Biosynthesis of Salivary Proteins in the Parotid Gland of the Subhuman Primate, Macaca fascicularis

CELL-FREE TRANSLATION OF THE mRNA FOR A PROLINE-RICH GLYCOPROTEIN AND PARTIAL AMINO ACID SEQUENCE AND PROCESSING OF ITS SIGNAL PEPTIDE*

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The major anionic proline-rich proteins in the parotid and submandibular secretions of subhuman primates and man perform the important biological function of inhibiting crystal growth of calcium phosphate salts from saliva, which is supersaturated with calcium phosphate salts, thereby preventing excess deposition of hydroxylapatite on tooth surfaces. The present work was initiated as a first step towards investigating proline-rich protein biosynthesis in parotid glands using the subhuman primate, Macaca fascicularis, as a model system. RNA was isolated from macaque parotid glands and separated into poly(A)-enriched and poly(A)-deficient fractions by chromatography on oligo(dT)-cellulose. The mRNAs in both fractions promoted incorporation of radiolabeled amino acids into polypeptides in an mRNA-dependent reticulocyte lysate translation system. Five major proline-rich polypeptides were detected and one of these was shown to be the in vitro precursor of the major anionic macaque proline-rich protein (MPRP), which is the structural and functional counterpart of the major anionic proline-rich proteins in the parotid and submandibular secretions of man (Oppenheim, F. G., Offner, G. D., and Troxler, R. F. (1982) J. Biol. Chem. 257, 9271–9282). Radiosequencing of the material in anti-MPRP immune precipitates showed that the in vitro precursor of MPRP contained an 18-residue signal peptide. The in vitro precursor of MPRP was processed in dog pancreas vesicles to a form with a lower apparent Mr, and with an NH₂-terminal amino acid sequence identical to that of native MPRP. The phenylthiohydantoin derivatives of Ala and Ile were detected at residue 9 and those of Val and Met were detected at residue 16 of the signal peptide. This indicated that the in vitro precursor of MPRP, which migrated electrophoretically as a single band in anti-MPRP immune precipitates, contained two different in vitro polypeptides derived from two different mRNAs. These results are discussed in the context of the genetic polymorphism among the major anionic proline-rich proteins in the parotid and submandibular secretions of man.

The major anionic proline-rich proteins constitute up to 30% of the protein in the parotid and submandibular secretions of man (1). The PRPs(*) comprise a family of homologous proteins that displays a genetic polymorphism with three phenotypes characterized by the presence of the protein pair, PRP I and III, the protein pair PRP II and IV, or all four PRPs (2). These proteins, together with the tyrosine-rich phosphopeptide, statherin (3), inhibit spontaneous precipitation of calcium phosphate salts and crystal growth of calcium phosphate salts in vitro (4). Since saliva is supersaturated with calcium phosphate salts, the biological function of the major anionic PRPs appears to be to maintain saliva supersaturated with respect to calcium phosphate and to prevent excess deposition of calcium phosphate on tooth surfaces (4, 5).

Little is known about the molecular events occurring during the biosynthesis of PRPs. Meunzer et al. (6, 7) reported that chronic treatment of rats with the β-agonist, isoproterenol, resulted in hypertrophy of the parotid glands and concomitantly, expression of the genes for six basic proline-rich proteins. The six basic proline-rich proteins were the primary translation products templated by poly(A⁺) mRNA from the parotid glands of isoproterenol-treated animals, whereas these proteins were essentially undetectable in translation reactions templated by poly(A⁺) mRNA from untreated animals (8). While the isoproterenol-treated rat parotid gland is an interesting system for investigating the effects of catecholamines on gene expression, the biological function of basic proline-rich proteins in the oral cavity of rats and man is not known.

We described the cell-free translation of the mRNAs for precursors of PRPs from a human submandibular gland and identified these precursors on the basis of cross-reactivity with immune serum specific for PRPs and preferential incorporation of radiolabeled proline (9). The disadvantages of using human parotid or submandibular glands reside in the limited availability of glandular tissue and the complexity of the system due to the genetic polymorphism among the PRPs (2).

We have isolated a proline-rich glycoprotein (MPRP) which is a major component in the parotid and submandibular secretion of the subhuman primate, Macaca fascicularis (10). The chromatographic and electrophoretic properties and amino acid composition of MPRP are very similar to those of

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(*) The abbreviations used are: PRP, proline-rich protein; MPRP, macaque proline-rich protein; Quadrol, N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine, HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; PTH, phenylthiohydantoin.

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the PRPs. MPRP and the PRPs display a 68% homology within the NH₂-terminal 66 residues (10, 11). Kouvelari et al. (12, 13) have shown immunohistochemically at the light and electron microscope level the various acinar cells of both human and macaque parotid and submandibular glands, lending support to the site of proline-rich protein biosynthesis. MPRP and the PRPs have comparable activities in the crystal growth inhibition assay (10), leaving little doubt that MPRP is the macaque counterpart of the major anionic PRPs of man. Further, *M. fascicularis* is known to develop a number of human diseases under experimental conditions including periodontitis and caries (14).

In the present investigation, isolation of translatable mRNA from the macaque parotid gland and characterization of the in vitro precursor of MPRP are described.

**EXPERIMENTAL PROCEDURES**

**Materials**—Parotid glands were surgically removed from adult animals within 1 h of sacrifice, frozen in liquid nitrogen, and stored at −80 °C until used.

**RNA Isolation**—Frozen tissue (approximately 8 g) was broken into small pieces with a pestle and mortar under liquid nitrogen and transferred to 20 ml of extraction buffer consisting of 0.02 M Tris-HCl, pH 8.0, 0.075 M NaCl, 0.025 M EDTA and 0.5% sodium dodecyl sulfate. Subsequently, 10 ml of phenol saturated with extraction buffer was added and the tissue was homogenized in a Polytron (Brinkmann Instruments) at constant temperature. After the addition of a further 30 ml of extraction buffer and 40 ml of phenol saturated with extraction buffer, the homogenate was kept on ice for 30 min, and centrifuged at 10,000 × g for 10 min. The aqueous phase was extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, v/v) and twice with chloroform:isoamyl alcohol (24:1, v/v). Nucleic acids in the resulting aqueous phase were recovered by ethanol precipitation overnight at −20 °C. The RNA was further purified by repeated precipitation in the presence of 3 M sodium acetate, pH 6.0, to remove DNA and low M₆ RNAs (15). The resulting RNA was ethanol-precipitated, twice extracted with 2 M lithium chloride in 50 mM sodium acetate, pH 6.0, and again ethanol-precipitated. The purified RNA was chromatographed on oligo(dT)-cellulose (Type II; Collaborative Research, Waltham, MA) as described by Aviv and Leder (16). The poly(A⁺)-enriched and poly(A⁻)-deficient fractions (subsequently referred to as poly(A⁺) and poly(A⁻) mRNAs, respectively) were ethanol precipitated and stored at −80 °C. The poly(A⁺) fraction was used for determining agarose gels (17) and shown to contain undegraded 18 and 28 S ribosomal RNAs.

In one experiment, the poly(A⁺) fraction was chromatographed on poly(U) agarose (P-L Biochemicals). The poly(A⁺) fraction was applied to the column in binding buffer consisting of 50 mM Tris-HCl, pH 7.5, 0.7 M NaCl, 10 mM EDTA, 0.5% sodium dodecyl sulfate, and 25% formamide (v/v). The poly(A⁺) fraction (not retained on the column in binding buffer) was recovered by the addition of 2 volumes of ethanol and stored at −20 °C.

**Cell-free Translation**—Reticulocyte lysates were prepared from rabbits made anemic with acetylphenylhydrazine and lysates were stored under liquid nitrogen. Prior to use, lysates were made mRNA-dependent by limited digestion with micrococlal nuclease (Boehringer Mannheim) as described by Peham and Jackson (18). The standard 20-μl translation reaction contained: 10 μl of nuclease-treated lysate, 12 mM HEPES buffer, pH 7.6, 8 μM hboin, 20 μg/ml of creatine phosphokinase, 5 mM creatine phosphate, 110 mM potassium acetate, 0.25 mM magnesium chloride, 120 μM unlabeled amino acids minus the radiolabeled amino acid used, 1-10 μg of RNA, and 1 μCi/μl of L-[^35]S]methionine (Amersham Corp. 1200-1500 Ci/mmol) or one of the following titrated amino acids: L-[^3]H]leucine, L-[^3]H]phenylalanine, L-[^3]H]tyrosine, L-[^3]H]histidine, or L-[^3]H]proline (Amersham Corp. 130 Ci/mmol).

Translation reactions were incubated for 35 min at 30 °C and incorporation of radiolabeled amino acids into high trichloroacetic acid-insoluble material was determined (19).

For analysis, 15 volumes of ice-cold acetone containing 2% concentrated HCl (v/v) were added to 1 volume of translation reaction and the resulting precipitate was washed with ice-cold acetone. The washed precipitates were taken up in 62.5 mM Tris-HCl, pH 6.8, containing 10% glycerol (v/v), 2% sodium dodecyl sulfate, and 5% β-mercaptoethanol (sample buffer), heated at 100 °C for 2 min, and examined electrophoretically.

**Gel Electrophoresis**—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to Laemmli (19) on 12.5% polyacrylamide gels in which the ratio of acrylamide to N,N'-methylenebisacrylamide was 1:18.7. Fluorography was performed by exposing the dried gels to Kodak XAR-5 film at −80 °C.

**Immunoprecipitation**—Preparation and specificity of rabbit antisera directed against MPRP and PRP I have been described previously (20). Poly(A⁺) mRNA from the macaque parotid gland was translated in vitro with rabbit reticulocyte lysates and the translation products were isolated by immunoprecipitation using program 121078 with 0.25 M Quadrol and a combined S₁ and S₂ wash. The background of each immunoprecipitation reaction was determined by using a wash step (no coupling reagent) followed by double coupling of Phenylthiohydantoin norleucine was added to each fraction collector tube to serve as an internal standard.

The butyl chloride in fraction collector tubes was evaporated to dryness under nitrogen. The residues were dissolved in 1.0 ml of butyl chloride and 0.5 ml was assayed for radioactivity in a liquid scintillation spectrometer. The remaining 0.5 ml of butyl chloride was evaporated to dryness and the dry residue was dissolved in 1.8-ml fractions and each fraction was assayed for radioactivity. The PTH derivatives of radiolabeled amino acids were identified by comparing the elution volume of radiolabeled PTH amino acid standards. Repetitive yields were determined from the recovery of the PTH derivatives of valine (steps 1 and 10) and leucine (steps 2, 9, and 11) of the sperm whale apomyoglobin standard.

**Processing of the in Vitro Precursor of MPRP in Dog Pancreas Vesicles**—Macaque parotid gland poly(A⁺) mRNA was translated in an mRNA-dependent reticulocyte lysate obtained commercially (Amersham Corp. Catalog No. N-90). The translation reactions contained: 14 μl of reticulocyte lysate, 2 μg of mRNA 1.2 μg of dog pancreas vesicles (New England Nuclear; 1:4 dilution of vesicles as supplied), and 1 μCi/μl of L-[^3]H]proline, L-[^3]H]methionine, or L-[^3]H]valine (Amersham Corp.). Translation reactions were incubated for 35 min at 30 °C, made 2% with respect to sodium dodecyl sulfate, and heated to 100 °C overnight. Heat-killed, formalin-fixed, *S. aureus* was added and the solid material was recovered by centrifugation through a 1 M sucrose solution (300 μl) in 1 × buffer. The pellet was washed repeatedly with the same buffer and translation products were eluted from the solid material in sample buffer for electrophoretic analysis or in 70% formic acid for radioscintillation.

**Automated Edman Degradation of Translation Products**—Sequencer chemicals were purchased from Beckman Instruments or Pierce Chemical Co. The immunoprecipitated translation products eluted from heat-killed, formalin-fixed *S. aureus* in 0.6 ml of 70% formic acid containing 1 μg of sperm whale apomyoglobin (Beckman Instruments) were applied to the spinning cup with sample application program 2772. Sequencing using Edman degradation (22) was carried out on a Beckman 890C Sequencer equipped with a cold trap using program 121078 with 0.25 M Quadrol and a combined S₁ and S₂ wash. The background of each radioscintillation reaction was reduced by performing a wash step (no coupling reagent) followed by double coupling of Phenylthiohydantoin norleucine was added to each fraction collector tube to serve as an internal standard.

3978

Translation of Macaque Parotid Gland mRNAs

F. G. Oppenheim and R. F. Troxler, unpublished data.
RESULTS

Cell-free Translation of the in Vitro Precursor of MPRP—
Macaque parotid poly(A⁺) mRNA and poly(A⁻) mRNA pro-
moted incorporation of L-[³H]proline, L-[³⁵S]methionine, and
L-[³H]isoleucine into acetone-insoluble polypeptides in an
mRNA-dependent reticulocyte lysate (Table I). The stimula-
tion of radiolabeled amino acid incorporation was approxi-
mately 5 times greater per µg of RNA with poly(A⁺) mRNA
template. Examination of the translation products showed
that the profile of radiolabeled polypeptides was essentially
the same with either poly(A⁺) mRNA or poly(A⁻) mRNA
templates (Fig. 1). Separate rechromatography of the poly(A⁺)
mRNA on oligo(dT)-cellulose, or of the poly(A⁻) mRNA on
poly(U) agarose, did not alter the profile of polypeptides
 templated by either fraction or decrease template activity
(counts/min incorporated/µg of RNA).

L-[³H]Proline was preferentially incorporated into 5 polypep-
dides, designated bands I–V (Fig. 1), which represent the
major proline-rich polypeptides whose mRNAs were extracted
from the parotid gland and translated in vitro. We show below
that the material in band V is the in vitro precursor of native
MPRP. MPRP is the single, major proline-rich protein in
macaque parotid secretion functionally equivalent to the hu-
mman PRPs (10). We have been unable to establish which of
the native proline-rich proteins in macaque parotid secretion
correspond to the in vitro polypeptides contained in bands I–
IV. However, it is certain that these polypeptides (bands I–
IV) are not the in vitro forms of macaque parotid gland
proteins analogous to PRPs I–IV in human parotid secretion
because MPRP is the only functionally equivalent proline-
rich protein in the macaque (10).

The apparent Mᵣ values for proline-rich proteins are erro-
neously high in gel filtration and electrophoretic systems
 calibrated with globular protein standards. Somewhat more
accurate Mᵣ values are obtained when such systems are cali-
brated with proline-rich polypeptide standards (10, 26). The
apparent Mᵣ values of MPRP, PRPs, and the polypeptides in
bands I–V, on slab gels calibrated with both globular protein
standards and chick skin collagen α₁ (1) chain CNBr peptides,
are given in Table II. These data show that the apparent Mᵣ
values of these proteins are about 1.5 times greater when
estimated by reference to the calibration curve for globular
protein standards versus collagen CNBr peptides. The appar-
ent Mᵣ values of native MPRP, native PRPs I or II, and
native PRPs III or IV were 19,800, 14,300, and 12,000, re-
spectively, when estimated with the collagen CNBr peptide
calibration curve. These values are in good agreement with
the Mᵣ values calculated from the amino acid composition or
amino acid sequences of these proteins (10, 27–29). For this
reason, the Mᵣ values of translation products subsequently
referred to are those obtained from gels calibrated with col-
lagen CNBr peptides.

Immune precipitates prepared with anti-MPRP immune
serum from the poly(A⁺) mRNA directed translation reactions
(Fig. 1, lanes 1–3) contained one major component with an

<table>
<thead>
<tr>
<th>Isotope</th>
<th>RNA</th>
<th>Fraction</th>
<th>Amount</th>
<th>Incorporation ratio: poly(A⁺)/poly(A⁻)</th>
<th>µg</th>
<th>cpm/µg RNA</th>
</tr>
</thead>
</table>
| L-[³⁵S]Methio-
   nine       | Poly(A⁺)| 1.6      | 261,000| 5.8                                   | 7.0  | 45,000     |
| L-[³H]Proline | Poly(A⁺)| 1.6      | 43,000 | 5.4                                   | 7.0  | 8,000      |
| L-[³H]Isoleuc-
   ine       | Poly(A⁺)| 1.6      | 18,000 | 4.5                                   | 7.0  | 4,000      |

* Values represent counts/min/µg of RNA incorporated into hot
  trichloroacetic acid-insoluble material in a 25-µl translation reaction.

![Fig. 1](image_url)
TABLE II

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Apparent M,</th>
<th>Globular protein standards</th>
<th>Collagen cyanogen bromide peptide standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPRP</td>
<td>28,600 ± 400</td>
<td>19,800 ± 100</td>
<td></td>
</tr>
<tr>
<td>PRP I or II</td>
<td>22,000 ± 500</td>
<td>14,300 ± 500</td>
<td></td>
</tr>
<tr>
<td>PRP III or IV</td>
<td>19,500 ± 400</td>
<td>12,200 ± 500</td>
<td></td>
</tr>
<tr>
<td>Band I</td>
<td>33,000 ± 800</td>
<td>22,800 ± 1000</td>
<td></td>
</tr>
<tr>
<td>Band II</td>
<td>26,200 ± 700</td>
<td>17,500 ± 900</td>
<td></td>
</tr>
<tr>
<td>Band III</td>
<td>23,000 ± 800</td>
<td>14,500 ± 800</td>
<td></td>
</tr>
<tr>
<td>Band IV</td>
<td>20,000 ± 600</td>
<td>12,400 ± 400</td>
<td></td>
</tr>
<tr>
<td>Band V</td>
<td>19,700 ± 300</td>
<td>11,500 ± 100</td>
<td></td>
</tr>
</tbody>
</table>

*Native MPRP is a phosphoglycoprotein in which the minimum M, of the polypeptide moiety is 15,340 and the minimum M, of the carbohydrate moiety is 4,150 (10, 11). The M, values of PRP I and III calculated from the amino acid sequence are 15,627 and 11,145, respectively (27, 28).

The M, values of globular protein standards and collagen CNBr standards are given in the legend to Fig. 1.

Fig. 2. Analysis of anti-MPRP immune precipitates from poly(A+) mRNA directed translation reactions by gel electrophoresis and fluorography. Lane 1, acetone-insoluble polypeptides from a translation reaction containing L-[3H]proline (53,000 cpm); lane 2, anti-MPRP immune precipitate from the translation reaction shown in lane 1 (8000 cpm); lane 3, anti-MPRP immune precipitate from a translation reaction containing L-[35S]methionine (8000 cpm); lane 4, anti-MPRP immune precipitate from a translation reaction containing L-[3H]isoleucine (8000 cpm).

Fig. 3. Mr values of CNBr peptides. Mr of CNBr peptides from the in vitro translation of poly(A+) mRNA are shown in lane 1 in the legend to Fig. 1.

The apparent Mr of 11,500 (Fig. 2). This component displayed an electrophoretic mobility identical to that of the polypeptide in band V. The anti-MPRP immune serum showed a weak affinity for the polypeptides in bands I–IV, but these components were quantitatively insignificant in the immune precipitates. The polypeptide in band V was the least abundant proline-rich translation product (Fig. 1, lanes 1 and 4) but the main component in the anti-MPRP immune precipitate, and on this basis was designated the in vitro precursor of MPRP. Anti-MPRP immune precipitates from translation reactions templated by poly(A) mRNA containing radiolabeled proline, methionine, or isoleucine were identical to those in Fig. 2 (data not given).

Automated Edman Degradation of the In Vitro Precursor of MPRP—Radiolabeled methionine was incorporated into the in vitro precursor of MPRP, but native MPRP does not contain methionine (30). This suggested that a methionine-containing signal peptide occurred at the NH2 terminus of the in vitro precursor of MPRP.

Macaque parotid gland poly(A+) mRNA was translated in reticulocyte lysates containing L-[35S]methionine, L-[3H]valine, L-[3H]isoleucine, L-[3H]alanine, or L-[3H]leucine and the material in anti-MPRP immune precipitates from these translation reactions was subjected to automated Edman degradation.

Radiosequencing of the material in the anti-MPRP immune precipitate from the translation reaction containing radiolabeled methionine showed that the PTH derivative of methionine occurred at steps 1, 13, and 16 (Fig. 3A). This is consistent with the premise that methionine occurred at residues 1, 13, and 16 of a signal peptide at the NH2 terminus of the in vitro precursor of MPRP.

Radiosequencing of the in vitro precursor of MPRP in the immune precipitate from the translation reaction containing radiolabeled valine revealed that the PTH derivative of valine was present at steps 8, 16, 23, and 28 (Fig. 3B). Valine is located at residues 5 and 10 of native MPRP (10, 11). Therefore, it was concluded that the PTH derivative of valine at steps 23 and 28 of the in vitro precursor of MPRP corresponded to valine at residues 5 and 10 of the native protein. If correct, this would mean that the in vitro form of MPRP contains an 18-residue signal peptide.

Radiosequencing of isoleucine- or alanine-labeled material in immune precipitates demonstrated that the PTH derivative of isoleucine was located at steps 4, 9, and 17, and that the PTH derivative of alanine occurred at steps 9 and 12 (Fig. 3C and D). The radioactivity observed at step 1 in the analysis of isoleucine-labeled material was an artifact because no radiolabeled PTH-isoleucine was detected when the sample was examined by high pressure liquid chromatography. The PTH derivative of leucine was detected at steps 2, 3, 5, 6, 10, and 11 by radiosequencing analysis of immune precipitate from a translation reaction carried out with radiolabeled leucine (Fig. 3E). From the foregoing results, a partial amino acid sequence of the signal peptide at the NH2 terminus of the in vitro precursor of MPRP was deduced (Scheme 1).

Both methionine and valine occurred at residue 16 and both alanine and isoleucine occurred at residue 9 of the MPRP signal peptide. This can be explained if two different MPRP precursors, derived from two different mRNAs, were isolated from translation reactions with anti-MPRP immune serum. The observed microheterogeneity precluded unambiguous determination of the complete amino acid sequences of either signal peptide by radiosequencing techniques, and further experiments to detect further microheterogeneity were not performed.

Processing of the In Vitro Precursor of MPRP—The suppression that the in vitro precursor of MPRP contains an 18-residue signal peptide is based on assignment of PTH-[3H] valine at steps 23 and 28 to residues 5 and 10 of the native protein. This was confirmed by showing that dog pancreas vesicles processed the in vitro MPRP precursor to a polypeptide with a smaller Mr, which lacked methionine and had an
NH$_2$-terminal sequence corresponding to that of native MPRP.

Macaque parotid gland poly(A$^+$) mRNA was translated in reticulocyte lysates with radiolabeled proline, methionine, or valine, with or without dog pancreas vesicles.

In the translation reaction containing radiolabeled proline and dog pancreas vesicles, processing of all proline-rich polypeptides (bands I–V) resulted in a decrease in the apparent $M_r$ from 11,500 to 10,500 (Fig. 4, lanes 1 and 2), and the apparent $M_r$ values of these polypeptides decreased from 200 to 1100 daltons when compared to proline-rich polypeptides from control translations without dog pancreas vesicles (Table III). Processing of the in vitro precursor of MPRP (band V) resulted in a decrease in the apparent $M_r$ from 11,500 to 10,500 (Fig. 4, lanes 3 and 4). In this experiment, processing of the MPRP precursor was about 80% complete as indicated by the presence of the $M_r = 10,500$ and 11,500 bands in a ratio of approximately 4:1 (Fig. 4, lane 4). The extent to which the in vitro form of MPRP was processed by dog pancreas vesicles in the experiments depicted in Figs. 4 and 5 was 80 and 50%, respectively. The explanation for this result is not clear.

With the commercial reticulocyte lysate (Amersham Corp.) the profile of proline-rich proteins was the same as that seen using the lysate prepared according to the procedure of Pelham and Jackson (18). However, the in vitro precursor of MPRP was a much more prominent band in the commercial lysate even though the poly(A$^+$) template was the same used with the other lysate (compare Fig. 1, lane 1 and Fig. 4, lanes 1 and 2). We cannot explain this observation.

Macaque parotid gland poly(A$^+$) mRNA was translated in reticulocyte lysates containing radiolabeled methionine, with or without dog pancreas vesicles. In the presence of dog pancreas vesicles, the polypeptides in bands I–V were difficult to detect whereas these polypeptides were present in translation reactions minus dog pancreas vesicles (data not given) and identical to those in Fig. 1 (lane 2). Furthermore, band V was not detected in the anti-MPRP immune precipitate from the translation reaction containing dog pancreas vesicles (data not given). These results provide indirect evidence that each proline-rich polypeptide (bands I–V) contains a signal peptide with one or more methionine residues and that the processed polypeptides lack this amino acid.

Macaque parotid gland poly(A$^+$) mRNA was next translated in a reaction containing L-[3H]valine and dog pancreas vesicles. Electrophoretic analysis of the anti-MPRP immune precipitate demonstrated that the in vitro precursor of MPRP had been partially processed due to the presence of M, = 10,500 and 11,500 bands in the immune precipitate. Automated Edman degradation of the material in the immune precipitate showed that the PTH derivative of valine occurred at steps 5, 8, 10, and 16 (Fig. 5). The interpretations of these data were that the PTH-[3H]valine at steps 5 and 10 corresponded to residues 5 and 10 of the in vitro MPRP precursor from which the signal peptide had been cleaved and that the PTH-[3H]valine at steps 8 and 16 corresponded to valine at residues 8 and 16 of the signal peptide.

**Discussion**

The present investigation is the first to demonstrate cell-free translation of the mRNAs for proline-rich proteins from the parotid gland of the subhuman primate, *M. fascicularis*. One of the proline-rich translation products (band V) was shown to be the in vitro precursor of MPRP on the basis of its apparent $M_r$, cross-reactivity with anti-MPRP immune serum, automated Edman degradation of the material in anti-
Translation of Macaque Parotid Gland mRNAs

MPRP immune precipitates, and processing in dog pancreas vesicles to a polypeptide with an NH₂-terminal amino acid sequence comparable to that of native MPRP. The in vitro precursor of MPRP contains a signal peptide at the NH₂-terminus as has been reported for numerous other secretory proteins (31). Microheterogeneity in the signal peptide of the MPRP precursor suggested that band V contained two chemically distinct translation products derived from different mRNAs in the macaque parotid gland.

Several findings in the present work deserve further comment. First, the profile of proline-rich polypeptides templated by poly(A⁺) mRNA and poly(A⁻) mRNA was nearly identical (Fig. 1). The poly(A⁺) mRNA fraction displayed about one-fifth as much template activity as did the poly(A⁺) mRNA (Table I), yet examination of the poly(A⁺) fraction on denaturing agarose gels showed this fraction contained predominantly ribosomal RNA. Since most of the RNA after poly(A) selection was recovered in the poly(A⁻) mRNA fraction, and template activity for proline-rich polypeptides in the poly(A⁺) fraction was about one-fifth that of the poly(A⁺) mRNA fraction, a significant portion of the message population for proline-rich polypeptides did not bind to oligo(dT)-cellulose. Chromatography of the poly(A⁺) fraction on poly(U) agarose, a resin known to have a greater affinity for short poly(A) sequences (32), did not alter the template activity, indicating that some mRNAs for proline-rich polypeptides contain very short poly(A) sequences, or none at all. It is possible that removal of poly(A) sequences from the 3' region of these mRNAs occurs during processing (33) or that these mRNAs are not polyadenylated initially. The present work may be among the first in which mRNAs for secretory proteins have been found in both the poly(A⁺) mRNA and poly(A⁻) mRNA fractions. Parenthetically, the genes for PRPs in man are located on chromosome 1 (34). Thus, it would be expected that the genes for macaque parotid gland proline-rich proteins are also encoded in nuclear DNA and that the mRNAs derived from these genes would at some point become polyadenylated.

Second, we identified 5 major proline-rich polypeptides (bands I–V) among the translation products templated by mRNAs from the macaque parotid gland, and the polypeptide(s) in band V was unmistakably characterized as the in vitro precursor(s) of MPRP. The identity of the in vitro polypeptides in bands I–IV is not known, although these could be the macaque counterparts of the basic proline-rich proteins (35) and the basic proline-rich glycoprotein (36) in the parotid and submandibular secretion of man.

**TABLE III**

<table>
<thead>
<tr>
<th>Band</th>
<th>Apparent Mr, of proline-rich polypeptides (bands I–V) templated by macaque parotid gland poly(A⁺) mRNA directed reticulocyte lysate containing L-[¹⁴C]proline in the presence or absence of dog pancreas vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>23,100</td>
</tr>
<tr>
<td>II</td>
<td>16,600</td>
</tr>
<tr>
<td>III</td>
<td>13,700</td>
</tr>
<tr>
<td>IV</td>
<td>11,600</td>
</tr>
<tr>
<td>V</td>
<td>11,500</td>
</tr>
</tbody>
</table>

*These data were obtained by analysis of the bands shown in lanes 1 and 2 of the fluorogram depicted in Fig. 4, using the calibration curve for collagen CNBr peptides.*

First, the profile of proline-rich polypeptides templated by poly(A⁺) mRNA and poly(A⁻) mRNA was nearly identical (Fig. 1). The poly(A⁺) mRNA fraction displayed about one-fifth as much template activity as did the poly(A⁺) mRNA (Table I), yet examination of the poly(A⁺) fraction on denaturing agarose gels showed this fraction contained predominantly ribosomal RNA. Since most of the RNA after poly(A) selection was recovered in the poly(A⁺) mRNA fraction, and template activity for proline-rich polypeptides in the poly(A⁺) fraction was about one-fifth that of the poly(A⁺) mRNA fraction, a significant portion of the message population for proline-rich polypeptides did not bind to oligo(dT)-cellulose. Chromatography of the poly(A⁺) fraction on poly(U) agarose, a resin known to have a greater affinity for short poly(A) sequences (32), did not alter the template activity, indicating that some mRNAs for proline-rich polypeptides contain very short poly(A) sequences, or none at all. It is possible that removal of poly(A) sequences from the 3' region of these mRNAs occurs during processing (33) or that these mRNAs are not polyadenylated initially. The present work may be among the first in which mRNAs for secretory proteins have been found in both the poly(A⁺) mRNA and poly(A⁻) mRNA fractions. Parenthetically, the genes for PRPs in man are located on chromosome 1 (34). Thus, it would be expected that the genes for macaque parotid gland proline-rich proteins are also encoded in nuclear DNA and that the mRNAs derived from these genes would at some point become polyadenylated.

Second, we identified 5 major proline-rich polypeptides (bands I–V) among the translation products templated by mRNAs from the macaque parotid gland, and the polypeptide(s) in band V was unmistakably characterized as the in vitro precursor(s) of MPRP. The identity of the in vitro polypeptides in bands I–IV is not known, although these could be the macaque counterparts of the basic proline-rich proteins (35) and the basic proline-rich glycoprotein (36) in the parotid and submandibular secretion of man.
precipitates from a poly(A+) mRNA directed translation reaction containing dog pancreas vesicles and L-[3H]valine. The amino acid sequences of the processed and unprocessed polypeptides are shown in the upper panel. Each Sequencer cycle was assayed as described under "Experimental Procedures" and the results are given in the lower panel. The PTH derivative of valine detected at steps 5 and 10 corresponds to valine at residues 5 and 10 of processed MPRP polypeptide; the PTH derivative of valine at steps 8 and 16 corresponds to valine at residues 8 and 16 of the signal peptide in the unprocessed precursor of MPRP. Approximately 20,000 cpm were applied to the Sequencer cup. The repetitive yield of the sperm whale apomyoglobin standard was 90%.

Fig. 5. Radiosequence analysis of the material in anti-PRP immune precipitates from a poly(A+) mRNA directed translation reaction containing dog pancreas vesicles and L-[3H]valine. The amino acid sequences of the processed and unprocessed polypeptides are shown in the upper panel. Each Sequencer cycle was assayed as described under "Experimental Procedures" and the results are given in the lower panel. The PTH derivative of valine detected at steps 5 and 10 corresponds to valine at residues 5 and 10 of processed MPRP polypeptide; the PTH derivative of valine at steps 8 and 16 corresponds to valine at residues 8 and 16 of the signal peptide in the unprocessed precursor of MPRP. Approximately 20,000 cpm were applied to the Sequencer cup. The repetitive yield of the sperm whale apomyoglobin standard was 90%.

Fig. 6. Immune precipitation of proline-rich polypeptides templated by macaque parotid gland poly(A+) mRNA and poly(A+) mRNA with immune serum directed against native PRP I or native MPRP. Lanes 1 and 2, anti-PRP I and anti-MPRP immune precipitates, respectively, from a poly(A+) mRNA directed translation reaction containing radiolabeled methionine. Lanes 3 and 4, anti-PRP I and anti-MPRP immune precipitates, respectively, from a poly(A+) mRNA directed translation reaction containing radiolabeled methionine. Lanes 1–4 each contained 30,000 cpm.

Third, radiosequence analysis of the material in anti-MPRP immune precipitates showed that the 18-residue signal peptide at the NH₂ terminus of the in vitro precursor of MPRP had alanine and isoleucine at residue 9, and methionine and valine at residue 16 (Fig. 3 and Scheme 1). The methionine-valine microheterogeneity can be explained by a 1-base change in the codon (third position), but the alanine/isoleucine microheterogeneity would require a change in the first 2 bases of the codons for the latter two amino acids. We have determined approximately 75% of the primary structure of native MPRP and have found no evidence for microheterogeneity in the amino acid sequence (10, 11). This suggests the possibility that the base sequence in the mRNAs for the two in vitro precursors of MPRP may be identical in the regions coding for the native protein whereas the base sequences coding for the signal peptides are clearly different. A somewhat similar situation has been described for mouse liver and salivary gland α-amylase mRNAs which have identical base sequences in the coding and nontranslated 3' region but different base sequences in the 5' untranslated regions (37, 38). Microheterogeneity in the amino acid sequences of the signal peptides of other secretory proteins such as rat preproinsulin (39) and canine pretrypsinogen (40) has been described.

Finally, electrophoretic analysis of the material in anti-MPRP immune precipitates from reticulocyte lysates (Fig. 2) and automated Edman degradation of native MPRP (10, 11) indicate the presence of a single component both in the translation reactions and in the protein found in the parotid secretion of M. fascicularis, respectively. It is interesting, therefore, that radiosequencing experiments (Fig. 3) showed that the in vitro precursor of MPRP comprises two polypeptides. This suggests the possibility that there may be two genes for MPRP in M. fascicularis, as might be predicted considering the genetic polymorphism among human PRPs.

REFERENCES
Translation of Macaque Parotid Gland mRNAs


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Biosynthesis of salivary proteins in the parotid gland of the subhuman primate, Macaca fascicularis. Cell-free translation of the mRNA for a proline-rich glycoprotein and partial amino acid sequence and processing of its signal peptide.

H S Belford, E G Triffleman, G D Offner, R F Troxler and F G Oppenheim


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