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ANDROGENIC REGULATION OF HEPATIC GENE EXPRESSION

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Summary—Androgen-dependent synthesis of α2u globulin in the rat liver has been used in our laboratory as a model for studying the effect of sex hormones on hepatic gene expression. α2u Globulin is a group of low molecular weight ($M_r \sim 18{,}000$) male specific urinary proteins synthesized and secreted by hepatocytes. In the male rat hepatic synthesis of α2u globulin begins at puberty (~40 days), reaches a peak level (~20 mg/day) at about 75 days and declines during old age. Androgens can induce α2u globulin in ovariectomized female rats *in vivo* and in the liver perfusion system *in vitro*. However, both prepubertal and senescent (>800 days) male rats not only do not produce α2u globulin but are also refractory to androgen administration. α2u Globulin is coded by a multigene family comprising about 20–30 gene copies per haploid genome. All of these gene copies seem to express translationally active mRNAs giving rise to individual isoforms of α2u globulin. Appearance and disappearance of the cytoplasmic androgen-binding protein (CAB) correlates with the androgen responsiveness of hepatocytes. Photoaffinity labeling of the hepatic cytosol shows that the biologically active binding protein, found in the cytosol of the mature male rat liver, has a molecular weight of 31 kDa. A molecular transition of the 31-kDa CAB to a biologically inactive 29-kDa form may be the basis of hepatic androgen insensitivity during prepuberty and senescence.

INTRODUCTION

The liver is the central regulatory organ for metabolic co-ordination. The metabolic needs of male and female animals especially during the reproductive periods vary considerably. Such variations in the sexually dimorphic hepatic metabolism are mediated by structural and functional alterations of liver cells. Sex hormones play key roles in orchestrating these sex-specific changes primarily via the regulation of hepatic gene expression. Some of the effects of the sex-steroids are exerted directly on the liver while others may be indirectly mediated through the pituitary hormones such as growth hormone and prolactin. The sexually dimorphic pattern of the hepatic gene expression in higher animals has been reviewed by Roy and Chatterjee [1] and by Gustafsson *et al.*[2]. Although the livers of all higher animals show enzymatic and other changes after exposure to sex steroids, the effect is highly conspicuous in the case of oviparous females and in male rodents. This is due to the high rate of synthesis of the egg-yolk protein vitellogenin in the female and the male-specific urinary proteins in rodents. The estrogenic regulation of vitellogenin gene expression in frogs and birds has been extensively studied in many laboratories and reviewed by several authors [3, 4]. Studies in our laboratory have led to the identification and characterization of the male rat urinary protein celled α2u globulin and we have undertaken an in-depth investigation of the mechanism of the hormonal regulation of α2u globulin synthesis in the rat liver [5]. Several other groups have also contributed towards an overall understanding of the hepatic synthesis of α2u globulin [6–8]. Hepatic synthesis of a similar urinary protein in the mouse has also been the subject of endocrinological interest [9]. In this article we will describe some of our findings concerning the androgenic regulation of α2u globulin gene expression in the rat liver with a special emphasis on the possible role of a cytoplasmic androgen-binding protein in androgen action.

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STRUCTURE AND EXPRESSION OF THE MULTIPLE COPIES OF α2U GLOBULIN GENE

Two-dimensional gel electrophoresis of the hepatocyte lysates pulse labeled *in vitro* with [^{35}S] methionine can identify sex-differences in the synthesis of major hepatic proteins. Results of such a study are presented in Fig. 1. The pattern of the newly synthesized proteins from normal male and female hepatocytes differs in two major molecular groups, one within the 28–68- and the other within the 18–22-kDa range. All four of the female specific proteins fall within the 28–68-kDa group (Fig. 1A). Within this size class, six male specific proteins can also be identified (Fig. 1B). Structural and regulatory features of this group of hepatic proteins are largely unknown. The other group, i.e. the 18–22-kDa component (Fig. 1B) is comprised almost totally of the various polymorphic forms of α2u globulin, the male rat urinary protein. This group is composed of five major and one minor isoelectric forms and within the same isoelectric forms some of the components show 2–3 molecular weight variants.

The synthesis of α2u globulin shows both androgenic induction and estrogenic repression. In addition, different isomorphic forms of α2u globulin show differential sensitivity to androgenic regula-

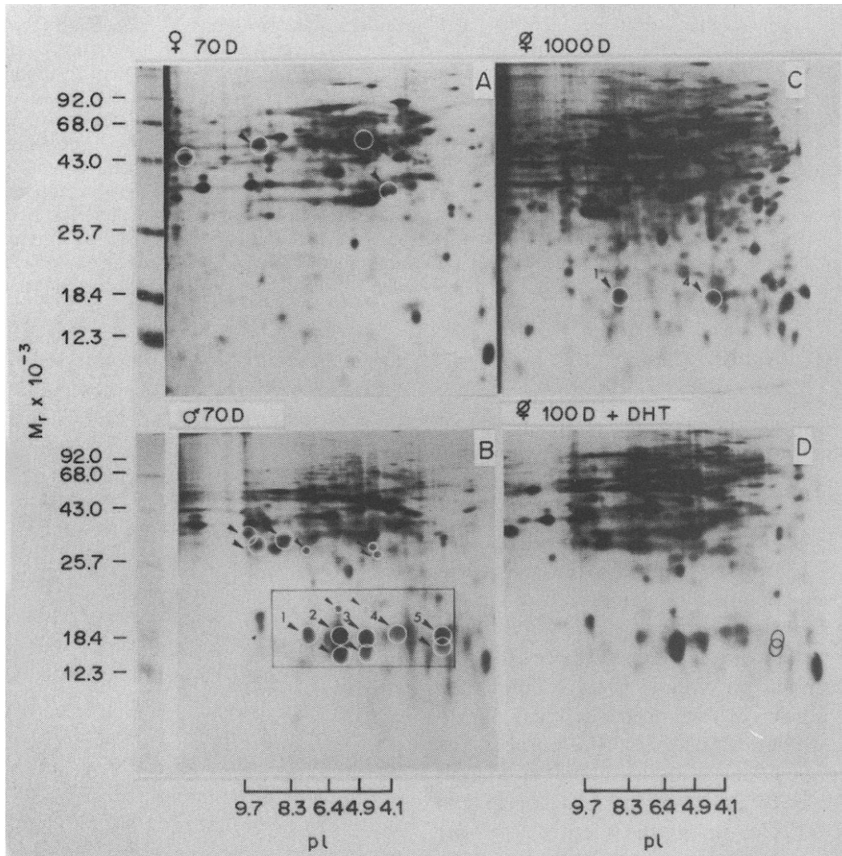

Fig. 1. Sex-specific synthesis of hepatic proteins. The picture shows autoradiograms of two-dimensional gel electrophoresis of radio-labeled hepatocyte lysates. Hepatocytes were derived from male and female rats as identified on the top of each frame. Hepatocytes were labeled *in vitro* for 2 h with [^{35}S]methionine. Frame A, 70-day-old female; Frame B, 70-day-old male; Frame C, 100-day-old female ovariectomized at 70 days of age; Frame D, 100-day-old female ovariectomized at 70 days of age and treated daily for 10 days with 5α-dihydrotestosterone (50 μg/100 g) before sacrifice. Sex-specific protein spots are identified with circles. In frame B, the α2u globulin complex is enclosed within the rectangular box.

tion. The major isoelectric forms 1 and 4 can be made to appear simply by removing the estrogenic influence from the female through ovariectomy (Fig. 1C) while the isoelectric form 5 shows the most stringent androgen requirement (Fig. 1D). The differential decline of the various isoforms of α2u globulin during aging has also been noted [10].

The variant forms of α2u globulin are produced from a multigene family composed of 20–30 gene copies per haploid genome [8, 11]. This gene cluster occupies about 720 kb of DNA. Both visual observation of the autoradiographic pattern in Fig. 1 and quantification of the five major isolectric forms presented in Fig. 2 show a more than 10-fold variation in the hepatic concentrations of individual isoforms of α2u globulin. Although differential expression of the individual isoforms is certainly one facet of the regulatory complexity of this gene family, much of the observed variations may be due to the multiple copies of some isoform gene copies with silent mutations. We have sequenced several cloned cDNAs corresponding to α2u globulin mRNA. Nucleotide sequences of two of the clones presented in Fig. 3 exemplify the point. Although these two mRNAs differ in seven positions, only three of these base substitutions alter the aminoacid codes. Substitution at codon 71 converts serine to alanine, that at 98 converts tyrosine to aspartic acid and the point mutation at 99 changes isoleucine to arginine. This example indicates certain gene copies may contain base substitutions which either do not change the amino acid code (as in codons 68, 78, 97 and 100) or change into amino acids of the same charge characteristic (as shown in codon 71) or even produce amino acid substitutions of opposite charges (as in codons 98 and 99), thus maintaining the same isoelectric point of the two individual gene products.

Taking the isoelectric variant 4 as the lowest denominator and possibly the product of a single copy of the α2u globulin gene, one can account for at least 27 functional gene copies within this gene family. Such a calculation, however, assumes that the contribution from differential regulation of the individual genes acts both in positive and negative manners and does not introduce any gross error into this calculation. Thus it is likely that none of the members of this gene family constitutes non-functional pseudogenes.

ANDROGENIC STIMULATION α2u GLOBULIN SYNTHESIS *IN VITRO*

Studies with whole animals have shown that in addition to androgen, growth hormone, insulin, glucocorticoid and thyroxine can synergistically promote the hepatic synthesis of α2u globulin [11]. Such *in vivo* observations of multihormonal interactions open the possibility of the indirect influence of some of the hormonal mediators. Because of the complex multicellular interactions in the hormonal induction of α2u globulin in the rat liver [12], isolated hepatocytes in culture have not proved to be a suitable system for studying the effect of individual hormones on the α2u globulin system. We have been able to circumvent this problem by utilizing the *in vitro* liver perfusion for studying the direct effect of the steroid and non-steroid hormones. In such experiments, liver with the circulatory system intact is removed from the animal and is perfused with the rabbit blood deficient in appropriate hormones [13]. Use of the heterologous blood allows an accurate quantification of the secreted α2u globulin into the circulating fluid through the sensitive radioimmunoassay. Figure 4 shows a rapid androgenic stimulation of α2u globulin production by the liver derived from castrated male rats. The stimulation is observed within 30 min after androgen administration, and within 120 min about a 10-fold higher level of α2u globulin accumulates in the androgen supplemented system as compared to the vehicle control. Labeling experiments with [^{35}S]methionine show that the androgen-mediated increase in α2u globulin is due to an accumulation of the newly synthesized protein. So far we have not been able to demonstrate the existence of nuclear androgen receptors in hepatocytes—thus the molecular mechanism of such a rapid and large increase in α2u globulin synthesis by the androgen *in vitro* still remains to be established.

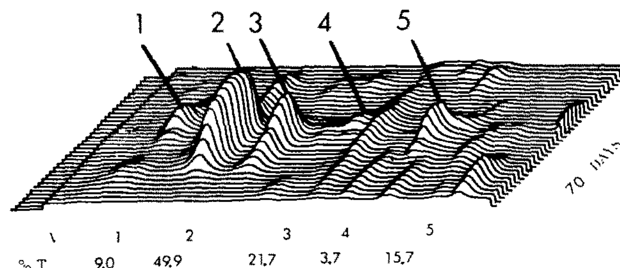

Fig. 2. Computerized scanning of the various isoforms of α2u globulin synthesized by hepatocytes derived from a mature male rat. The major isoelectric variants are numbered 1–5. The relative percentage (% *T*) of each major isoelectric variant (*V*) are presented at the bottom.

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NUCLEOTIDE SEQUENCE OF

α2u GLOBULIN cDNA

CTG CTG CTG CTG TGT CTG CGC CTG ACA CTG GTC TGT GGC CAT GCA GAA

GAA GCT AGT TCC ACA AGA GGG AAC CTC GAT GTG GCT AAG CTC AAT GGG

GAT TGG TTT TCT ATT GTC GTG GCC TCT AAC AAA AGA GAA AAG ATA GAA

GAG AAT GGC AGC ATG AGA GTT TTT ATG CAG CAC ATC GAT GTC TTG GAG

AAT TCC TTA GGC TTC AAG TTC CGT ATT AAG GAA AAT GGA GAG TGC AGG

GAA CTA TAC TTG GTT TCC TAC AAA ACG CCA GAG GAT GGT GAA TAT TTT
          T           G                             C
GTT GAG TAT GAC GGA GGG AAT ACA TTT ACT ATA CTT AAG ACA GAC TAC
                                                              T
TAC ATA TAC GTC ATG TTT CAT CTC ATT AAT TTC AAG AAC GGG GAA ACC
G   G     T
TTC CAG CTG ATG GTG CTC TAC GGC AGA ACA AAG GAT CTG AGT TCA GAC

ATC AAG GAA AAG TTT GCA AAA CTA TGT GAG GCG CAT GGA ATC ACT AGG

GAC AAT ATC ATT GAT CTA ACC AAG ACT GAT CGC TGT CTC CAG GCC CGA

GGA TGA AGA AAG GCC TGA GCC TCC AGT GCT GAG TGG AGA CTT CTC ACC

AGG ACT CTA GCA TCA CCA TTT CCT GTC CAT GGA GCA TCC TGA GAC AAA

TTC TGC GAT CTG ATT TCC ATC CTC TGT CAC AGA AAA GTG CAA TCC TGG

TCT CTC CAG CAT CTT CCC TAG TTA CCC AGG ACA ACA CAT CGA GAA TTA

AAA GCT TTC TTA AAT TTC TCT TGG CCC CAC CCA TGA TCA TTC CGC ACA

AAT ATC TTG CTC TTG CAG TTC AAT AAA TGA TTA CCC TTG CAC TTT
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Fig. 3. Nucleotide sequence of two α2u globulin mRNAs. Base substitutions at positions 71, 98 and 99 alter the amino acid code as indicated at the bottom. The rest of the point mutations do not alter the amino acid sequence. Despite all of these base substitutions the protein products of these two mRNAs are expected to comigrate on two-dimensional gel electrophoresis.

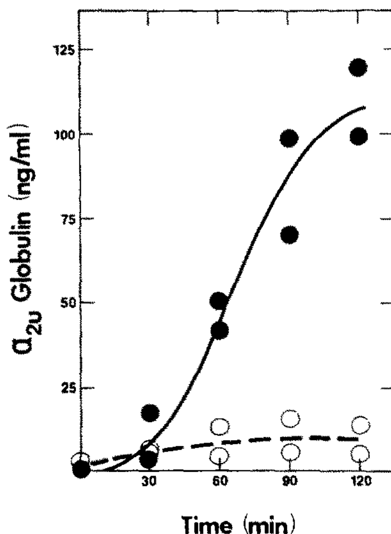

Fig. 4. Cumulative levels of α2u globulin in the perfusates of castrated male rat livers with and without *in vitro* androgen supplementations. 5α-Dihydrotestosterone (300 μg) ●—●, or the vehicle (5 μl ethanol) ○—○, was added to the perfusion fluid at 0 min. Samples were taken at 30-min intervals and α2u globulin concentrations were determined by radioimmunoassay. Data points are from two separate sets of experiments (4 animals).

POSSIBLE ROLE OF THE CYTOPLASMIC ANDROGEN-BINDING PROTEIN IN THE HEPATIC SYNTHESIS OF α2u GLOBULIN

Although we have not been able to demonstrate the presence of the nuclear androgen receptor in hepatocytes, studies in our laboratory have identified a cytoplasmic androgen binding protein (CAB) whose presence correlates with the ability of the androgen to stimulate α2u globulin synthesis [5]. Recently, by means of photoaffinity labeling with tritiated methyl-trienolone (R-1881), we have further characterized this cytoplasmic androgen binding moiety and have obtained indications for its molecular alteration during transition from the androgen-sensitive to androgen-insensitive state as observed during aging. Specific androgen-binding proteins present within the liver cytosols derived from prepubertal adult and senescent male and adult female rats are presented in Fig. 5. Among these various types only the liver of the adult male rat is readily responsive to androgenic stimulation for the synthesis of α2u globulin. A major 31-kDa androgen-binding protein can be demonstrated within the cytosol of the adult male rat. This band is absent in the prepubertal and senescent males which are androgen insensitive and also within the hepatic

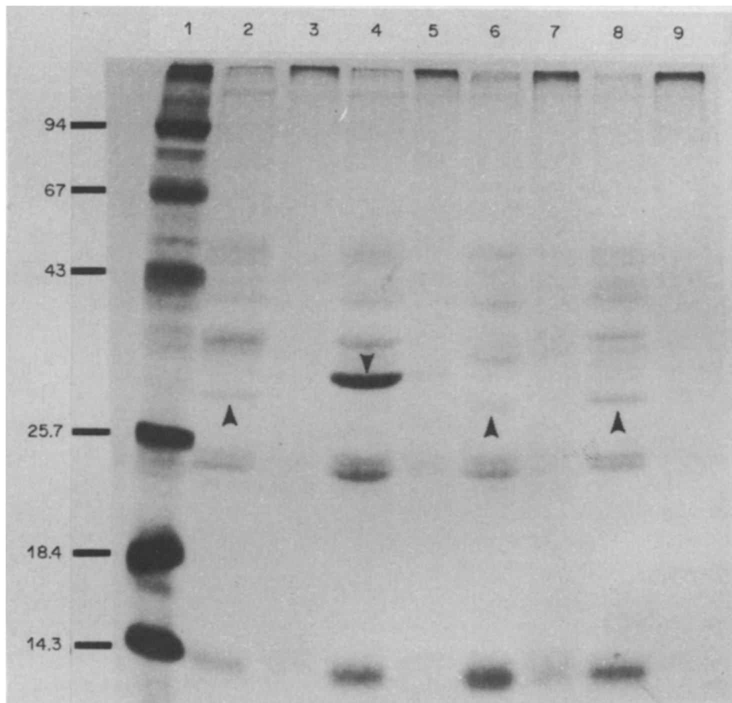

Fig. 5. Electrophoresis of proteins labeled with [^{3}H]R-1881 in hepatic cytosols derived from animals of different ages and sexes. Lane 1, labeled molecular weight markers; Lanes 2 and 3, 30-day-old male without and with a 500-fold excess unlabeled R-1881; Lanes 4 and 5, 100-day-old male; Lanes 6 and 7, 850-day-old male; Lanes 8 and 9, 100-day-old female. The downward arrow marks the 31-kDa binding protein and the upward arrows mark the 29-kDa binder. The binding components which do not show any age- or sex-differences may represent steroid-metabolizing enzymes.

cytosol of the female rats which normally do not produce α2u globulin [5]. Interestingly, however, in all of the latter three cases, the 31-kDa binding component is replaced by another binding component of a slightly lower molecular weight (29-kDa). At present we do not have any evidence concerning the interconvertibility of the 31- and 29-kDa androgen-binding components, but if further studies prove this to be the case, it will provide a novel mechanism for the regulation of the tissue specific androgen sensitivity during maturation and aging.

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