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Allele-specific expression of the PSP gene in the mouse sublingual glands

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Summary

There are two known alleles of the mouse parotid secretory protein (PSP) gene: *Psp*^{a} and *Psp*^{b}. *Psp*^{a} is carried by DBA/2J mice and *Psp*^{b} is carried by C57BL/6J mice. Eighty-eight mice derived from a F_1(C57BL/6J × DBA/2J) to DBA/2J backcross were analysed for PSP mRNA expression in the sublingual glands. Expression was found in heterozygous mice only. This indicates that only *Psp*^{b} is expressed in this tissue. Furthermore, it maps the allele-specific sublingual gland determinant within 3·4 cM of *Psp*. Previous analysis of *Psp*^{b} identified an enhancer-like region in position −4·6 to −3·1 kb that was necessary for transgene expression in the sublingual glands. Here it is shown that the corresponding region in *Psp*^{a} enhances transgene expression in the sublingual glands as efficiently. The implications for regulation of PSP mRNA expression in the sublingual glands are discussed.

1. Introduction

The mouse has both major and minor salivary glands. The three major ones are the paired parotid, submandibular and sublingual glands. Only a few genes have been analysed by transgenesis for expression in the salivary glands, including the human salivary amylase 1C gene (Ting *et al*., 1992), the rat proline-rich protein gene *RP15* (Tu *et al*., 1992), the mouse proline-rich protein C1 and A1 genes (Zhou *et al*., 1997) and the mouse parotid secretory protein (PSP) gene (Mikkelsen *et al*., 1992; Larsen *et al*., 1994; Laursen & Hjorth, 1997), and selective gene activation in this tissue is not well understood (reviewed by Samuelson, 1996).

The PSP gene is expressed selectively in the salivary glands (Poulsen *et al*., 1986; Shaw & Schibler, 1986): at a high level in the parotid glands (Hjorth, 1979; Owerbach & Hjorth, 1980; Madsen & Hjorth, 1985) and at a lower level in the sublingual glands (Mikkelsen *et al*., 1992). Therefore, the PSP gene is useful for analysing both general salivary gland expression and specific expression in the various glands.

Two alleles of the PSP gene, *Psp*^{a} and *Psp*^{b}, have been described by analysis of inbred mouse strains (Owerbach & Hjorth, 1980). *Psp*^{a} is carried by DBA/2J, A/J and NZB mice whereas *Psp*^{b} is carried by C57BL/6J, CBA/J, C3H/Tif and BALB/cJ mice.

Here it is indicated that only those strains that carry *Psp*^{b} express PSP mRNA in the sublingual glands. Furthermore, analysis of N2 mice from a C57BL/6J to DBA/2J backcross substantiated that PSP mRNA expression in the sublingual glands is specific for the b-allele and that the determinant maps very close to and possibly within the PSP locus. Nevertheless, analysis in transgenic mice indicated that this difference in the expression of *Psp*^{a} and *Psp*^{b} maps outside the only identified sublingual regulatory region in the PSP gene.

2. Materials and methods

(i) *Animals*

All mice from inbred strains and F_1(C57BL/6J × DBA/2J) mice were purchased from Bomholtgaard Breeding & Research Centre A/S.

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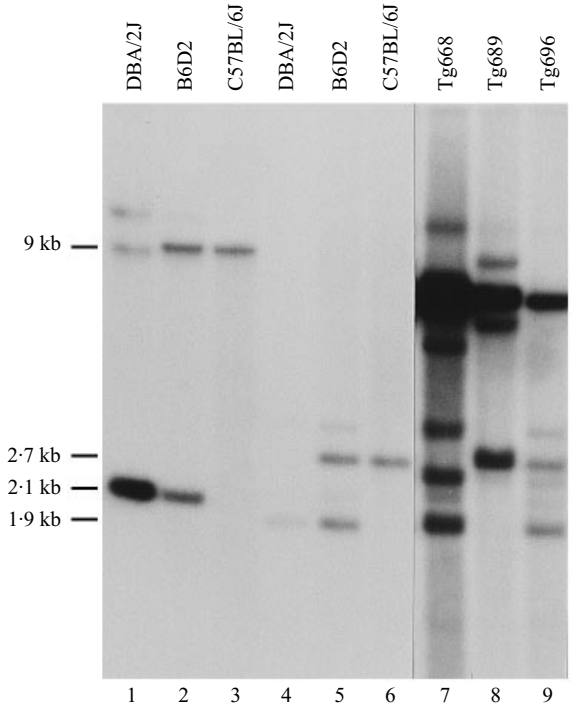

Fig. 1. Identification of *Psp*^{a}, *Psp*^{b} and transgenic mice by Southern blotting analysis. B6D2: F_1(C57BL/6J × DBA/2J). Eight micrograms of genomic DNA digested with *Bam*HI (lanes 1–3) or *Eco*RI (lanes 4–9) as prescribed by the manufacturer (Amersham) was applied to each lane. The probe was a ^{32}P-dATP random labelled 0·8 kb genomic fragment spanning the proximal promoter region in the PSP gene. This probe is illustrated in Fig. 3 as a black bar.

(ii) *Transgene construct*

DBA/2J genomic DNA was partially digested with *Sau*3A and ligated into the *Bam*HI restricted arms of λ47·1 (Loenen & Brammar, 1980). Positive clones were identified using as probe the same promoter fragment used for the Southern analysis shown in Fig. 1. A 6·0 kB *Hin*dIII fragment was subsequently subcloned into pBluescript SK. A 3·9 kb *Eco*RI fragment derived from this clone was ligated into an *Eco*RI opened Lama vector (Mikkelsen *et al*., 1992). Lama was built from *Psp*^{b} sequences derived from the congenic C3H.AMY^{YBR} strain (Nielsen, 1982). DBA3·9/Lama2·5 was purified for microinjection as a 6·4 kb DNA fragment by *Xho*I restriction followed by agarose gel electrophoresis and gene-clean procedures as described (Larsen *et al*., 1994).

(iii) *Transgenic mice*

Transgenic founder mice carrying DBA3·9/Lama2·5 were produced by pronuclear microinjection in F_1(C57BL/6J × DBA/2J) × F_1(C57BL/6J × DBA/2J) 1-cell embryos as described (Hogan *et al*., 1986).

(iv) *Southern blotting*

Genomic DNA was purified from tails as described (Hogan *et al*., 1986) and digested with the relevant restriction enzyme. Following size-separation by electrophoresis in a 1% agarose gel, the DNA was blotted onto a nitrocellulose membrane by capillarity. The final stringency in the washing procedures was 0·1% 20 × SET (3 M-NaCl, 0·02 M-EDTA and 0·4 M-Tris[hydroxymethyl]aminomethane) (pH 7·8), 0·1% (w/v) SDS and 0·1% (w/v) $Na_4P_2O_7.H_2O$. All manipulations were as described (Sambrook *et al*., 1989).

(v) *Northern blotting*

Total RNA was purified by a small-scale version of the single-step method of guanidinethiocyanate–phenol–chloroform extraction (Chomczynski & Sacchi, 1987) or by use of the RNA Isolator kit (Genosys) and size-separated by electrophoresis in a 2·2 M formaldehyde, 1·4% agarose gel in a MOPS (3-(*N*-morpholine)propane-sulphonic acid) running buffer and blotted onto a nylon membrane by capillarity as described (Sambrook *et al*., 1989). Hybridizations with random labelled probes were performed as described (Church & Gilbert, 1984) except that bovine serum albumin (BSA) was omitted. The final stringency in the washing procedures was 1 mM-EDTA (pH 8·5), 20 mM-Na_2HPO_4 (pH 7·2), 1% SDS (w/v) at 68 °C.

3. Results

(i) *Psp*^{b} *but not Psp*^{a} *is expressed in the sublingual glands*

C57BL/6J, CBA/J, C3H/Tif, BALB/cJ, DBA/2J, NZB and A/J mice were analysed for PSP mRNA expression in the sublingual glands. PSP mRNA was consistently and only detected in the sublingual glands of mice carrying *Psp*^{b} (Fig. 2*a*). This suggested that only *Psp*^{b} is expressed in this tissue. To investigate this phenomenon further we made a backcross between C57BL/6J and DBA/2J: F_1(C57BL/6J × DBA/2J) × DBA/2J. The offspring were analysed for genotype using the *Bam*HI-dependent restriction fragment length polymorphism shown in Fig. 1 lanes 1–3, where the a-allele is indicated by a 2·1 kb band and the b-allele by a 9 kb band. The exact nature of the less intense bands is presently unknown. Altogether 88 such N2 mice were analysed (summarized in Fig. 2*b*). All 44 mice homozygous for *Psp*^{a} lacked expression in the sublingual glands whereas all 44 mice that carried both *Psp*^{a} and *Psp*^{b} expressed PSP mRNA in this tissue. This confirmed that only *Psp*^{b} is expressed in

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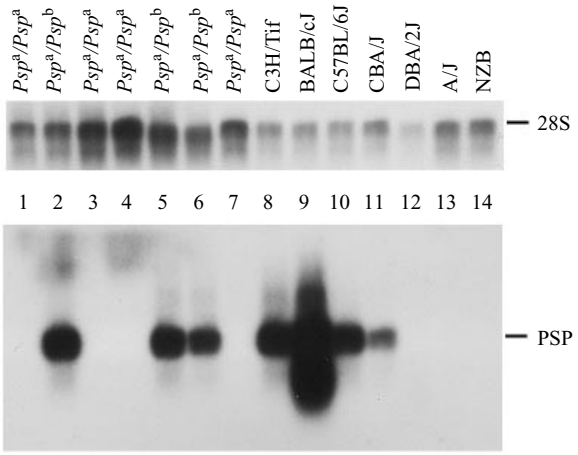
(*a*)

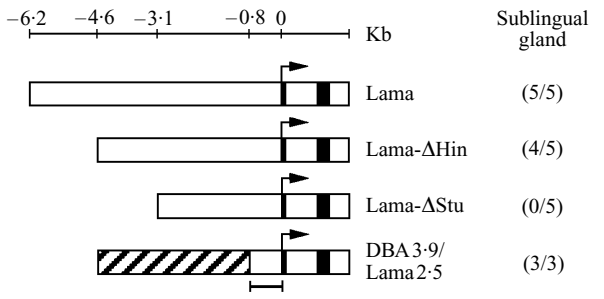


(*b*)

Genotype	Expression	Mice
Psp^a/*Psp*^{a}	+	0
	–	44
Psp^a/*Psp*^{b}	+	44
	–	0

Fig. 2. Allele-specific expression of PSP mRNA in the sublingual glands. (*a*) Northern blot analysis of total RNA from the sublingual glands of mice derived from a F_1(C57BL/6J × DBA/2J) to DBA/2J backcross (lanes 1–7) and from seven inbred strains (lanes 8–14). The RNA was probed with a ^{32}P-dATP random labelled 0·9 kb PSP cDNA fragment (Madsen & Hjorth, 1985). Upper panel: the filter was rehybridized with a probe that recognizes 28S rRNA (Maden *et al.*, 1987) to verify efficient transfer of the RNA. (*b*) summary of the results obtained from analysis of 88 mice from the F_1(C57BL/6J × DBA/2J) to DBA/2J backcross.

(*a*)



(*b*)

Transgenic line	Tg668	Tg689	Tg696
Sublingual gland	2023%	41%	43%

Fig. 3. Structure and expression of the DBA3·9/Lama2·5 construct. (*a*) Lama, Lama-ΔHin and Lama-ΔStu were analysed by Mikkelsen *et al.* (1992). Black boxes represent exons. The striped box represents *Psp*^{a}-derived sequences. The number of expressing lines versus the number of analysed lines is indicated in parentheses. DBA3·9/Lama2·5 was built from Lama and sequences derived from the DBA/2J genome (see Section 2). (*b*) The level of DBA3·9/Lama2·5 expression was calculated as an average percentage of that of the endogenous PSP gene in Tg689 and Tg696 after correction for the number of *Psp*^{b} alleles (i.e. 2 and 1, respectively – see Fig. 1, lanes 8 and 9). Expression levels were estimated by analysis of the Northern blot illustrated in Fig. 4*b*. Loading errors were corrected by use of the 28S rRNA signal. Quantifications were performed using a PhosphorImager apparatus (General Dynamics) and the ImageQuant software version 3·3.

the sublingual glands and furthermore indicates that the determinant for this allele-specific expression pattern maps within 3·4 cM from the *Psp* locus at the 95% confidence limit ((1–0·034)^{88} = 5%).

Interestingly, whereas the level of PSP mRNA in the sublingual glands is highly variable between inbred lines carrying *Psp*^{b} (compare lanes 8–11 in Fig. 2*a*) there was only a low level of variability between individual N2 backcross mice (e.g. compare lanes 2, 5 and 6 in Fig. 2*a*). This may suggest that the level of *Psp*^{b} expression is influenced by putative strain-specific differences within PSP gene regulatory regions or in a closely linked locus. Alternatively, the level of sublingual gland expression is influenced by an unlinked modifier locus and C57BL/6J and DBA/2J mice carry an identical or at least functionally equivalent allele at this putative locus.

(ii) *Both Psp^b and Psp^a carry a sublingual gland enhancer between −4·6 and −3·1 kb*

The analysis presented above of PSP mRNA expression in inbred strains and in (C57BL/6J × DBA/2J) × DBA/2J mice does not resolve whether the difference between *Psp*^{a} and *Psp*^{b} is acting in *trans* or *cis*. However, since the determinant maps very close to *Psp*, a *cis*-determination is a distinct possibility.

PSP gene regulation has been analysed by transgenesis (Mikkelsen *et al.*, 1992; Larsen *et al.*, 1994; Laursen & Hjorth, 1997). In the sublingual glands, expression of a *Psp*^{b}-derived minigene named Lama (Fig. 3*a*) was shown to depend on an enhancer-like region between −4·6 and −3·1 kb (Mikkelsen *et al.*, 1992). The Lama construct was expressed at 10–100% of the level of endogenous PSP mRNA in this tissue. Thus, the region between −4·6 and −3·1 kb seems to be a major sublingual gland regulatory region in the PSP gene. This region therefore appeared to be a likely candidate for harbouring the sublingual gland-specific difference between the two alleles.

To investigate this possibility we built a hybrid construct, DBA3·9/Lama2·5, carrying Lama-derived sequences downstream from −0·8 kb and *Psp*^{a}-derived sequences from position −4·6 to −0·8 kb (Fig. 3*a*). Transgenic founder mice carrying DBA3·9/Lama2·5 were crossed to F_1(C57BL/6J × DBA/2J) mice and transgenic offspring were identified by Southern blotting analysis (Fig. 1, lanes

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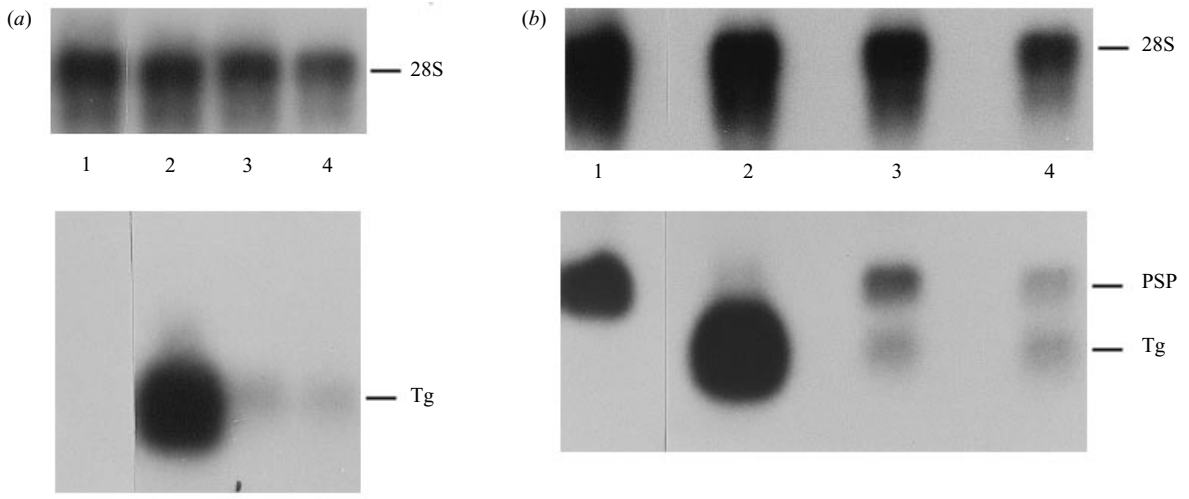

Fig. 4. The sequence between −4·6 and −0·8 kb in *Psp*[a] and *Psp*[b] is functionally equivalent. Northern blot of sublingual gland total RNA from transgenic mice carrying the DBA3·9/Lama2·5 construct. Three micrograms of RNA was loaded in each lane. Upper panel: the filter was rehybridized with a probe that recognizes 28S rRNA (Maden *et al.*, 1987) to verify efficient transfer of the RNA. (*a*) The probe was a ^{32}P-end-labelled transgene specific oligonucleotide (854). The oligonucleotide and the hybridization conditions have been described (Mikkelsen *et al.*, 1992). (*b*) The probe was a ^{32}P-dATP random labelled PSP cDNA fragment spanning 199 bp of exon h and i. This sequence is also present in DBA3·9/Lama2·5.

4–9). The 2·7 and 1·9 kb bands indicate the b- and a-allele of the PSP gene, respectively. F_1 transgenic mice from three independently derived lines were analysed for expression by Northern blotting using a transgene specific oligonucleotide as probe. The results indicate that DBA3·9/Lama2·5 was expressed in the sublingual glands of all three lines (Fig. 4*a*). To compare the level of transgene expression in the sublingual glands with that of the endogenous PSP gene, a PSP cDNA fragment was used as probe (Fig. 4*b*). This indicated that DBA3·9/Lama2·5 was expressed at between 40% and 2000% of the level of the endogenous PSP gene (Fig. 3*b*). This level of expression overlaps that of the previously analysed *Psp*[b] derived constructs (Mikkelsen *et al.*, 1992), demonstrating that the region between −4·6 and −0·8 kb in *Psp*[a] is equally functional to that in *Psp*[b] in the transgenic assay. This suggests that the sublingual gland function of the enhancer-like region between −4·6 and −3·1 kb is equivalent in *Psp*[a] and *Psp*[b]. It is unclear why DBA3·9/Lama2·5 is expressed at such high levels in line Tg668 but the high transgene copy number (see Fig. 1, lane 7) and/or transgene integration near positive-acting sequences are likely explanations.

4. Conclusions

In 88 mice carrying either *Psp*[a] alone or one copy each of *Psp*[a] and *Psp*[b], PSP mRNA was only detectable in the sublingual glands of those carrying *Psp*[b]. This indicates a specific lack of *Psp*[a] expression in this tissue and maps the determinant within 3·4 cM (95% confidence limit) of *Psp*. The only regulatory region identified in the PSP gene was cloned from *Psp*[a] and analysed in transgenic mice. In this assay, the *Psp*[a]-derived sequences appeared to enhance sublingual gland expression as efficiently as those derived from *Psp*[b]. It is therefore unlikely that the difference in sublingual gland expression is caused by differences between the two alleles in the −4·6 to −0·8 kb region.

In light of these results, two general models may explain the specific lack of expression of *Psp*[a] in the sublingual glands: (1) it could be caused by a *cis*-determinant within the PSP locus (i.e. either *Psp*[a] carries a negative-acting element outside the region between −4·6 and −0·8 kb or *Psp*[b] carries a positive-acting element that is present in DBA3·9/Lama2·5 outside −4·6 to −0·8 kb) or (2) it could be caused by a determinant at another locus located within 3·4 cM (95% confidence limit) of *Psp*. In this case a positive-acting allele is coupled to *Psp*[b].

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