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Tissue-Specific Expression of the Rat Alpha$_{2u}$ Globulin Gene Family

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The rat α_{2u} globulin gene family encodes approximately 20 low-molecular-weight (20,000) proteins with pIs ranging from 4.5 to 7.9. α_{2u} globulin protein isoforms were detected in the liver and in the submaxillary, lachrymal, preputial, and mammary glands of Sprague-Dawley rats. The hormonal and developmental regulation of α_{2u} globulin synthesis in each of these tissues was unique, and it appears that different α_{2u} gene sets were transcribed in the various tissues.

The α_{2u} globulins are a group of closely related low-molecular-weight proteins of unknown function synthesized in the rat. α_{2u} globulin was first found in the urine of mature males and was later shown to be synthesized in the liver (13, 14). Synthesis of hepatic α_{2u} globulin is under complex hormonal and developmental control (4, 5, 9) and has proven to be an excellent model system for the study of hormonal control of gene expression.

The α_{2u} globulins are encoded by 20 to 25 genes clustered on chromosome 5 (6). These genes have a very high degree of sequence homology (4, 6). Laperche et al. (10) showed that different members of the α_{2u} globulin gene family are expressed in the liver and submaxillary gland. We examined the expression of individual members of the α_{2u} globulin gene family by studying the protein isoforms expressed in different tissues. In addition to those in the liver and submaxillary gland, we were able to detect and characterize α_{2u} globulins in three other tissues, the lachrymal, mammary, and preputial glands. The levels of α_{2u} mRNA and protein varied greatly in the different tissues as did the hormonal regulation of α_{2u} globulin synthesis.

Rats were purchased from Taconic Farms, Germantown, N.Y. The animals were maintained on a 12-h light-dark cycle and fed laboratory chow ad libitum. Tissues were removed and placed in liquid nitrogen, and S100 fractions were prepared as described previously (16). α_{2u} globulin antiserum was prepared as described previously (7).

From 20 to 150 g of total protein S100 extract was applied to ultrathin isoelectric focusing (IEF) gels (Serva, Garden City, N.Y.) in accordance with the instructions of the manufacturer. After electrophoresis, the proteins were fixed by immersing the gels in 20% trichloroacetic acid for 1 min. The gels were then processed by using a modification of the Western blotting procedure of Towbin et al. (17). The gels were blocked for 1 h in phosphate-buffered saline (PBS) containing 3% gelatin (Norland, New Brunswick, N.J.). The gels were then incubated in PBS plus 0.5% Tween 20 (Fisher Scientific Co., Pittsburgh, Pa.) containing column-purified α_{2u} globulin antiserum (1:1,000) for 6 to 18 h and washed for 2 h in 5 changes of a buffer containing 0.5% deoxycholic acid, 0.5% Triton X-100, and 0.1% sodium dodecyl sulfate in PBS. After being washed, the gels were incubated in PBS plus 0.5% Tween 20 containing 10^6 cpm of ^{125}I-labeled protein A per ml (New England Nuclear Corp., Boston, Mass.; specific activity, 100 mCi/ml) for 1 h, washed as described above, dried, and autoradiographed in the presence of intensifying screens for 12 to 48 h.

The preparation of tissue RNA and analysis by Northern or dot blotting was described previously (6, 7). The dot and Northern blots were hybridized with a nick-translated α_{2u} cDNA probe described by Kurtz and Nicodemus (9).

Messenger RNA was translated by using a New England Nuclear translation kit containing dog pancreatic microsomes in accordance with the instructions of the manufacturer. α_{2u} globulin was recovered from the translation cocktail by immunoprecipitation, as described previously (7). Immunoprecipitated ^{35}S-labeled proteins were eluted in urea buffer and analyzed by two-dimensional gel electrophoresis by the method of O'Farrell (11).

Multiple α_{2u} isoforms from the various tissues were resolved with a one-dimensional IEF system. From 7 to 18 α_{2u} globulin isoforms with pIs ranging from 4.5 to 7.9 were detected in the liver and in the lachrymal, submaxillary, preputial, and mammary glands (Fig. 1). To determine whether these isoforms resulted from the expression of multiple genes or from posttranslational modification,

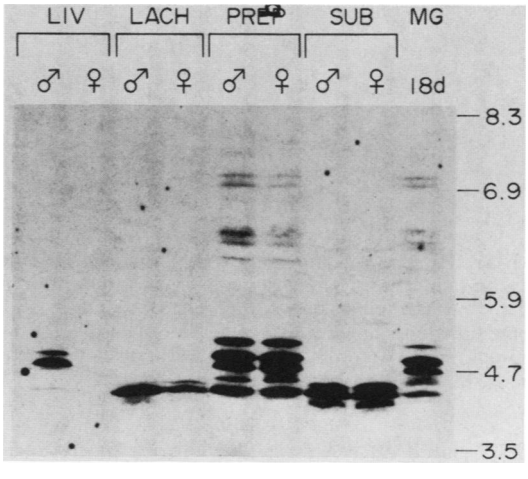

FIG. 1. Total protein (S100) extract from the preputial gland (PREP) (20 μg) and from the liver (LIV), lachrymal gland (LACH), submaxillary gland (SUB), and mammary gland (MG) (150 μg each) was applied to an IEF gel (pH 3 to 10) and subjected to electrophoresis. The α_{2u} globulin isoforms were visualized by Western blotting with column-purified α_{2u} antibody and ^{125}I-labeled protein A, as described in Materials and Methods. The positions of pI marker proteins (Serva) are indicated on the right. d, Days.

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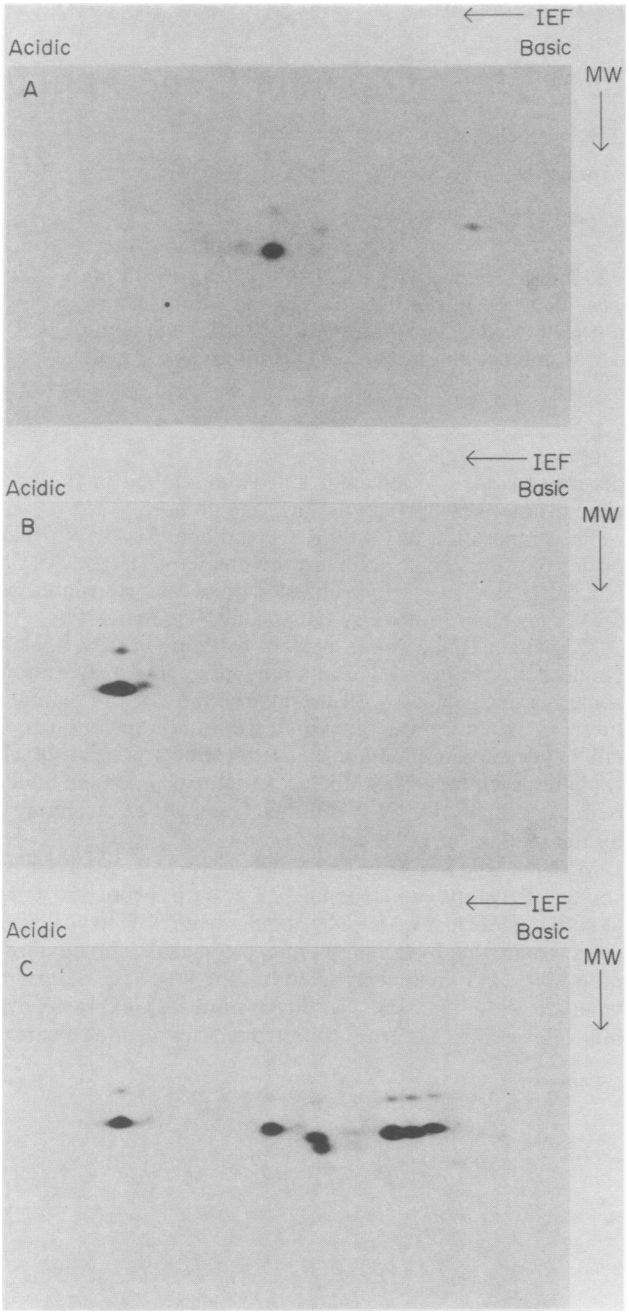

FIG. 2. IEF of α_{2u} globulins. mRNA (2 μg each) from the liver (A), lachrymal gland (B), and preputial gland (C) of the male was translated in the presence of dog pancreatic microsomes, immunoprecipitated, subjected to two-dimensional electrophoresis, and visualized by autoradiography, as described in Materials and Methods. MW, Molecular weight.

polyadenylated mRNA from the various tissues was translated and immunoprecipitated, and the in vitro-translated proteins were analyzed by two-dimensional gel electrophoresis (Fig. 2). The number and pIs of the in vitro-translated proteins corresponded closely with those of the α_{2u} globulin isoforms detected in tissue S100 extracts (Fig. 1). Although many isoforms were common to more than one tissue, each tissue expressed a unique subset of isoforms (Fig. 1).

In addition to differences in the number and pIs of the α_{2u} isoforms, dot blot analysis indicated that the overall levels of α_{2u} globulin mRNA varied approximately 60-fold in the different tissues (Fig. 3A and Table 1). Northern blots showed that in all tissues except the salivary gland a single α_{2u} globulin mRNA with a size of approximately 1 kilobase was present (Fig. 3B); the salivary gland appeared to contain two α_{2u} mRNA species, probably arising from differential splicing (10). Several other tissues (brain, kidney, lung, and heart) had no detectable α_{2u} globulin mRNA.

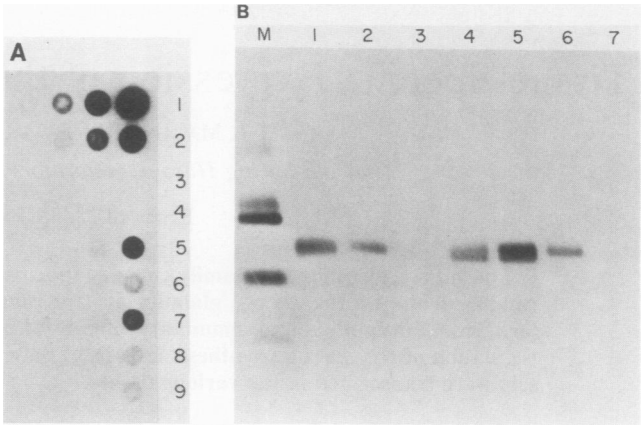

FIG. 3. Dot blot analysis of α_{2u} globulin RNA abundance. (A) Fivefold dilutions (from 1.0 to 0.08 μg) of mRNA from the adult-male preputial gland (1), the adult-male liver (2), the adult-female liver (3), the adult-male kidney (4), a 25-day-old submaxillary gland (5), a 60-day-old submaxillary gland (6), the adult-male lachrymal gland (7), the adult-female lachrymal gland (8), and a mammary gland of a female 18 days pregnant (9) were applied to nitrocellulose. The bound RNA was hybridized with α_{2u} cDNA and visualized by autoradiography, as described in Materials and Methods. (B) Northern blot of tissue mRNA. From 0.2 to 2.0 μg of mRNA was subjected to electrophoresis on a 1.5% gel, transferred to nitrocellulose, and hybridized with a nick-translated probe, p*a*176, as described in Materials and Methods. Shown are molecular weight standards (M), 0.1 μg of preputial gland mRNA (1), 0.3 μg of male liver mRNA (2), 2.0 μg of female liver mRNA (3), 2 μg of salivary gland mRNA (4), 2 μg of lachrymal gland mRNA (5), 10 μg of mammary gland mRNA from a female 18 days pregnant (6), and 10 μg of male kidney mRNA (7).

TABLE 1. Relative amounts of α_{2u} globulin mRNA in the liver and in the submaxillary, lachrymal, preputial, and mammary glands[a]

Tissue	% of α_{2u} globulin mRNA in adult-male liver
Adult preputial	330
Adult-male liver	100
Adult-female liver	0
Adult-male kidney	0
Adult-male lachrymal gland	30
Adult-female lachrymal gland	10
30-day-old submaxillary gland	25
Adult submaxillary gland	7
18-day-gestation mammary gland	5

[a] Total RNA from the tissues was analyzed by dot blotting as described in Materials and Methods. The relative amounts were calculated from the slopes of the lines generated by plotting counts per minute versus micrograms of RNA.

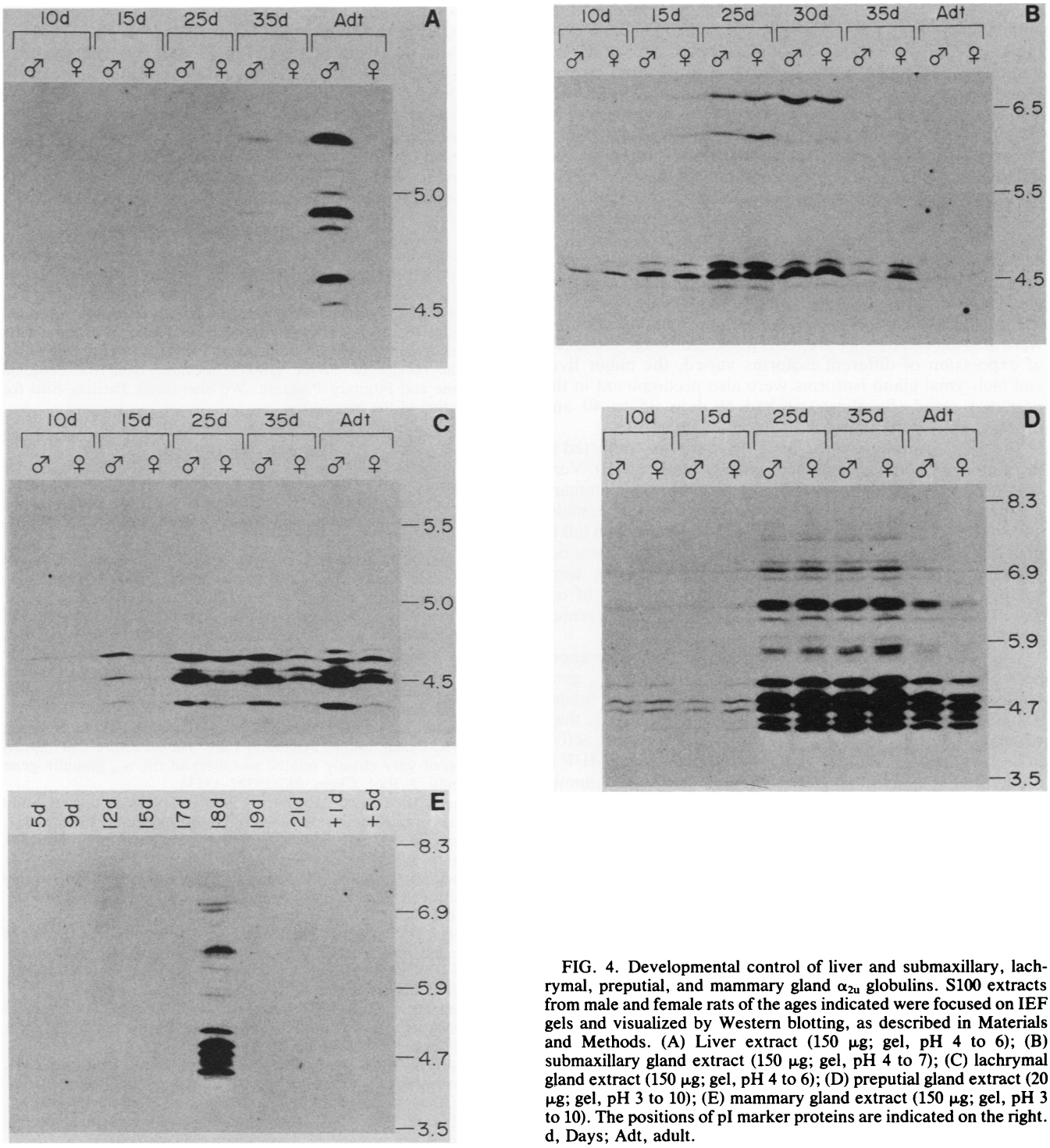

FIG. 4. Developmental control of liver and submaxillary, lachrymal, preputial, and mammary gland α_{2u} globulins. S100 extracts from male and female rats of the ages indicated were focused on IEF gels and visualized by Western blotting, as described in Materials and Methods. (A) Liver extract (150 μg; gel, pH 4 to 6); (B) submaxillary gland extract (150 μg; gel, pH 4 to 7); (C) lachrymal gland extract (150 μg; gel, pH 4 to 6); (D) preputial gland extract (20 μg; gel, pH 3 to 10); (E) mammary gland extract (150 μg; gel, pH 3 to 10). The positions of pI marker proteins are indicated on the right. d, Days; Adt, adult.

Four major and three minor isoforms of α_{2u} globulin were detected in the mature male rat liver (Fig. 4A). As previously reported (3, 7), α_{2u} globulin could not be found in the livers of intact females or in juvenile males. Low levels of α_{2u} globulin was first detected in the male at about 35 days. Synthesis increased as the animal matured, reaching maximal adult levels at about day 60. All isoforms of α_{2u} globulin appeared to be coordinately induced in the liver.

The submaxillary gland expressed a more acidic subset of the α_{2u} proteins (Fig. 1). In contrast to those in the liver, submaxillary α_{2u} globulins were detected as early as day 10 in both males and females (Fig. 4B). The level of expression in both sexes was similar, and there was a marked increase in synthesis during development, reaching maximal levels at day 30, followed by an abrupt decrease at puberty.

The lachrymal glands also expressed an acidic subset of

α_{2u} globulins (Fig. 1). The lachrymal isoforms appeared to be almost identical to the submaxillary α_{2u} globulins; however, the regulation of expression in the two tissues was very different. Like those in the submaxillary gland, lachrymal α_{2u} globulins were detected as early as day 10 (Fig. 4C). There was a small increase in the level of synthesis with development, but there was no decrease in expression at puberty. At all ages, synthesis in the male was three- to five-fold higher than that in the female, although the same subset of isoforms was expressed in both sexes.

Very high levels of α_{2u} globulin were present in the preputial glands of both male and female rats. Eighteen isoforms could be detected in both males and females after day 25 (Fig. 4D). Except for the two most acidic lachrymal gland isoforms, all of the α_{2u} globulins expressed in the liver and in the submaxillary, lachrymal, and mammary glands were also detected in the preputial gland. Although the levels of expression of different isoforms varied, the major liver and lachrymal gland isoforms were also predominant in the preputial gland. Synthesis peaked at days 35 to 40 and dropped slightly in adults.

The 18 preputial gland α_{2u} isoforms were also detected in the mammary glands of pregnant female rats (Fig. 4E). Very low levels of α_{2u} globulin could be detected in the mammary gland at days 15 to 16 of gestation in some groups of animals, and synthesis peaked sharply at days 18 to 19 and then fell to undetectable levels by days 20 to 21. α_{2u} globulins were not detected in the nursing animals or after the pups were weaned. A more detailed analysis of the regulation of α_{2u} globulin synthesis in the mammary gland will be presented elsewhere.

There are several similarities and some major differences between the tissue-specific regulation of the rat α_{2u} gene family and that of the mouse analog of α_{2u}, MUP (major urinary polypeptide). The most striking difference is that, whereas the preputial gland in rats is by far the most active in α_{2u} synthesis (5 to 10% of the total mRNA), no MUP is found in mouse preputial glands (B. Held, personal communication). If α_{2u} globulins and MUP are indeed behavioral cues (or carriers thereof), as has been suggested (15), the preputial gland would be a logical source for such proteins, since it is known that male rat preputial gland secretions are responsible for the acceleration or synchronization of estrus in females (2).

The hormonal and developmental induction of α_{2u} globulins and MUP is very similar in both the lachrymal and submaxillary glands. In the lachrymal gland, there is a sexual dimorphism in expression such that males synthesize three to five times more than females do. In both the rat and mouse submaxillary glands, protein synthesis peaks in juveniles and drops sharply at puberty. In rats the lachrymal and submaxillary α_{2u} proteins seem to be encoded largely by one gene set, distinct from the gene set expressed in the liver; in contrast, in mice the submaxillary MUP gene family appears to be a subset of the liver family and the lachrymal gene family is quite distinct from both.

The regulation of α_{2u} globulins and MUP in the mammary gland appears to be similar; both peak just past midgestation then fall sharply at parturition. However, whereas the rat mammary gland α_{2u} globulins include 18 to 20 isoforms, only one MUP isoform is found in the mouse mammary gland.

The number of α_{2u} isoforms found in the preputial and mammary glands (18 to 20) is close to the number of α_{2u} genes present in the rat genome. It appears likely that the entire repertoire of α_{2u} genes is transcriptionally active in these tissues. A specific subset of these genes is apparently expressed in the liver, and another subset is apparently expressed in the submaxillary and lachrymal glands. There have been many recent reports (1, 12, 18) on the existence of tissue-specific enhancers, i.e., a DNA sequence near (or sometimes in) a gene which greatly increases transcription when the gene is introduced into the proper tissue type. The α_{2u} globulin gene family affords the opportunity to identify such sequences for several different tissues. Experiments are under way to establish the molecular basis for the tissue-specific expression of the α_{2u} globulin gene family.

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