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Tissue-specific expression in the salivary glands of transgenic mice

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ABSTRACT

Using a DNA construct, named Lama, derived from the murine parotid secretory protein (PSP) gene, we have obtained salivary gland specific gene expression in transgenic mice. Lama is a PSP minigene and allows analysis of the PSP gene 5′ regulatory region by transgenesis. We show here that the regulatory region included in Lama with 4.6 kb of 5′ flanking sequence is sufficient to direct expression specifically to the salivary glands. The expression level in the parotid gland is only about one percent of the PSP mRNA level, while that of the sublingual gland is near the PSP mRNA level. This suggests significant differences in the PSP gene regulation in the two glands. In addition, Lama is a secretory expression vector in which cDNAs or genomic fragments can be inserted. We demonstrate that the Lama construct can direct the expression of a heterologous cDNA encoding the C-terminal peptide of human factor VIII to salivary glands and that the corresponding peptide is secreted into saliva.

INTRODUCTION

Many uses have been found for transgenic animals produced by microinjection of cloned genes into the nuclei of fertilized eggs. Numerous genes have been introduced and expressed by this method. By using constructs containing specific regulatory regions, expression can be directed to a number of specific tissues (1–3).

Much attention is given to the use of transgenic animals as 'bioreactors' that express large amounts of, e.g., medically important proteins, which can be harvested and purified with ease and at low cost. A number of considerations, such as the ability of different tissues to modify proteins appropriately, and the expected ease of harvesting the expressed proteins, have led to the choice of the mammary gland as a suitable organ in this respect (4). Expression of transgenes controlled by mammary gland specific regulatory regions has led to production and secretion of medically important proteins such as alpha-1-antitrypsin and blood coagulation factor IX in the milk of mice, rabbits, and sheep (5–7).

The salivary glands constitute potential model systems for expression and secretion of heterologous proteins in transgenic animals. Saliva in relative large amounts can be collected easily from adult rodents following hormone stimulation (8, 9), with no restrictions as to sex. In addition, the genes expressed specifically in mammary glands seem to have regulatory properties in common with those expressed in salivary glands (see, e.g., 5, 10, and 11), and both the morphogenetic development and the protein secretory mechanisms of the two glands appear to be similar.

Several proteins are expressed in substantial amounts in the salivary glands (9, 12). The most abundantly expressed protein in the parotid gland of mice is the parotid secretory protein, PSP (13). Its mRNA too is very abundant in this gland (13, 14). The gene is expressed also in the sublingual gland, although at approximately 1/10 of the level found in the parotid gland. The gene coding for PSP has been cloned and characterized (14, 15), whereas the function of the protein remains unknown.

In the present work, we investigated the possibility of expressing proteins specifically in the salivary glands of the mouse by using a construct, Lama, consisting of flanking and structural sequences from the PSP gene. We show that Lama directs expression specifically to the salivary glands of transgenic mice, and that 4.6 kb of 5′ flanking DNA is sufficient for this expression. We demonstrate that the regulatory regions necessary for normal PSP expression in the parotid and the sublingual glands are not identical, as the sublingual expression of Lama mRNA approaches 100% of the PSP mRNA level, whereas the parotid gland expression does not exceed 1% . Furthermore, we show that Lama can be used to express the heterologous cDNA encoding the human blood coagulation factor VIII C-terminal peptide in transgenic mice and direct the corresponding peptide to the saliva.

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MATERIALS AND METHODS

DNA constructs

The Lama constructs were built by ordinary cloning techniques (16) using Lambda clones (15), cDNA clones (13), M13 mp18/mp19 and the plasmid pPoly III-I (17).

The reading frame trimmings adjacent to the unique cloning site were performed by using T4 DNA polymerase and adding either dGTP, dATP or dCTP in separate reactions, resulting in a specific termination at the first G, A and C, respectively. The protruding 5′ termini were removed with S1 nuclease. In this way three versions of the Lama construct were generated each matching a different reading frame

The cDNA fragment encoding the human FVIII light chain was obtained from a *Bgl* II / *Pvu* II digest of the plasmid pSVF8-80 (18). The cDNA fragment was made blunt ended by using Klenow enzyme and was cloned into the *Sma* I site of Lama.

The fragments used for microinjection were gel purified using the GeneClean kit (bio 101 la Jolla).

Animals and DNA microinjections

Pronuclei in eggs from F1(C57BL/6×DBA/2) females, fertilized by F1(C57BL/6×DBA/2) males, were microinjected with DNA (19) and manipulated embryos were subsequently implanted into pseudopregnant foster mothers. Transgenic lines, except from F250, were bred by crossing transgenic founders or heterozygous animals with inbred strains.

Southern blot analysis

Genomic DNA prepared from mouse tail (19) was digested with the appropriate enzymes. The resulting fragments were separated according to size by 1% agarose gel electrophoresis, blotted onto nitrocellulose filters, and hybridized with a DNA fragment labelled with ^{32}P-dATP by nick-translation (16). The post-hybridization wash was at 67°C in 0.1×SSC with 0.1% pyrophosphate and 0.1% SDS. All DNA manipulations were as described (16).

Northern blot analysis

Total RNA was prepared by a small-scale version of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (20). RNA was electrophoresed in 1.4% denaturing agarose gels containing 2.2 M formaldehyde and blotted onto nitrocellulose filters (16).

Hybridization with DNA fragments labelled with ^{32}P-dATP by nick-translation (16), and the post-hybridization wash were as described above for Southern blot analysis.

Hybridizations with end-labelled oligonucleotide probes were carried out in 6×SSC, 5× Denhardt's solution and 0,05% sodium pyrophosphate at 68°C for 16 hours. Before exposure, the filters were washed in 6×SSC at room temperature for 5 minutes followed by two washes, each for 30 minutes at 68°C. 30 pmol oligonucleotide was end-labelled by T4 polynucleotide kinase as described (16) and separated from unincorporated nucleotides by chromatography through a 10 ml Sephadex G-25 column.

Primer extension analysis

The primer extension analyses were made essentially as described in (16).

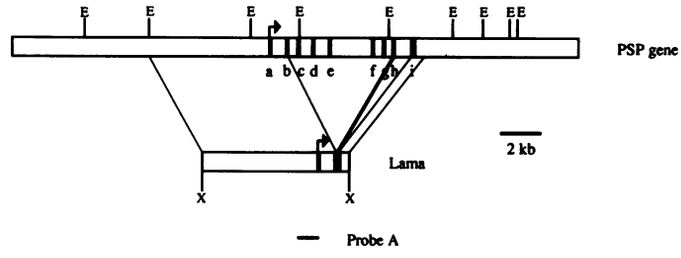

Figure 1. The Lama construct and its relationship to the PSP gene. Black boxes indicate exons (from **a** to **i** in the PSP gene), 'E' indicates *Eco* RI restriction sites, 'X' indicates *Xho* I sites, and the arrows indicates the start and direction of transcription (15). The Lama construct contains exon α, the first intron, part of exons β and h, exon i, and the 5′ and 3′ flanking sequences indicated, all derived from the PSP gene. The second *Eco* RI site from the left on the map of the PSP gene is included in the Lama construct. The location of probe A, used in the analyses of integrations, is indicated. The Lama construct consists of the following sequences: A large 5′ flanking genomic sequence (6.2 kb), exon a (untranslated), intron 1, 85 bp of intron β (the intron β sequences include 8 bp 5′ untranslated sequence, the signal peptide coding sequence (60 bp), and 17 bp coding for the N-terminal of the mature, processed PSP protein), and 14 bp derived from the M13mp18 polylinker used in the cloning steps. A *Sma* I site, derived from M13mp18, constitutes a unique cloning site, which can be used for cloning heterologous cDNAs into the expression cassette. The M13mp18 derived sequence is flanked, on the 3′ side, of 13 bp PSP protein coding sequence before the translation stop (derived from exon h), followed by 212 bp 3′ nontranslated sequence (remaining exon h and exon i; the 225 bp fragment was derived from the cDNA). Finally, 550 bp of the 3′ flanking genomic sequence were included in the construct. The final Lama construct, with a total length of 7.9 kb, was assembled in the vector pPolyIII-I (17).

ELISA test for FVIII

The mice were anaesthetized, then salivation was induced by injecting isoproterenol (5 ng per g body weight, i. p.). Saliva was collected over a 30 min. period and instantly frozen at −70°C . For ELISA measurements the saliva collections (around 0.05 ml) were diluted in 0.5 ml of 0.15 M Tris (pH 7.4), supplemented with (final concentrations): NaCl 0.15 M, Benzamidine 2 mM, Aprotinin 100 KIU/ml, NaN_3 0.02%, EDTA 5 mM, PMSF 2 mM, and N-Ethylmaleimid 5 mM. After centrifugation for 10 min. at 12,000×g the total amount of human FVIII antigen in the supernatants were determined by ELISA, utilizing two human haemophilic antibodies (21).

RESULTS AND DISCUSSION

The Lama construct

A PSP minigene, Lama, which can also be used as an expression vector for foreign coding sequences, was built from sequences derived from the PSP gene (Fig. 1; (15)). It contains a 6.2 kb 5′ flanking genomic region with the putative regulatory sequences, the first two exons a and b separated by the authentic 0.8 kb intron, a unique *Sma* I site followed by most of exon h and the whole of exon i of the PSP gene, and 0.5 kb of 3′ flanking genomic region. Exons a and b encode the 5′ untranslated mRNA region and the signal peptide of the PSP protein. The exon h and i sequences encode the 3′ region of the PSP mRNA, including the polyadenylation signal. This PSP minigene can be used for studies of the regulatory region of the PSP gene by analysing, e.g., expression of deletion constructs in transgenic mice. The mRNA expressed from the minigene will be discernible from the endogenous mRNA on the basis of size and the sequence

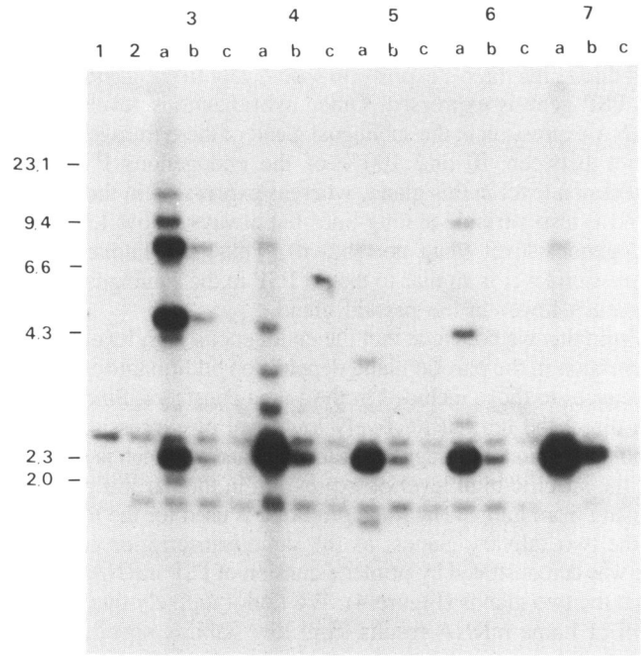

Figure 2. Southern blot analysis of the Lama integrations. 5 μg *Eco* RI digested genomic DNA was applied in each lane, gel-electrophoresed, blotted onto nitrocellulose filter, and hybridized with probe A (see Fig. 1). Lanes 1 and 2 contain DNA from a homozygous and heterozygous non-transgenic mouse, respectively, for comparison. Lanes 3–7 contain DNA from transgenic animals: Tg213 (3), F250 (4), Tg251 (5), Tg261 (6), and Tg262 (7). Lanes marked with an 'a' contain undiluted DNA from the transgenic animals indicated, whereas lanes marked 'b' and 'c' contain DNA from transgenic animals, diluted with DNA from non-transgenic animals (1:10 and 1:50, respectively). Comparison of hybridizing intensity of endogenous and transgene specific fragments allows a copy number estimation. Migration distances of DNA markers (*Hin* dIII cut lambda DNA; sizes in kb) are indicated at the left.

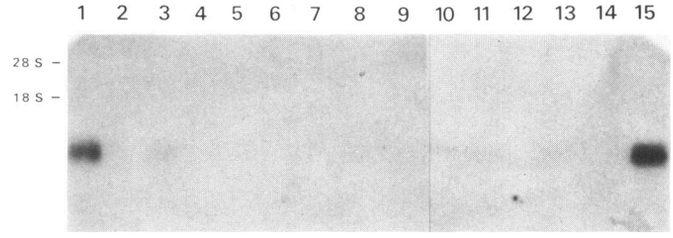

Figure 3. Expression analysis of the transgene integration in line Tg213. Total RNA from tissues of a Tg213 mouse was isolated, electrophoresed through denaturing gels, blotted onto nylonfilters, and hybridized with a specific oligonucleotide with the sequence 5'-GGGCAAGTTGTGATCCCCGGGTA-CCCC-3'. The first part of this sequence (eleven nucleotides) is identical to part of PSP exon h sequence, the next fourteen nucleotides cover part of the polylinker of cloning vector M13mp18, and the last two nucleotides are identical to part of PSP exon b. Thus, this probe specifically detects a messenger transcribed from the integrated transgenes, and does not hybridize to the endogenous PSP mRNA. RNA (amount in μg in brackets) was isolated from: lanes 1 and 15: sublingual gland [2], lane 2: submaxillary gland [20], lane 3: parotid gland [20], lane 4: pancreas [20], lane 5: liver [20], lane 6: uterus [20], lane 7: kidney [20], lane 8: lachrymal gland [10], lane 9: lung [10], lane 10: muscle [5], lane 11: lymph node [5], lane 12: spleen [10], lane 13: brain [10], and lane 14: heart [10]. The migration distances of 28S and 18S ribosomal markers are indicated at the left. The size of the hybridizing species has been estimated to be approximately 500 nucleotides.

around the cloning site. Heterologous cDNAs or genomic fragments can be inserted into the Lama construct in the proper reading frame. They are expected to be expressed in transgenic mice in the same tissues as Lama, and to result in hybrid proteins equipped with the secretory signal peptide derived from Lama. Thus, the protein specified by the heterologous DNA should be directed to saliva. A detailed description of the Lama construct is given in the legend to Fig. 1.

Transgenic mice carrying Lama

Our primary task was to investigate whether the PSP minigene Lama could be expressed in transgenic mice. Fertilized mouse eggs were microinjected with a 7.9 kb *Xho* I fragment consisting of the entire Lama construct (Fig. 1). By Southern blot analysis (data not shown) we identified five transgenic founders: F213, F250, F251, F261, and F262. Genomic DNA from F250 and F1 offspring from the other founders (denoted Tg) was digested with *Eco* RI and analyzed by Southern blotting to evaluate the integrity, organization, and copy number of the integrations (Fig. 2). The probe used spans the proximal 0.8 kb of the 5' flanking sequence (probe A in Fig. 1). The results indicate that the integrations contained intact copies, organized predominantly in head-to-tail tandem repeats. Thus, the strongly hybridizing *Eco* RI fragment of about 2.5 kb is a linker fragment between the 3' end of one copy and the 5' end of the following copy. Although the integration in F213 seems to have a more complex structure, it also displays the 2.5 kb hybridizing fragment (Fig. 2). Other hybridizing fragments presumably represent junctions between transgenic and chromosomal sequences. We estimate that each of the five integrations contain between 10 and 50 copies (see legend to Fig. 2 for details).

Lama is expressed specifically in the salivary glands of transgenic mice

The expression of Lama was assayed by isolating total cellular RNA from a number of tissues. RNA preparations were Northern blotted and hybridized with a 27 nucleotide probe spanning the junction sequences between PSP exon β, part of the M13mp18 polylinker, and PSP exon h. This sequence is unique for the predicted transcript from Lama (see legend to Fig. 3), and this probe does not hybridize to the mRNA from the endogenous PSP gene. From mice carrying each of the five integrations, RNA was isolated from the three major salivary glands (parotid, submaxillary, and sublingual glands) as well as from liver, spleen, and muscle. Furthermore, a number of additional tissues were analyzed from Tg213 and Tg251 mice.

A representative Northern blot is presented in Figure 3, showing the tissue specific expression of Lama in line Tg213. An approximately 500 nucleotide hybridizing RNA is seen in the parotid and sublingual glands, but not in the other tissues. This size is in agreement with the expected size of the Lama transcript (370 nt + poly A tail). Line Tg213 shows relatively high expression in the sublingual gland (lane 1+15). In contrast, relatively low expression is seen in the parotid gland (lane 3). Note that ten times more parotid than sublingual RNA was applied in these lanes. Analysis of the other transgenic lines demonstrated expression in the sublingual glands in all lines and revealed one additional line, Tg251, with expression in the parotid gland at a relatively low level. Lama expression was not found in liver, spleen, muscle and the submaxillary gland of any of the lines

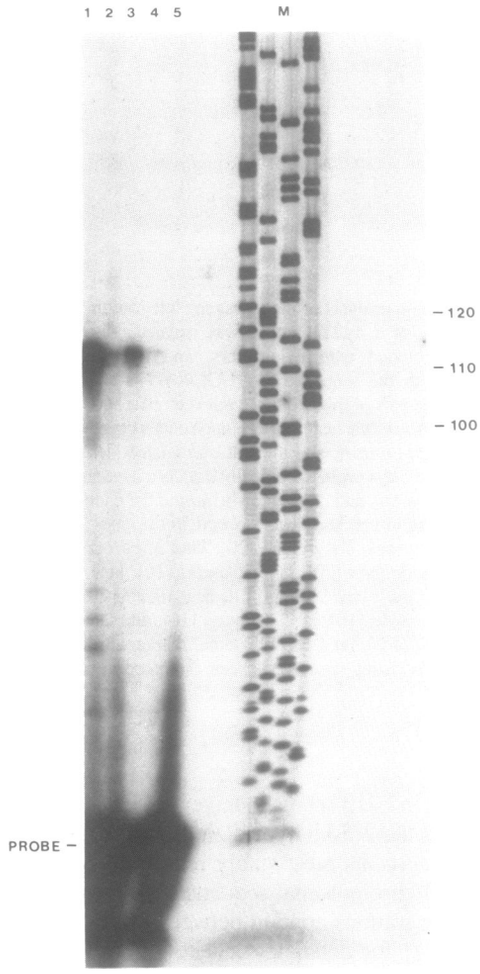

Figure 4. Primer extension analysis of the PSP mRNA present in the parotid gland and the sublingual gland. Lanes 1 and 3 contain extended products obtained when using total RNA isolated from the parotid gland, lanes 2 and 4 contain extended products obtained when using total RNA from the sublingual gland, and lane 5 is a negative control (total muscle RNA was used). **m** indicates the sequencing ladder used as marker. The primer used was complementary to positions 85 to 115 in the PSP cDNA sequence and had the sequence 5'- GACTCTG-AGTTCCCAATGAGCAGGCCACAC−3'.

(data not shown). There seems to be no correlation between the transgene copy number and the expression level. Overall, we find that Lama directs expression specifically to the glands where the PSP gene is expressed. Other hybridizations revealed that mRNA expression in the sublingual gland of these transgenic lines varies between 10 and 100% of the endogenous PSP gene expression level in this gland, whereas expression in the parotid gland is also variable among lines but always below 1% of the endogenous level (data not shown). Thus, the Lama mRNA expression level is similar to that of PSP in the sublingual gland, but much lower in the parotid gland.

From this we conclude that the endogenous high level of PSP expression in the parotid gland depends on additional regulatory sequences to those included in the Lama construct. Since Lama can direct the normal relatively low level of expression in the sublingual gland, this suggests that an enhancer, which is required for the specific high level of expression in the parotid gland, is missing from Lama. The same promoter is used for the PSP gene in the two salivary glands, as the same transcription initiation site was demonstrated by primer extension of PSP mRNA isolated from the two glands (Figure 4). We find it unlikely that the low level of Lama mRNA results from low stability specifically in the parotid gland, although we have not excluded the possibility. The differences in expression levels among the five integrations are interpreted as position effects imposed by the genomic DNA surrounding each integration. However, the Lama expression in the two glands appears to respond differently, i. e., the levels do not vary in parallel.

Deletion analysis of the Lama construct

To define 5' flanking regions responsible for salivary gland specific gene expression, transgenic mice were produced carrying Lama deletion constructs in which progressively larger fragments were deleted from the 5' end, using restriction sites present in this region. Integrations were analyzed as previously described (Southern blots; data not shown), and this led us to the conclusion that all the lines had intact copies of the transgene integrated. Northern blot analyses using the Lama-specific oligonucleotide as probe provided us with expression data (data not shown). All transgenic lines were analysed for expression in the parotid gland and in the sublingual gland. In addition, RNA from submaxillary gland, liver, spleen, and muscle was analyzed for most lines.

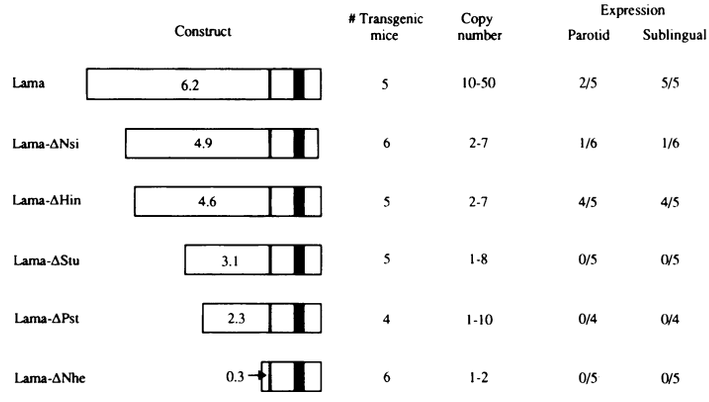

Figure 5. Deletion analysis of the Lama construct. Summary of the results from analyses of transgenic mice carrying 5' deletion constructs. For each construct, the following are indicated: a schematic drawing indicating (in kb) the extent of the 5' region present, the number of transgenic lines analyzed, the estimated number of transgene copies present in the lines, and presence/absence of expression of the construct in the sublingual gland and the parotid gland in the lines obtained. Black boxes in schematic drawings indicate exon sequences (see also Fig. 1).

No expression was ever observed in any tissues other than the parotid gland and the sublingual gland (data not shown). The results of these analyses are summarized in Figure 5, which shows the extent of the 5′ region included in each construct, the number of transgenic animals made with each construct, the number of integrated transgene copies, and the expression seen in these lines.

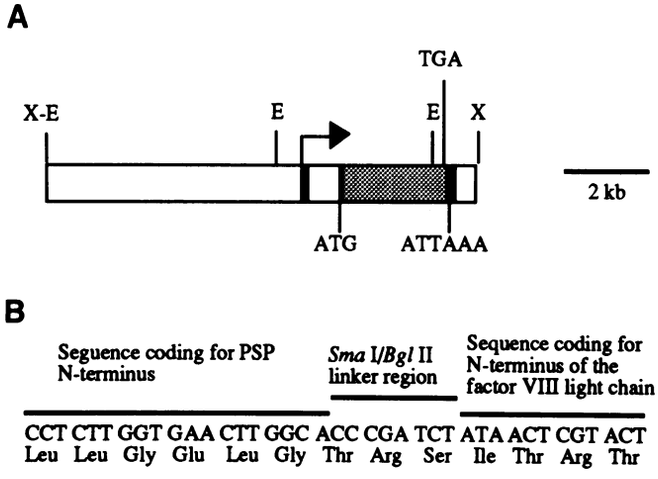

Figure 6. The Lama/FVIII-C hybrid gene. (A) Non-transcribed sequences and intron 1 derived from the PSP gene are represented as open boxes, exons derived from the PSP gene are shown as black boxes and the inserted *Bgl* II /*Pvu* II fragment of the FVIII cDNA (18) is shown as a dotted box. Recognition sites for restriction enzymes *Eco* RI (E), *Xho* I (X), the PSP gene transcription initiation site (arrow), and the stop codon (TGA) derived from the FVIII cDNA are indicated above the map. The PSP derived translation initiation codon (ATG) and polyadenylation signal (ATTAAA; unpublished data) are shown below the map. (B) Structure of the 5′ linker region between Lama and the inserted cDNA fragment. The predicted structure of the amino terminus of the secreted polypeptide is shown below the nucleotide sequence, and the origin of the sequences are indicated above.

Using constructs containing 5′ flanking regions from the transcription start site up to positions −0.3 kb, −2.3 kb or −3.1 kb, we observed no expression in any of the transgenic lines (4−6 lines analyzed for each construct, see Fig. 5). The construct having 4.6 kb 5′ sequence, Lama-ΔHin, was expressed in 4 out of 5 lines in both the parotid and the sublingual glands. Expression in the sublingual gland was always higher than that in the parotid gland, as described above for the Lama construct with the 6.2 kb 5′ region (data not shown). The fact that one line did not express this construct is presumably a result of position effects. Lama-ΔNsi, which has 4.9 kb 5′ flanking sequence, was expressed in only one out of six transgenic lines. The single expressing line, Tg596, was unique in the sense that expression in the parotid gland was much higher than that observed in any other line (data not shown). Therefore, we regard absence of expression as the typical observation for this construct and the aberrant line Tg596 to have the transgene inserted near a chromosomal enhancer. Due to the presence of a *Nsi* I site in the 3′ flanking region of Lama, the Lama-ΔNsi construct is lacking approximately 150 bp in the 3′ end of Lama.. Therefore at present, we are not able to determine whether the general lack of expression of this construct is caused by a negative acting element in the 5′ flanking region (between −4.6 and −4.9 kb), or by the deletion of a positive element in the 3′ flanking region. This deletion analysis indicates that sequences present in the 5′ region up to −4.6 kb are sufficient for tissue specific expression of the PSP gene.

Mice carrying a Lama/FVIII C-terminus hybrid gene

To investigate the potential use of Lama to express and direct peptides to the saliva, we inserted into the *Sma* I cloning site of the Lama construct an end-filled *Bgl* II /*Pvu* II fragment of the cDNA encoding the C-terminal light chain of the human blood coagulation factor VIII, FVIII (18). The resulting construct is shown in Figure 6. A two base pair deletion immediately 5′ to the *Sma* I site ensured the correct reading frame. In this construct

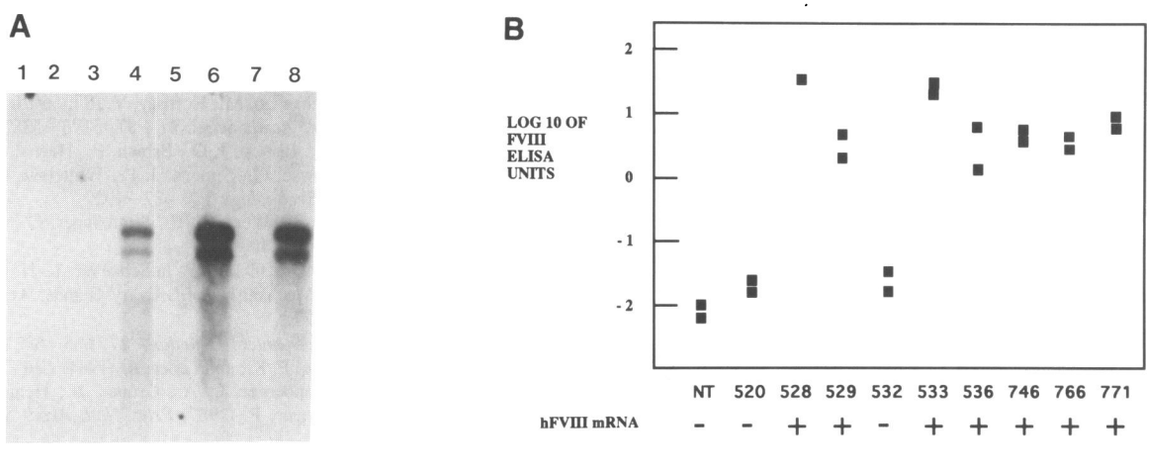

Figure 7. Expression from the Lama/FVIII-C construct. (A) Total RNA from the parotid and the sublingual glands was analyzed by Northern blotting using the nick-translated cDNA fragment described in Fig. 6A, legend. Lane 1−4 contain RNA from the parotid gland, lane 5−8 contain RNA from the sublingual gland. The numbers indicated in brackets refer to the amount of RNA (μg) applied in each lane. Lane 1: line Tg520 [20], lane 2: line Tg529 [20], lane 3: line Tg532 [20], lane 4: line Tg533 [20], lane 5: line Tg520 [6], lane 6: line Tg529 [5], lane 7: line Tg532 [5], and lane 8: line Tg 533 [4]. (B) Amount of human FVIII antigen in samples of induced saliva, collected over a 30 min. period from individual mice. Each point represents an individual ELISA measurement. The transgenic lines are indicated by their numbers; **NT** is a non-transgenic litter mate; + indicates the presence of FVIII mRNA in the salivary glands of the line based on Northern blot analyses. One unit equals the ELISA measurement of FVIII antigen present in 1 ml of normal human plasma.

the polyadenylation signal is derived from Lama. The predicted polypeptide product from the construct differs from the authentic FVIII light chain in the amino terminus by the addition of 9 amino acids (see Fig. 6)

The construct was excised as a 10.1 kb *Xho* I fragment and microinjected into fertilized mouse eggs. Transgenic mice were identified by Southern blot analyses of genomic tail DNA using a nick-translated FVIII cDNA fragment as probe (see legend to Fig. 6) and 9 lines were established (data not shown). Southern blot analyses confirmed the presence of intact transgenes in the lines (data not shown).

Expression from the Lama/FVIII-C construct

To analyse the expression in the lines carrying the Lama/FVIII-C construct, total RNA was isolated from the three major salivary glands, as well as from liver, spleen and muscle and analysed by Northern blotting. The blots where hybridized with the FVIII probe used for the Southern blot (see legend to Fig. 6). Only RNA from the parotid gland and the sublingual gland showed hybridization (data not shown). Figure 7A shows a Northern analysis of RNA purified from four of these transgenic lines, two of which express the transgene. Among the 9 transgenic lines, transcription of the transgene was detected in 7 lines, in both the sublingual gland and the parotid gland. The relative expression levels resemble those obtained when using the PSP minigene, Lama, and deletions from it, i.e., relatively high expression in the sublingual gland and low in the parotid gland. Interestingly, we detect two different hybridizing RNA species. The larger of these, about 2900 nucleotides, has the predicted size of an mRNA transcribed from the Lama/FVIII-C construct (expected 2635 nucleotides + poly A tail). The origin of the smaller RNA, which has a size of approximately 2500 nucleotides, is as yet unknown. These results demonstrate that Lama is able to drive the transcription of a heterologous cDNA in a tissue specific manner.

To analyse whether the FVIII light chain was produced and secreted into the saliva of the transgenic animals, isoproterenol provoked saliva was collected (9) and analysed for the presence of FVIII antigen by ELISA. A considerable amount of FVIII antigen was found in the saliva from transgenic lines transcribing the construct (Fig 7B). The expressing lines secrete an amount of FVIII light chain per salivation (about 0.05 ml of saliva) of about 10 units, which corresponds to the amount of FVIII in 10 ml of normal human plasma (22). In contrast, the non-transgenic litter mates have FVIII antigen at levels that are two orders of magnitude lower. This demonstrates that the Lama expression construct is able to direct a significant amount of the encoded peptide to the saliva.

CONCLUDING REMARKS

Here we report that a murine PSP minigene can be expressed in a correct tissue specific manner in the salivary glands of transgenic mice. A construct based on the PSP gene, Lama, containing 4.6 kb of 5′ flanking genomic sequence, exon α, intron 1, parts of exons β and h, exon i and 0.5 kb 3′ flanking genomic sequence is specifically expressed in two of the three major salivary glands, the sublingual and the parotid gland. Thus, the Lama construct is expressed in the same tissues as the endogenous PSP gene. The expression level of Lama mRNA in the sublingual gland is similar to the endogenous PSP mRNA level, whereas the level of Lama expression is much lower than the PSP expression in the parotid gland. This indicates the existence of a parotid specific enhancer for the PSP gene which is not included in the Lama construct, and we are currently searching for this putative enhancer.

The mouse salivary amylase gene is also expressed at a high level in the parotid gland. When this gene, including 5 kb 5′ and 10 kb 3′ flanking regions was established in 2 different lines of transgenic mice, no expression was detected in the parotid glands (23). This suggests that sequences located at a considerable distance from the gene are required for parotid specific expression, which agrees with our findings for the PSP gene. Recently, the same group has demonstrated expression in salivary glands of transgenic mice with a 1 kb 5′ flanking sequence from the human salivary amylase gene *AMY1C* (M. Meisler, personal communication).

Salivary gland expression of the Lama/FVIII-C hybrid gene and the demonstrated secretion of the encoded peptide into saliva opens new opportunities. It promotes the salivary glands as useful experimental model systems for investigations such as testing and optimizing transgene constructs for secretion of proteins, analysis of secondary modifications of heterologous synthesized proteins expressed in salivary glands, and finally as a unique possibility to study the effects of altered composition of saliva on for instance digestion and uptake of foodstuffs in transgenic animals.

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