BPI-Like Proteins in Oral and Airway Epithelia

Psp and Smgb: a model for developmental and functional regulation in the rat major salivary glands

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Abstract

This paper summarizes past work detailing the developmental expression, cell and organ localization and biochemical features of the proteins parotid secretory protein (PSP) and isoforms of submandibular gland protein B (SMGB), and describes the molecular characterization of the genes that encode them, Psp and Smgb. These genes appear to be related to the BPI (bactericidal/permeability-increasing protein)/LBP (lipopolysaccharide-binding protein)/PLUNC (palate, lung and nasal epithelial clone) gene family found in the oral and respiratory organs of humans, rodents and cattle. We have emphasized the diverse patterns of expression of these genes among the submandibular, sublingual and parotid salivary glands of the rat, and their potential usefulness in defining and identifying genomic regulatory mechanisms of salivary development. While Psp is expressed similarly in the mouse, the putative Smgb gene of the mouse seems not to be expressed, apparently due to the insertion, between exons 1 and 2, of a gene for a retroviral protein.

Expression of parotid secretory protein (PSP) and related proteins in the major salivary glands of the rat

Parotid gland

PSP was first identified as band M1 on native polyacrylamide gels of parotid saliva from adult rats [1], and was subsequently identified in DEAE-Sephadex or DEAE-cellulose fractions as a leucine-rich protein constituting the predominant component of saliva [2,3]. In the mouse, a similar protein was identified as the major component of parotid saliva and called PSP. The Psp gene was localized to chromosome 2 [4], and encodes a protein of derived molecular mass 23 kDa and high leucine content (23%) [5,6]. Polyclonal antibodies raised against purified rat M1 protein were equally reactive against 23.5 kDa bands on Western blots of rat and mouse parotid secretions, suggesting that the rat protein is the homologue of mouse PSP [7]. In both mouse and rat, PSP appears during secretory granule formation in the first postnatal week, and increases by 21 days to the high adult level ([8,9]; W.D. Ball, unpublished work).

Submandibular gland (SMG)

Perinatal stage of differentiation

The protein products of the Psp gene and of a related gene, Smgb, were identified in secretion from the perinatal rat SMG. Initially, these proteins were called SMG protein A (SMGA), SMGB1 and SMGB2 (23.5, 26 and 27.5 kDa) and were referred to collectively as the B1-immunoreactive proteins (B1-IPs) or B1 proteins, since all were reactive with antibodies raised against protein SMGB1, the first of the
group to be isolated. Subsequent studies showed that SMGA is the sole protein encoded by the Psp gene, and that SMGB1 and SMGB2 are differently glycosylated forms of SMGB [8].

Although PSP and SMGB are predominant products of the neonatal SMG, these and most other major secretory proteins of the parotid gland are not expressed in the seromucous acinar cells of the adult gland. During SMG development, two novel and transient secretory cell types appear: the Type III (or pro-acinar) cells become the mature acinar cells, and the Type I (or terminal tubule) cells form the intercalated ducts [10]. Type III (pro-acinar) cells have ultrastructurally novel secretory granules containing the protein products of the Psp and Smgb genes. Their secretion was elicited specifically by β-adrenergic agonists, indicating that a functional signal transduction system is present within a few days of birth [11–13].

Type I (terminal tubule) cells have typical dense secretion granules and no expression of the B1-IPs. Secretion is stimulated in response to cholinergic agonists and they express a major secretory protein, protein SMGC, at 89 kDa on electrophoretic gels. Polyclonal antibodies raised to biochemical isolates of this protein helped to clearly identify the two cell types. Indeed, by the time secretion granules are apparent in the prenatal gland (18–19 days post-conception), the cell types are distinguishable by electron microscopic immunocytochemistry [14]. It should be pointed out that this distinct separation of the pro-acinar to acinar cell lineage may not occur in the SMG of the perinatal mouse, which also shows developmentally regulated PSP expression in the perinatal gland ([8]; W.D. Ball, unpublished work). Denny and collaborators have reported the appearance of immunoreactive adult mucin in both secretory cell types of the perinatal mouse SMG [15,16].

Maturation of the SMG

In the first postnatal week, the Type III cells begin to differentiate directly into mature acinar cells. These synthesize Glx-rich proteins (GRPs) and SMG mucin, which are the major secretory proteins of the mature acini [17–20]. GRPs and mucin accumulate first within pro-acinar cells in intermediate secretion granules that also contain PSP and SMGB. Through the second and third postnatal weeks, GRP mRNA and protein increase to adult levels, as estimated from Western and Northern blots. Expression of PSP and SMGB, as assessed by Western blots, decreases markedly over 30 days, but faint bands of SMGB are still visible on Western blots into adulthood [10].

In the adult, B1 reactivity is no longer detectable in the acinar cells by immunocytochemistry, raising the question of which cell types contain the reactivity seen on Western blots. B1-IP reactivity is seen in some cells of intercalated ducts. Also present are occasional islands of cells with the immunocytochemical reactivities and ultrastructural features of perinatal pro-acinar cells, e.g. immunoreactivity with anti-B1-IP antibodies but none for the adult markers, mucin and GRP. These have a higher labelling index after administration of [3H]thymidine than do the typical adult acinar cells. We have speculated that these are acinar replacement cells originating in intercalated ducts, which have the highest labelling index of any parenchymal component in adult rat SMG [21,22].

Sublingual gland (SLG)

The development of the SLG is more rapid and direct than that of the other major salivary glands. During embryogenesis, the SLG develops mucous acini and serous demilunes, and by birth their arrangement is essentially the same as that seen in the adult [23]. Smgb is expressed in the demilunes at a high level through adulthood as the protein SMGB, which differs from SMGB1 and SMGB2 in its carbohydrate composition (see below). Psp mRNA and PSP protein are expressed at low levels in the neonate, and very low levels of mRNA transcripts can be detected in the adult [8,13,24].

Expression in other organs of proteins immunologically cross-reactive with the B1-IPs

Polyclonal antibodies raised to the perinatal protein species of the SMG were used to evaluate their expression in several organs, using light and electron microscopic immunocytochemistry. Anti-B1 antibodies, which recognize the products encoded by both Psp and Smgb, detected reactive species in the serous von Ebner’s glands of the tongue, with reactivity localized mostly in ducts and not in acini. A different pattern was seen in lacrimal glands, where anti-B1 labelled both ducts and acini, and in the submucosal glands of the trachea, where anti-B1 reactivity was found primarily in acini (A.R. Hand and W.D. Ball, unpublished work). More recent studies using specific anti-PSP antibodies detected PSP in only a few von Ebner’s gland acinar cells, indicating that SMGB is responsible for the ductal labelling [25]. We were unable to detect PSP or SMGB transcripts (lacrimal gland) or protein (von Ebner’s gland, tracheal glands) by Northern or Western blotting. This is probably due to low levels of gene expression and/or to a low proportion of expressing cells. A survey of stomach, intestine, pancreas, liver, kidney, thymus, muscle, brain, testis, adrenal, pituitary and thyroid disclosed no immunoreactivity for the B1-IPs, indicating that the immunocytochemical procedures provided relevant disclosure of protein antigens ([13]; A.R. Hand and W.D. Ball, unpublished work).

Molecular characterization of rat PSP and SMGB

We had recognized the likelihood that the rat leucine-rich protein was the homologue of mouse PSP, and that the rat SMG B1-IPs were also related. A mouse Psp cDNA (PS5; [26]) was used to isolate Psp clones from an adult rat parotid gland cDNA library. Full-length rat and mouse Psp mRNAs are 975 and 972 nucleotides respectively, and have 82% overall sequence similarity. The mouse Psp clone was also used to
isolate an Smgb cDNA, originally called ZZ3, from a neonatal SMG cDNA library [27]. Full-length Smgb transcripts are 869 nucleotides in length, and have two domains of similarity to rat and mouse Psp mRNA. The first domain corresponds to the 5' untranslated region and signal peptide-encoding portion of Smgb, and has 88% identity with rat Psp. The second domain, corresponding to the secreted portion of SMGB plus the 3' untranslated region, has overall 58% identity with rat Psp, with greatest similarity in the 3' untranslated regions. These two domains are separated by 102 bp that are present in Psp but not in Smgb. The Psp gene encodes only the 23.5 kDa protein product of the SMG, SLG and parotid gland that is reactive with the original anti-B1 IgG. The other B1-IPs, including SMGB1, SMGB2 and several smaller minor bands in the SMG, the 28.5 kDa protein of the parotid gland, and the 27 kDa and 18.5 kDa species of the SLG, are encoded by Smgb. All of these are derived from a single polypeptide chain by differential glycosylation and/or post-translational proteolysis [8].

The most highly conserved regions of the secreted forms of SMGB and PSP are around the two cysteine residues (SMGB amino acids 138 and 181) that contribute to the three-dimensional structure shared among members of the BPI (bactericidal/permeability-increasing protein)/LBP (lipopolysaccharide-binding protein)/PLUNC (palate, lung and nasal epithelial clone) gene family [28,29]. SMGB contains two potential N-glycosylation sites, at residues 96–98 (Asn-Ala-Ser) and 144–146 (Asn-Thr-Ser), and the SMGB proteins are sensitive to peptide N-glycosidase F [8,27]. Rat Psp has no N-glycosylation consensus sequences, and does not stain with periodate–Schiff [11], although mouse PSP is positive for this stain [4]. These findings suggest that SMGB and mouse PSP are glycoproteins, whereas rat PSP is not.

Expression patterns of SMGB and PSP during development
Preparation of specific probes for mRNAs of the rat Smgb and Psp genes and production of specific antisera to their encoded proteins has allowed comparison of their expression during development. This varies among glands and cell types, and we compared Smgb and Psp gene expression in the parotid gland, SMG and SLG at different postnatal ages. In the parotid gland, both PSP and SMGB are major secretory products of the immature acinar cells of the neonatal gland. PSP increases by 21 days to high adult levels, while SMGB expression declines and shifts from acini to ducts between 14 and about 25 days, and then remains at very low levels through adulthood. In the SMG, both genes are expressed strongly at birth, and later show similar patterns of decline to very low levels of PSP by 20 days and SMGB by 30 days postnatally. Low transcription of Smgb remains in the adult, but we cannot detect Psp expression in adult glands. In the SLG, both PSP and SMGB are products of the serous demilune cells, and their expression patterns are markedly different. At all ages, Psp is transcribed at very low levels, but Smgb transcription begins to increase before birth, and SMGB is a major secreted protein in the adult [24,30].

Genomic organization of Smgb and Psp genes
In the rat, the close conservation of PSP and SMGB signal peptides and 3' untranslated regions, as well as their gland-specific expression patterns, suggested that their genes arose by duplication, and that regulatory regions were conserved. In situ hybridization demonstrated that Psp and Smgb are tightly linked at 3Q41–3Q42 on rat chromosome 3 [31]. The complete coding sequences are present on a 52 kb P1 phage genomic clone, aligned in head-to-tail orientation, with Smgb approx. 21 kb downstream from Psp (Figure 1, upper). Both Psp and Smgb are encoded by nine exons, spanning 8.3 and 11.8 kb respectively.

Sequence analysis around the rat Psp and Smgb transcription start sites demonstrates close conservation of the 5' untranslated regions from exon 1, as well as a high degree of sequence similarity within the first 550 bp upstream [31]. Despite the close sequence conservation of the upstream nucleotides, experiments in transgenic mice from the Hjörth laboratory [32–34] and our unpublished data have demonstrated that the region from −1 kb to the transcription start site of either mouse Psp or rat Smgb did not direct transgene expression to any adult salivary gland. The Hjörth
laboratory has identified an SLG enhancer at 4.6 kb [32] and a parotid enhancer at approx. 8 kb [33,34] upstream of the mouse Psp transcription start site.

Presence of an Smgb gene in the mouse
The close sequence similarity and conserved expression patterns of rat and mouse Psp suggested that there might also be a mouse Smgb gene; however, we had been unable to detect any evidence of SMGB protein or RNA in any mouse salivary gland. Mouse Psp is located on chromosome 2, in a cluster of BPI/LBP/PLUNC family genes including Plunc and von Ebner’s minor salivary gland protein (www.ncbi.nlm.nih.gov; accession number NW_000179.1). At 29 kb downstream of Psp is an open reading frame encoding a BPI family member identified as ‘similar to Smgb’ (Figure 1, lower). The predicted protein has 42% identity/60% similarity with rat Smgb, making it less similar to rat Smgb than rat compared with mouse Psp (69%/81%) or rat compared with mouse PLUNC (76%/81%). This gene is likely to be mouse Smgb, because (1) rat and mouse Smgb are at corresponding positions in the BPI/LBP/PLUNC family gene clusters, (2) mouse Smgb is slightly more similar to rat Smgb than to any other member of this gene family in the rat, and (3) there is extensive sequence similarity between the rat Smgb upstream region plus exon 1 (accession number AF153355) and a ≈600 bp region upstream of mouse Smgb.

Mouse Smgb has the same intron/exon structure as rat Smgb, with one major difference. Rat Smgb exons 1 and 2 are separated by 3.2 kb, whereas in mouse Smgb, exons 1 and 2 are 11 kb apart. Within mouse Smgb intron 1 is the complete open reading frame of a protein that appears to be a retrovirus nucleocapsid protein, p24. Apparently, insertion of the retrovirus gene has separated exons 2–9 of mouse Smgb from exon 1 and its regulatory sequences, rendering it silent.

Future comparisons of Smgb and Psp regulation
The tightly linked genomic organization of the Psp and Smgb genes (Figure 1), coupled with their expression patterns, presents interesting examples of developmental divergence that are useful for investigating salivary cytodifferentiation. Three are given here. (1) In parotid gland acinar cells, expression of Smgb mRNA is markedly down-regulated over 14–25 days post partum, when Psp transcription is elevated to high adult levels [9,30]. (2) In serous demilune cells of the SLG, Smgb transcription increases rapidly before birth, and postnatally SMGB becomes a major adult secretory protein. Psp expression is very low at all stages of development [24,30]. (3) In the serous demilune cells of the SLG, the large developmental increase in Smgb transcription is in striking contrast to the decline in expression seen in the pro-acinar cells of the neonatal SMG as these become mature acinar cells [10,30]. This is particularly interesting given the similarity and close physical relationship between the sublingual and submandibular epithelial rudiments as they emerge from the oral epithelium only a few cells apart during embryogenesis [35]. It suggests a possible close relationship between the controlling elements of Smgb/Psp expression and the determination of specific cellular phenotype. Characterization of genomic regulatory elements in the Psp/Smgb complex should help to identify factors through which salivary gland differentiative pathways are controlled.

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References

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