preparation procedures. The 42 identified proteins (see http://toothprint.otago.ac.nz) were common to other cell types, with the exception of the major enamel proteins (amelogenins). Anatomically, many of these identified proteins could be assigned to cytoskeleton, ER, and mitochondria, consistent with the dominance of these structures in ameloblasts. Functionally, associations with secretory protein production, calcium regulation or energy metabolism were apparent in most cases. The proteomically derived phenotype was therefore consistent with the major protein-secretory and calcium-handling roles of enamel cells, both of which are likely to be energetically demanding [21,23]. These findings lend support to the rat tooth model, since all identified proteins have highly conserved orthologues in human. However it remains to determine whether the vastly compressed time-scale of murine tooth formation (3 weeks versus over a year in human) is reflected by differences at the proteome level.

The enamel-specific proteins, amelogenins, were predominant constituents of secretory enamel (Fig. 3C) and also readily detected in the soluble fraction of enamel cells. We did not detect ameloblastin or enamelin with Coomassie staining, consistent with previous evidence that these enamel proteins are produced in lower abundance or turned over more rapidly than amelogenins [16,31]. The trace amounts of actin and albumin (Fig. 3C) might reflect residual cellular debris (e.g., Tome's processes) and serum contamination, respectively [32,33].

5.5. Proteomic discoveries from enamel cells

Having identified some key protein machinery of enamel cells, we compared secretion- and maturation-phase proteomes with the aim of deriving functional correlations. This comparative approach led to several interesting findings, some of which we chose to investigate in more detail as reviewed more fully elsewhere [6,23,34].

In brief, our first new objective was to reevaluate the function of a high-affinity calcium-binding protein named calbindin$_{28kDa}$. Unexpectedly we found that calbindin$_{28kDa}$ was strongly down-regulated during maturation, the major stage of calcium transport into enamel. This observation contradicted the dogma that calbindins act as transcellular ‘‘calcium ferries’’ in calcium-transporting epithelia. A second surprise was finding substantial amounts of calbindin$_{28kDa}$ in the particulate fractions of enamel cells (Fig. 3B) and brain, again challenging the widely held notion of a mobile calcium buffer. These findings led us to hypothesise that calbindin$_{28kDa}$ interacts with immobile target proteins, and perhaps transduces calcium signals like calmodulin [19,20,22,35]. Our recent investigations have tentatively identified one such calbindin target as a protein kinase (JCK and MJH, unpublished results).

Another new direction was to pursue alternative calcium-transport mechanisms, now that a calbindin-based cytosolic route seemed unlikely. Two prominent calcium-binding proteins were found up-regulated at maturation, and identified as residents of the ER lumen. Consequently, we hypothesised that calcium follows an ER-based route across enamel cells. Since the ER is known to tolerate high calcium concentrations, such a ‘‘calcium transcytosis’’ mechanism seemed both feasible and highly advantageous for avoiding the problems of excess cytosolic calcium. Recent evidence that the ER calcium pump is similarly up-regulated at maturation supports this notion [6,20,28,29].

Finally, our proteomic discovery of ERp29 (Section 5.3) introduced the challenge of finding a functional role for this novel ER protein. ERp29 appears to have general biological importance, being highly conserved and ubiquitously expressed in animal cells. While the specific function of ERp29 remains unknown, a variety of findings suggest a unique role in the production of secretory proteins. Ameloblasts are amongst the most ERp29-enriched cells identified to date, whereas odontoblasts contain strikingly lower amounts of ERp29 [24,26,27,30]. Dental cells might therefore be pivotal in uncovering the function of ERp29, as they were in its discovery.

6. *ToothPrint*, a proteomic database for dental tissues

We have established *ToothPrint* as an online proteomic database for dental tissues, accessed on the World Wide Web at http://toothprint.otago.ac.nz [36]. This bioinformatic resource should facilitate

dissemination of dental proteomic information, and benefit its integration into the general biological knowledgebase. Initially implemented for developing rat enamel, *ToothPrint* provides a variety of proteomic data types including 2-D gel maps (Fig. 3), searchable lists of identified proteins, developmental regulation, ligand binding, subcellular fractionation and immunohistochemistry. Supplementary information about protein and DNA sequences, 2-D gel patterns in other tissues, protein structures and the published literature is accessible through links to remote databases. *ToothPrint* has been designed in anticipation of including proteomic data from other dental tissues as it becomes available, so providing a comprehensive bioinformatic resource for the dental biology field.

7. Conclusions

We have found that enamel development is readily accessible to proteomics using standard 2-DE-based approaches. Tissue-specific difficulties, such as mineralisation and sample scarcity, have required only relatively minor modifications to conventional procedures. Functional value has been increased by incorporating subcellular fractionations and a variety of complementary protein assays in our studies. Our initial view of the enamel cell proteome has been informative, both by establishing a molecular fingerprint that complements existing knowledge and by revealing some features that were quite unexpected. It is now evident that enamel cells use common protein machinery for their principal specialised tasks (i.e., secreting a unique protein matrix and handling calcium in bulk). Rewardingly, this means that findings can now be translated from enamel cells to general cell types with increased confidence. However, it should be remembered that, although different cells might share the same protein tools, they may not use them in the same physiological ways. Cataloguing expressed proteins is a major focus of current proteomics, and it is likely that a much more extensive coverage of the enamel cell proteome can be achieved using cutting-edge mass spectrometers in combination with PAGE- and LC-based microseparations [34]. While this remains an important goal, thought should be directed to how the ensuing data will be used. Indeed, a quite remarkable amount of useful information is associated with the 42 proteins we identified to date, and understanding the functional significance of our discoveries is proving a much greater challenge than the original protein identifications. We expect that dentine and cementum formation will also be amenable to proteomic investigation, albeit requiring individual adaptations as for enamel. Consequently, proteomics has great potential to underpin a comprehensive understanding of tooth formation, to the benefit of several scientific fields.

Nomenclature

2-DE	two-dimensional gel electrophoresis
ER	endoplasmic reticulum
IEF	isoelectric focussing
IPG	immobilised pH gradient
MALDI	matrix-assisted laser desorption ionisation
PAGE	polyacrylamide gel electrophoresis
SDS	sodium dodecyl sulfate

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References

[1] H. Warshawsky, CRC Crit. Rev. Anat. Cell Biol. 1 (1988) 425.
[2] J.P. Simmer, A.G. Fincham, Crit. Rev. Oral Biol. Med. 6 (1995) 84.
[3] W.T. Butler, Eur. J. Oral Sci. 106 (Suppl. 1) (1998) 204.
[4] N.E. Saygin, W.V. Giannobile, M.J. Somerman, Periodontology 24 (2000) 73.
[5] B.F. Trump, I.K. Berezesky, FASEB J. 9 (1995) 219.
[6] M.J. Hubbard, Crit. Rev. Oral Biol. Med. 11 (2000) 437.
[7] F. Bronner, D. Pansu, J. Nutr. 129 (1999) 9.
[8] J.M. Lee, G.J. Zipfel, D.W. Choi, Nature 399 (1999) A7.

[9] M.S. McDonagh, P.F. Whiting, P.M. Wilson, A.J. Sutton, I. Chestnutt, J. Cooper, K. Misso, M. Bradley, E. Treasure, J. Kleijnen, Br. Med. J. 321 (2000) 855.
[10] P. Papagerakis, M. Peuchmaur, D. Hotton, L. Ferkdadji, P. Delmas, S. Sasaki, T. Tagaki, A. Berdal, J. Dent. Res. 78 (1999) 20.
[11] L.S. Chen, R.I. Couwenhoven, D. Hsu, W. Luo, M.L. Snead, Arch. Oral Biol. 37 (1992) 771.
[12] P.K. DenBesten, C. Gao, W. Li, C.H. Mathews, D.C. Gruenert, Eur. J. Oral Sci. 107 (1999) 276.
[13] J.A. D'Errico, H. Ouyang, J.E. Berry, R.L. MacNeil, C. Strayhorn, M.J. Imperiale, N.L. Harris, H. Goldberg, M.J. Somerman, Bone 25 (1999) 39.
[14] J.D. Termine, A.B. Belcourt, M.S. Miyamoto, K.M. Conn, J. Biol. Chem. 255 (1980) 9769.
[15] J.D. Termine, A.B. Belcourt, P.J. Christner, K.M. Conn, M.U. Nylen, J. Biol. Chem. 255 (1980) 9760.
[16] C.E. Smith, A. Nanci, Anat. Rec. 245 (1996) 186.
[17] L. Guo, R.M. Davidson, J. Bone Miner. Res. 14 (1999) 1357.
[18] J. Gao, A.L. Symons, H. Haase, P.M. Bartold, J. Periodontol. 70 (1999) 951.
[19] M.J. Hubbard, Eur. J. Biochem. 230 (1995) 68.
[20] M.J. Hubbard, Eur. J. Biochem. 239 (1996) 611.
[21] M.J. Hubbard, Connect. Tissue Res. 38 (1998) 17.
[22] R.J. Sayer, C.I. Turnbull, M.J. Hubbard, Cell Tissue Res. 302 (2000) 171.
[23] M.J. Hubbard, Electrophoresis 19 (1998) 1891.
[24] S.D. Shnyder, M.J. Hubbard, J. Histochem. Cytochem. 50 (2002) 557.
[25] C.E. Smith, Crit. Rev. Oral Biol. Med. 9 (1998) 128.
[26] M.J. Hubbard, N.J. McHugh, D.L. Carne, Eur. J. Biochem. 267 (2000) 1945.
[27] M.J. Hubbard, N.J. McHugh, Electrophoresis 21 (2000) 3785.
[28] M.J. Hubbard, N.J. McHugh, FEBS Lett. 391 (1996) 323.
[29] I.K. Franklin, R.A. Winz, M.J. Hubbard, Biochem. J. 358 (2001) 217.
[30] J. Demmer, C.M. Zhou, M.J. Hubbard, FEBS Lett. 402 (1997) 145.
[31] J.D. Bartlett, J.P. Simmer, Crit. Rev. Oral Biol. Med. 10 (1999) 425.
[32] W.Y. Chen, A. Nanci, C.E. Smith, Calcif. Tissue Int. 57 (1995) 145.
[33] R.I. Couwenhoven, C. Davis, M.L. Snead, Calcif. Tissue Int. 45 (1989) 367.
[34] M.J. Hubbard, Proteomics (2002) in press.
[35] M.J. Hubbard, N.J. McHugh, FEBS Lett. 374 (1995) 333.
[36] M.J. Hubbard, M.J. Faught, B.H. Carlisle, P.A. Stockwell, Proteomics 1 (2001) 132.