Volume 9 Number 14 1981 Nucleic Acids Research

Abundant androgen regulated mRNAs in mouse submandibular gland: cell-free translation of renin precursor mRNA

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Received 4 March 1981

ABSTRACT

Submandibular glands of male mice contain at least four abundant mRNAs that occur at low concentrations in glands of females. The male-specific mRNAs code for polypeptides of 48,000, 43,000, 29,000, and 27,000 MW. Androgenic regulation of these mRNAs is illustrated by their apparent absence in glands of castrate males and by their accumulation in glands of females treated with testosterone. Selective hybrid-arrested translation experiments also indicate reduced levels of these male-specific sequences in female gland cytoplasm. The 48,000 MW male-specific polypeptide is reduced in translation products directed by gland mRNA from C57BL10/J mice (variants deficient in salivary renin), suggesting the corresponding mRNA codes for a renin precursor. The identity of this polypeptide is confirmed by immune selection with renin-specific antibody.

INTRODUCTION

The submandibular gland of the mouse is sexually dimorphic in morphology and function.[1-3] In mature males, columnar epithelium in the convoluted tubules is typified by extensive rough endoplasmic reticulum, golgi apparatus, and secretory granules - structures that are very poorly developed in the same cells of female, and of immature and castrate male mice.[3-5] The secretory products of this androgen-regulated epithelium provide the saliva of male mice with a unique pattern of exocrine proteins. For example, salivary contents of nerve growth factor (NGF), epidermal growth factor (EGF), and renin are 30-90 fold greater in male mice than in females. Historically, submandibular gland has provided the richest source for purification of these proteins.[6-9] Other growth factors such as thymocyte transforming factor and two mesodermal growth factors, F-2 and MGF, also are found in the adult male gland.[10,11] Although the physiologic

role of these proteins in male saliva is not understood,[12-14] the submandibular gland is unequivocally a site of NGF, EGF, and renin synthesis in male mice.[15-19]

Preliminary to examining the biosynthesis of renin and growth factors in mouse submandibular gland and in other organs, we have compared the coding function of mRNA from the male and female gland and have found at least four mRNAs that are abundant only in the male gland. The size of some male-specific, mRNA-directed translation products correlates well with the established molecular weights of proteins synthesized and secreted in large quantity by the male gland. Furthermore, we have determined that one of these mRNAs codes for the renin precursor.

METHODS

Animals and maintenance

Unless specified, mice were male, female and castrate male CD-1 retired breeders purchased from Charles River Breeding Laboratories (Wilmington, MA). Where indicated, females received 15 daily subcutaneous injections of 1 mg testosterone propionate in 75 µl polyethylene glycol-400. Male mice of the SWR/J and C57BL/10J strains, both 35 days old, were purchased from Jackson Laboratories (Bar Harbor, ME). Animals had free access to food and water and were kept in alternating 12 hour cycles of light and dark.

Preparation of mRNA

Mice were killed by cervical dislocation, and excised submandibular glands from 2-4 mice were disrupted immediately in 4 ml ice-cold 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 3 mM $MgCl_2$, and 10% (w/v) sucrose using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY). To clear the cytoplasm of nuclei and cell debris, homogenates were sedimented for 2 min at 800 rpm through 4 ml 20% (w/v) sucrose in 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 3 mM $MgCl_2$, and 1% (w/v) Nonidet P-40.[20] RNA and ribonucleoproteins precipitated from the supernatant with 2 vol 95% ethanol at -20°C for 1 hour or more were deposited by centrifugation for 20 min at 4000 rpm in a Sorvall SS-34 rotor. Nucleic acids in precipitates dissolved in 5 ml of 6 M guanidine-HCl and 25 mM EDTA, adjusted to 0.1 M

potassium acetate (pH 5.0) were selectively precipitated for at least 2 h with 0.5 vol 95% ethanol at -20°C.[21-23] Precipitated RNA deposited by centrifugation for 20 min at 9000 rpm in a Sorvall SS-34 rotor was extracted with guanidine twice more. RNA from the third guanidine extraction was dissolved in 0.2 M potassium acetate (pH 5.0), precipitated with 2 vol 95% ethanol to remove residual guanidine-HCl and dissolved in 10 mM Tris-HCl (pH 7.4), 0.3 M NaCl, 10 mM EDTA, and 0.2% sodium dodecyl sulfate before deproteinization was completed by extraction with phenol/chloroform/isoamyl alcohol (25:23:2).[24] RNA was stored at -20°C in 2 vol 95% ethanol.

Poly(A)-containing (poly(A)$^+$) mRNA was isolated by oligo(dT)-cellulose chromatography.[21,25] Deproteinized RNA dissolved in 10 mM Tris-HCl (pH 7.4), 0.5 M NaCl, and 0.1% sodium Sarkosyl (Ciba-Geigy Corp., Greensboro, NC) was chromatographed on 2 ml columns (packed volume) of oligo(dT)-cellulose (Type T-2, Collaborative Research, Inc., Waltham, MA). Poly(A)$^+$ mRNA eluted from the column with 10 mM Tris-HCl (pH 7.4) was adjusted to 0.2 M potassium acetate (pH 5.0) and precipitated with 2 vol 95% ethanol. Poly(A)$^+$ mRNA dissolved in water was quantitated by absorbance at 260 nm.

mRNA-directed cell-free translation

Poly(A)$^+$ mRNA was translated in the mRNA-dependent reticulocyte lysate[26] purchased from New England Nuclear (Boston, MA). Reaction mixtures (12.5 µl) contained 80 mM potassium acetate, 0.65 mM magnesium acetate, 22 µCi [^{35}S]methionine (1118 Ci/mmol) or 17 µCi of [^{3}H]leucine (145 Ci/mmol) and the following components at concentrations provided in the kit: HEPES, spermidine, creatine phosphate, dithiothreitol, and GTP. Incorporation of [^{35}S]methionine or [^{3}H]leucine into protein was measured as alkali-stable, acid-insoluble radioactivity.[26] One µl of translation mixtures was added to 200 µl of 0.125 M H_2O_2, 0.25 M NaOH and 1 mg/ml unlabeled methionine or leucine, incubated for 15 min at 37°C, adjusted to 6% (w/v) TCA by addition of 100 µl 20% (w/v) TCA and incubated at 0°C for 20 min. Precipitates were collected on nitrocellulose filters (Type DA, Millipore Corp., Bedford, MA), dried, and counted in toluene-based scintillation fluid containing 0.4% (w/v)

Omnifluor (New England, Boston, MA).

Electrophoretic analysis of mRNA-directed translation products

mRNA-directed translation products were separated in linear 6-15% (w/v) polyacrylamide slab gels (1.5 mm thick) using a discontinuous buffer system.[27] Translation mixtures adjusted to 62.5 mM Tris-HCl (pH 6.8), 5% (w/v) sodium dodecyl sulfate, 10% (v/v) glycerol, 5% (v/v) mercaptoethanol and 0.001% (w/v) bromphenol blue were boiled 3 min immediately before electrophoresis at 37° for 165 min at 120 constant volts. Gels fixed 20 min at 37°C with 0.25% (w/v) acetic acid, 20% (v/v) methanol were soaked in Enhance (New England Nuclear, Boston, MA) to increase the sensitivity of autoradiography.[28]

Preparation of cDNA

cDNA transcribed from poly(A)$^+$ mRNA using AMV reverse transcriptase (provided by Dr. Joseph Beard, Life Sciences Corp., Fort Lauderdale, FL).[25,26] Reaction mixtures containing 10 µCi [^{3}H]dCTP (pH 8.3), 70 mM KCl, 10 mM $MgCl_2$, 4 mM sodium pyrophosphate, 2.5 mM dithiothreitol, 20 µg oligo(dT)$_{20}$ and 0.5 mM each of the four deoxynucleoside triphosphates were incubated 15 min at 0°C, and incubated an additional 15 min at 0°C after addition of 65 units reverse transcriptase and then for 30 min at 42°C. Reactions were terminated by heating 5 min at 60°C in 0.1 M NaOH, adjusted to 10 mM Tris-HCl (pH 7.4), 0.3 M NaCl, 10 mM EDTA and 0.2% (w/v) sodium dodecyl sulfate and deproteinized by phenol/chloroform/isoamyl alcohol extraction. After chromatography on Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with the same buffer, combined fractions containing cDNA were precipitated with 2 vol 95% ethanol. cDNA dissolved in water was quantitated by absorbance at 260_{nm}.

Selected hybrid-arrested translation

cDNA (0.5 µg) was hybridized at 50°C with 9 µg of unfractionated cytoplasmic RNA in 3 µl 10 mM HEPES (pH 7.4) and 180 mM KCl in sealed capillary tubes for 0, 30, or 60 minutes.[31-33] Contents of these reactions were added to translation mixtures as described in the appropriate legends.

Immunochemical analysis of cell-free synthesized renin

Renin, purified to homogeneity from submandibular glands of adult, retired breeder male mice (8) was used to immunize

rabbits in preparation of anti-mouse renin antibody. Rabbit R1721 produced antirenin antiserum which inhibited renin activity (1 milliGoldblatt unit) at a titer of 1:10,000. Renin specificity of the antiserum was demonstrated by Ouchterlony analysis.

Cell-free translation mixtures were diluted 5-fold with buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 M NaCl, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 0.1% Nonidet P-40, 0.25 mM phenyl methyl sulfonyl fluoride, 0.25 mM sodium tetrathionate. After addition of immune or control rabbit serum (10 µl serum per 25 µl translation products), samples were incubated 18 h at 4^{o}. Immune complexes were purified by adsorption with. S. aureus (IgG Sorb, New England Enzyme Center, Boston, MA) that had been preincubated with 100,000 x G supernatant from female mouse submandibular gland and washed twice with the same dilution buffer. This pretreatment was the most effective means of eliminating non-specific adsorption of translation products to protein A^{58} on the surface of S. aureus. Pretreated bacteria were added to translation product:antiserum mixtures and, after 15 min at 4^{o}, samples were centrifuged 30 sec in the Brinkman Microfuge. Bacterial-immune complex pellets washed 3 times with 1 ml volumes of buffer were deposited through 0.2 ml 1 M sucrose in buffer. Pellets resuspended in 62.5 mM Tris-HCl (pH 6.8), 5% (w/v) sodium dodecyl sulfate, 10% (v/v) brom phenol blue were boiled 5 min, centrifuged 5 min in the Brinkmann microfuge, and supernatants were analyzed in polyacrylamide gradient slab gels (see above).

RESULTS

Enhanced translational efficiency of male gland mRNA

Poly$(A)^{+}$ mRNA from male submandibular gland consistently directed protein synthesis to a 30% greater extent and at approximately twice the rate of mRNA from glands of females (Fig. 1). From the initial slopes of the [^{3}H]leucine incorporation curves (Fig. 1A), initial rates of translation were determined at 17.5 pmol leucine/min/µg male gland mRNA and 7.5 pmol leucine/min/µg female gland mRNA. Furthermore, the extent of amino acid incorporation directed by male gland mRNA exceeded

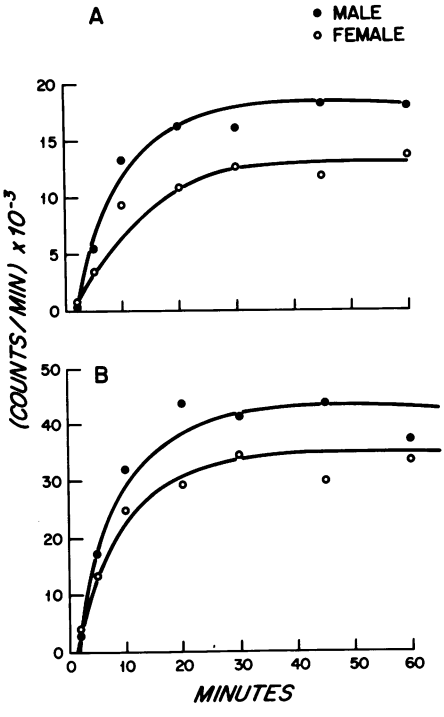

Figure 1. Kinetics of submandibular gland mRNA-directed translation. Translation mixtures (25 µl) containing 1.6 µg of poly(A)$^+$ mRNA and 22 µCi [^{35}S]methionine and 17 µCi [^{3}H]leucine were assayed for alkali-stable, acid-precipitable radioactivity (Methods) in 2 µl aliquot samples. Data were corrected for 44% spill of ^{35}S radioactivity in the ^{3}H channel. Incorporation curves were drawn by inspection. Panel A, incorporation of [^{35}S]methionine; directed by mRNA from male (•) or female (o) gland; panel B, incorporation of [^{3}H]leucine directed by mRNA from male (•) or female (o) gland.

that directed by female gland mRNA by at least 30% for every preparation of mRNA examined. Since this difference was observed for incorporation of [^{3}H]leucine (Fig. 1B) as well as [^{35}S]methionine (Fig. 1A), dissimilar amino acid content of polypeptides coded by male and female gland mRNA is unlikely. Similar results were observed for poly(A)$^+$ mRNA and for unfractionated cytoplasmic gland RNA, eliminating differential contamination of male and female gland poly(A)$^+$ mRNA with RNA as a trivial explanation for the differences in kinetics of reaction. Selective degradation of female gland mRNA during isolation also is unlikely, since the cell-free translation products directed by male and female gland mRNA are equivalent in size. Furthermore, the weight average molecular weight and the modal sedimentation coefficient are equivalent for submandibular gland poly(A)$^+$ mRNA from both sexes (data not shown).

Abundant mRNA-directed translation products unique to male submandibular gland

Translation products directed by male gland mRNA contained

four abundant polypeptides of 48,000, 43,000, 29,000 and 27,000 MW not detected in the products of female gland mRNA (Fig. 2). Resolution of the 29,000 MW and 27,000 MW polypeptides was obtained by applying 50% less translation mixture to gels and by decreasing the radiographic exposure time by 50% (Fig. 3).

The sexually dimorphic mRNA pattern was apparent in translations directed both by poly(A)$^+$ mRNA (Figs. 2 & 3) and by unfractionated cytoplasmic RNA (Fig. 4, tracks A,G). In addition to the male-specific translation products, approximately 50 polypeptides coded by mRNA from glands of both sexes were resolved in one-dimensional polyacrylamide gels. Two common polypeptides, 57,000 MW and 35,000 MW, were the most abundant cell-free synthesized proteins. The protein of higher molecular weight corresponds to salivary amylase precursor (MW 57,000), a protein secreted in large amounts by submandibular gland.[41-42] The sensitivity of polyacrylamide gel analysis of cell-free translation products is limited to biologically-active abundant and

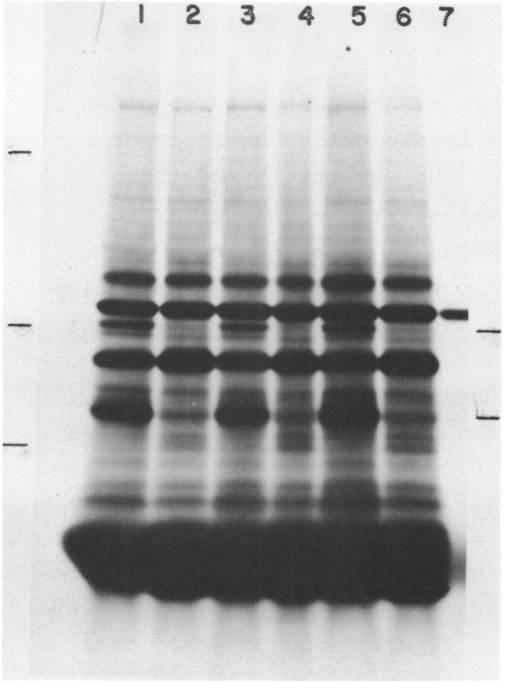

Figure 2. Electrophoretic analysis of translation products directed by male and female gland mRNA. [^{35}S]methionine-labeled cell-free translation products directed by poly(A)$^+$ mRNA from submandibular glands were analyzed in linear 6-15% gradient polyacrylamide gels, and labeled polypeptides were visualized radiographically (Methods). Translation products directed by: 0.4 µg mRNA from males (track 1) and from females (track 2), 0.5 µg mRNA from males (track 3) and from females (track 4), 0.6 µg of mRNA from males (track 5), and from females (track 6), no mRNA (track 7). In each case, translation products from male gland mRNA contained 88,000 cpm and those from female gland mRNA contained 70,000 cpm. The fluorogram was exposed for 4 days.

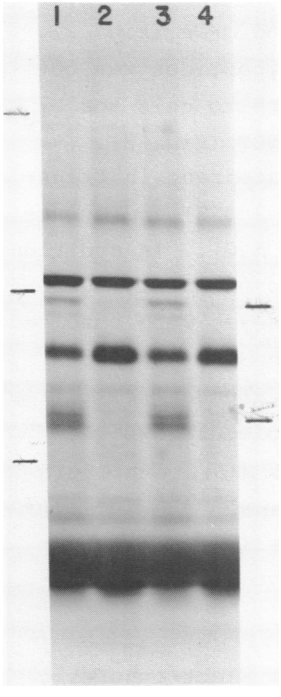

Figure 3. Electrophoresis of submandibular gland mRNA-directed translation products. Conditions are as in Fig. 2, tracks 1-4, except the quantity of sample and the radiographic exposure time both were reduced by 50%. Bars at left indicate the position of the molecular weight standards located by Coomassie Brilliant Blue staining. The standards were phosphorylase A, 92,500 MW, ovalbumin, 45,000 MW, and trypsinogen, 24,500 MW. Bars at right indicate the male-abundant translation products.

mid-abundant mRNAs, and therefore, mRNAs apparently specific to male gland (Fig. 2) may exist in female gland but at lower intracellular concentrations beyond the sensitivity of these techniques. Because of this possibility, we will refer to these apparently male-specific mRNAs as *male-abundant*.

Analysis of male-abundant mRNAs by hybrid-arrested translation

Hybrid-arrested translation experiments established that female gland does not contain biologically-inactive, male-abundant mRNA sequences at concentrations similar to those in male gland.[31,33] Unfractionated cytoplasmic RNA from male and female gland was translated after incubation at 50°C with cDNA transcribed from female gland poly(A)$^+$ mRNA (cDNA^f) to a $C_o t$ of 1.66 mol·sec/l. Hybridization of cDNA with mRNA selectively inhibits translation of mRNA in duplex, and only mRNAs lacking cDNA complememts continue to be translated.[31-33] Thus, if male-abundant mRNAs were absent or at low concentration in female gland poly(A)$^+$ mRNA, their translation would persist after reaction with cDNA^f.

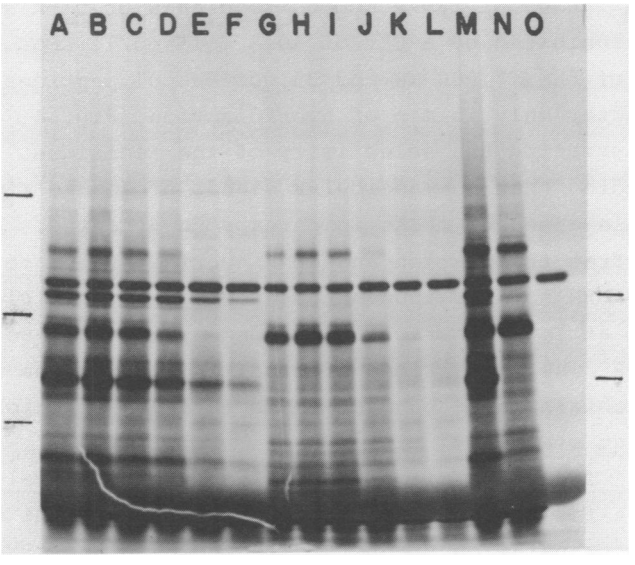

Figure 4. Selective hybrid-arrested translation of submandibular gland mRNA. Unfractionated cytoplasmic RNA (19 µg) from glands of male and female mice was incubated at 50°C with 0.5 µg cDNA transcribed from female gland poly(A)$^+$ mRNA. Contents of hybridization reactions were added to 12.5 µl translation mixtures (Methods) and the resulting translation products were analyzed in linear 6-15% gradient polyacrylamide gels (Fig. 2). Tracks A-C, male gland RNA incubated without cDNA for 0, 30, or 60 min, respectively, each contained 88,000 cpm; tracks D-F, male gland RNA incubated with cDNA for 0, 30, or 60 min, respectively, and radioactivity ranged from 48,000 to 9,000 cpm; tracks G-I, female gland RNA incubated without cDNA for 0, 30, or 60 min, respectively, radioactivity ranged from 24,000 to 8,000 cpm; track M, male gland poly(A)$^+$ mRNA without cDNA or 50° incubation (160,000 counts/min); track N, female gland poly(A)$^+$ mRNA without cDNA or 50° incubation (120,000 counts/min); track O, no mRNA addition. The fluorogram was exposed 5 days.

Translation of male-abundant mRNAs persisted, although to a slightly lower extent, even after 1 h incubation with cDNA^f (Fig. 4, tracks D-F). Non-specific inhibitory effects of cDNA^f on the translation system may have reduced the extent of male-abundant mRNA translation or the female gland poly(A)$^+$ mRNA template may contain low levels of male-abundant mRNA. Incubation of mRNA at 50°C without cDNA^f had no effect on the translation of common or male-abundant mRNAs (Fig. 4, tracks A-C) or of female gland mRNAs (Fig. 4, tracks G-I). In the presence of

cDNA^f, translation of mRNAs common to glands of both sexes was completely inhibited by a $C_o t$ of ~0.3 mol·sec/l. For example, appearance of the 57,000 MW and 35,000 MW polypeptides was abolished after only 15 min of hybridization (Fig. 4, tracks D-F), demonstrating the selectivity of the technique.

Concluding that submandibular glands of female mice totally lack the male-abundant mRNAs is perhaps unwarranted. First, since cell-free translation and cDNA hybridization are limited to abundant mRNAs, male-abundant mRNAs may exist in glands of females but at lower intracellular concentrations. Second, because cDNA^f was transcribed from poly(A)$^+$ mRNA, the female gland may contain male-abundant mRNAs in a non-functional poly(A)-lacking state.

Testosterone induction of male-abundant mRNAs in female mice

To test for hormonal regulation of male-abundant mRNAs, we compared translation products directed by gland mRNA from castrate mice and from female mice injected with testosterone (Fig. 5). Daily administration of 1 mg testosterone for 15 days induced accumulation of male-abundant mRNAs to levels comparable to those in male mice (Fig. 5, tracks E-G). Similar studies showed male-abundant mRNAs were present in glands of castrate males up to 4 weeks after operation but at greatly diminished levels. Daily injection of castrate males with 1 mg testosterone restored the cytoplasmic concentration of male-abundant mRNAs to previous levels within 5 days (data not shown). These data illustrate that cytoplasmic content of these male-abundant mRNAs is regulated, perhaps only in part, by testosterone.

Male-abundant mRNAs in variants deficient in salivary renin

Male mice of strain C57BL/10J, specifically deficient in submandibular gland renin, contain less mRNA encoding the male-abundant 48,000 MW polypeptide. Enzymatically-active renin from mouse submandibular gland is ~37,000 MW,[8] but the immediate product of translation is a precursor of ~50,000 MW.[18] Since male C57BL/10J mice secrete approximately 100-fold less salivary renin than SWR/J control males but normal levels of other male-abundant salivary products such as NGF and EGF,[43,44] we compared gland mRNA-directed translation products from these inbred strains of mice. The 48,000 MW male-abundant polypeptide was

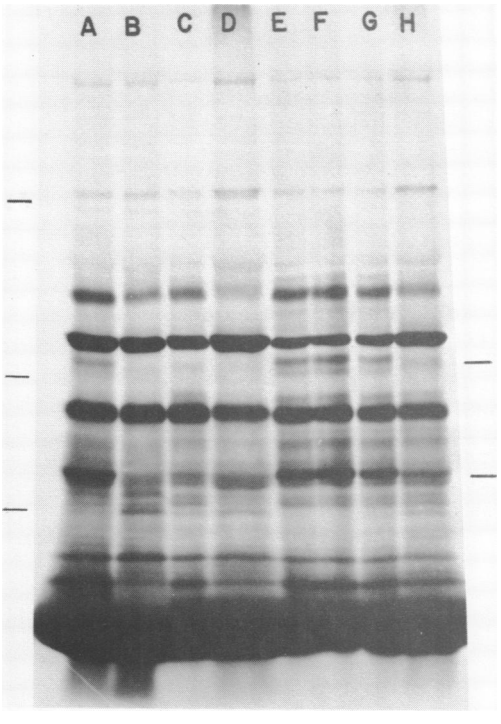

Figure 5. Testosterone regulation of male-abundant mRNAs. [^{35}S]methionine labeled cell-free translation products directed by poly(A)+ mRNA from submandibular glands were analyzed in linear 6-15% gradient polyacrylamide gels (Fig. 2). Track A, products directed by male gland mRNA; track B, products directed by female gland mRNA; tracks C & D, products directed by gland mRNA from female mice injected daily with 75 µl polyethylene glycol 400 for 15 days; tracks E-G, products directed by gland mRNA from female mice injected daily with 1 mg testosterone propionate in 75 µl polyethylene glycol 400 for 15 days; track H, products directed by gland mRNA from castrate male mice 28 days after operation. Each track contained 240,000 cpm; the fluorogram was exposed 2 days.

abundant in products coded by mRNA from SWR/J (control) mice and not in those of the renin deficient variants (Fig. 6). mRNA coding the 48,000 MW polypeptide appears greatly diminished in glands of low-renin C57BL/10J males, but other male-abundant mRNAs encoding the 43,000 MW, 29,000 MW and 27,000 MW polypeptides occur in the variants at concentrations similar to those in male SWR/J controls. These results suggested that the mRNA coding for the 48,000 MW male-abundant polypeptide corresponds to renin precursor mRNA.

Immunochemical identification of cell-free synthesized renin precursor

Immune selection of translation products directed by gland mRNA from male SWR/J and C57BL/10J mice with specific anti-renin antiserum identified the 48,000 MW male-abundant polypeptide as renin precursor (Fig. 7). Incubation of SWR/J gland translation products with immune serum resulted in selection of 2% of input radioactivity (not shown) and only two polypeptides (track G), a

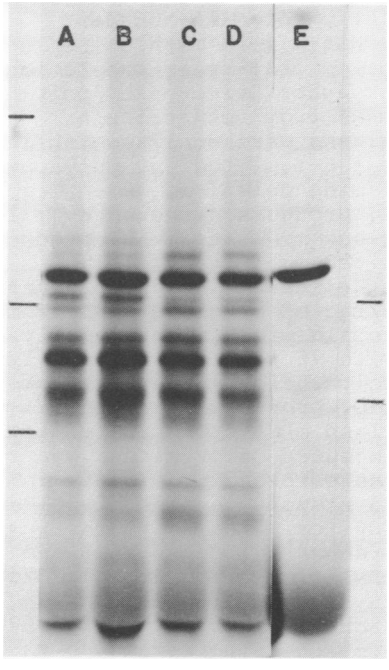

Figure 6. Assay of 48,000 MW male-abundant polypeptide in gland translation products of mice deficient in salivary renin. [^{35}S]methionine-labeled cell-free translation products directed by submandibular gland poly(A)$^+$ mRNA isolated from male SWR/J (control) and C57BL/10J (low salivary renin) mice were analyzed as in Fig. 2. Tracks A & B, products directed by gland mRNA from SWR/J mice; tracks C & D, products directed by gland mRNA from C57BL/10J mice; track E, no mRNA addition. Tracks A-D contained 160,000 cpm; the fluorogram was exposed 2 days.

major polypeptide of 48,000 MW and a minor band of 43,000 MW. The specific binding of these polypeptides to antirenin antiserum was blocked by pure, authentic submandibular gland renin. Identical analysis of C57BL/10J gland translation products revealed an absence of antirenin-selected proteins (track E). Furthermore, no cell-free synthesized polypeptides were selected by a pre-immune, control rabbit serum (tracks D & F). These data unequivocally identify the 48,000 MW polypeptide as renin precursor and demonstrate that renin precursor mRNA is abundant in the submandibular gland of male mice.

DISCUSSION

Mouse submandibular gland contains several abundant, androgen-regulated mRNAs. Evidence for this contention derives from electrophoretic comparison of translation products directed by mRNA from male and female gland and from specific hybrid-arrested translation. The induced accumulation of these male-abundant mRNAs in females treated with testosterone and the disap-

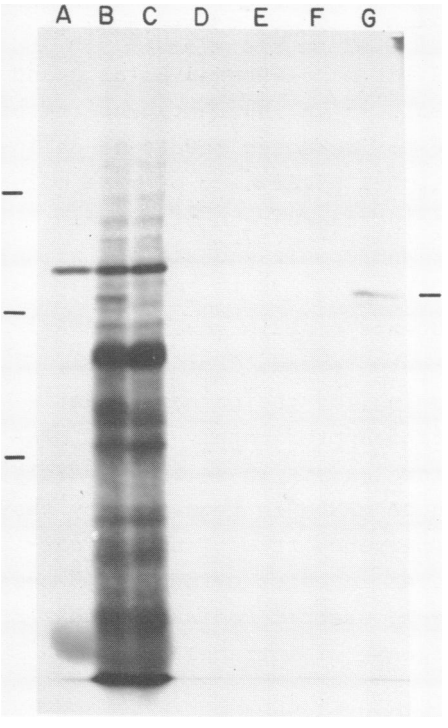

Figure 7. Immune selection of renin precursor polypeptide with renin-specific antiserum. [^{35}S]methionine-labeled cell-free translation products directed by gland mRNA from male SWR/J and C57BL/10J mice were incubated with immune or control serum, and immune complexes were purified by adsorption with S. aureus (Methods). After separation of immune complexes from bacteria (Methods), samples were analyzed electrophoretically as in Fig. 2. Track designations are: A, total products of lysate without mRNA addition; B, total products of SWR/J gland mRNA; C, total products of C57BL/10J gland mRNA; D & E, products of C57BL/10J gland mRNA selected with control and immune serum, respectively; F & G, products of SWR/J gland mRNA selected with control and immune serum, respectively. Tracks A-C contained 3 µl of translation mixture (~80,000 cpm for B,C); tracks D-G contained products immune selected from 8 µl of translation mixture. The fluorogram was exposed 3 days. Bar at right denotes the location of the 48,000 MW renin precursor; bars at left show positions of molecular weight standards.

pearance of these mRNAs from glands of castrate males, illustrates the pattern of androgenic regulation.

The increased relative translational efficiency of total male gland mRNA has at least two tenable explanations. First, mRNAs possibly specific to the male gland may be more efficient templates than gland mRNAs common to both sexes. Second, since intact 5' terminal caps appear to be required for efficient initiation of mRNA,[34] deficiencies in cap structure or the incidence of a greater fraction of incompletely processed mRNAs could account for the reduced biological activity of female gland mRNA. Elevated rates of protein synthesis previously observed *in situ* for organs under steroidal stimulation[35,36] have

been attributed to increased organ mRNA content and to increased initiation factor activities.[35,37-40] However, the elevated translational activity we observed for male submandibular gland mRNA relative to that from the female is an inherent property of isolated poly(A)$^+$ mRNA and not due to increased levels of cellular mRNA. Thus, enhanced translational efficiency of mRNA molecules *per se* may contribute to the increased rate of protein synthesis reported for cells under androgenic and steroid stimulation.

Accessory sex glands of male rodents contain several mRNAs whose expression is regulated by testosterone. For example, rat prostate contains 3-4 mRNAs coding for the primary constituents of seminal fluid, constituting approximately 50% of mRNA mass in that organ; a similar pattern exists in seminal vesicle. In both cases, gland content of these mRNAs is regulated by testosterone as judged by their disappearance from glands of castrate mice.[45-47] In these organs, as in chick oviduct[48-50] and male liver[38,51] primed with estrogen, steroid-mediated gene regulation occurs by selective increase of intracellular mRNA concentration.

Androgen-regulated mRNAs have been described not only in accessory sex glands of male rats but also in mouse liver and kidneys, organs without a direct reproductive role. For example, the mRNA coding for a Major Urinary Polypeptide, an α_2 globulin of 20,000 MW synthesized by mouse liver and excreted in large quantity, occurs at 5-fold higher concentrations in male liver compared to livers of female mice.[31,33] Similarly, kidneys of male mice contain 10 times more mRNA that encodes an abundant 20,000 MW protein than found in female kidney.[32] In these two instances of sexually dimorphic gene expression, the gene products seem unrelated to reproductive function, but cellular mRNA content is influenced strongly by testosterone and possibly by other androgens. In this respect, the situation in submandibular gland seems analogous to that in liver and kidney, because the salivary proteins regulated by androgens appear to be without reproductive roles. The apparent absence of these androgen-regulated mRNAs from submandibular glands of female mice suggests the proteins they encode are unimportant in

digestive function.

The sexually dimorphic pattern of abundant submandibular gland mRNAs correlates well with the coordinate pattern of renin and growth factor secretion by male mouse gland. The rise and fall of gland renin, NGF, and EGF contents in response to testosterone levels in male and female mice is documented thoroughly.[6-9] We have observed the regulation by testosterone of abundant mRNA coding polypeptides of 48,000, 43,000, 29,000, and 27,000 MW, values in agreement with the molecular weights of submandibular gland renin precursor (50,000 MW),[18] α-NGF (27,000 MW),[52] γ-NGF (26,000 MW)[54] and EGF binding protein (29,000 MW).[56] Furthermore, genetic and immunochemical evidence confirm that the male-abundant polypeptide corresponds to mouse salivary renin precursor mRNA. This contention is also supported by affinity chromatography of male gland mRNA translation products on pepstatin-Sepharose,[57] which retained a single cell-free synthesized polypeptide of 48,000 MW coincident with authentic mouse salivary renin (manuscript in preparation). Although immunochemical evidence for other androgenically-regulated proteins is still forthcoming, we believe the submandibular gland will be a useful system for examining coordinate regulation of abundant and androgenically-regulated mRNAs that code for polypeptides of biological importance.

ACKNOWLEDGEMENTS

We thank Mrs. Elizabeth Howell and Ms. Laurie Sturzenbecker for excellent technical assistance and Drs. Ronald A. Malt and John T. Fallon for helpful discussions. This research was supported by NIH grants AM-12769 and HDO-7092-05, by the Stanley Thomas Johnson Foundation, by R.J. Reynolds Industry, and by grant 13-504-789 from the American Heart Association, Massachusetts Affiliate, Inc. V.J.D. is a recipient of Clinical Investigator Award, NIH (5 KO8 00750) and R.P. is the Samuel A. Levine Fellow of the AHA, Massachusetts Affiliate.

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