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Detection of Early Changes in Androgen-Induced Mouse Renal β-Glucuronidase Messenger Ribonucleic Acid Using Cloned Complementary Deoxyribonucleic Acid†

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ABSTRACT: β-Glucuronidase mRNA was purified from androgen-induced mouse kidney by immunoadsorption of polysomes to protein A–Sepharose. Cell-free translation of mRNA isolated from the protein A bound RNA followed by immunoprecipitation revealed that β-glucuronidase mRNA represented approximately 2% of the purified mRNA fraction. This mRNA preparation was used to produce complementary DNA clones by recombination with pBR322. Clones containing sequences that were enriched during the purification procedure were selected by differential colony hybridization. These were further screened for homology with β-glucuronidase mRNA by hybrid-selected translation. A β-glucuronidase cDNA clone, designated pGUS7, was identified by these criteria. With this plasmid, the abundance of β-glucuronidase mRNA in total poly(A) mRNA from androgen-induced mouse kidney was estimated to be less than 0.04%. The β-glucuronidase cDNA plasmid hybridized to a mRNA of 2.6 kb in length, which was induced in an androgen receptor dependent fashion over a time course of 21 days. Treatment of female mice with a single dose of testosterone (10 mg) revealed that β-glucuronidase mRNA concentration begins to increase between 12 and 24 h after hormone administration.

Testosterone and other androgenic steroids stimulate the proximal convoluted tubules of mouse kidney, causing renal hypertrophy and increased RNA and protein synthesis (Bardin & Catterall, 1981). The synthesis of several enzymes is specifically enhanced, the best characterized of which is β-glucuronidase (EC 3.2.1.31) (Swank et al., 1978, Bardin et al., 1978). Studies in testicular feminized animals (Tfm/Y), which lack functional androgen receptor, have shown these changes to be androgen receptor dependent (Bullock & Bardin, 1974; Bardin et al., 1978). Induction of β-glucuronidase activity occurs over an unusually long period, reaching maximal levels in 20–30 days during chronic androgen treatment. There is also a lag of approximately 2 days in the rise of enzyme activity in response to the hormone (Swank et al., 1978; Pajunen et al., 1982).

The response of β-glucuronidase to androgens is controlled by a cis-acting genetic regulatory locus, Gus-r (Paigen, 1979). The availability of Gus-r genetic mutants is a feature of the mouse renal β-glucuronidase model system that is unique among steroid hormone regulated genes. Studies of the effect of the genetic regulator on β-glucuronidase mRNA have been hampered by the lack of a direct assay for mRNA concentration. The kinetics of induction have been studied by using a catalytic assay of β-glucuronidase mRNA activity (Watson et al., 1981). However, this assay cannot measure mRNA concentration in the absence of biological function, and it is not sensitive enough to measure changes in mRNA activity in the early phase of the induction cycle. Recombinant DNA clones containing β-glucuronidase complementary DNA (cDNA) would greatly facilitate, and improve the resolution of, these studies. In addition, structural studies made possible by cloning the cDNA will allow correlation of altered gene structure with mutant phenotype.

In this report, we describe the purification of β-glucuronidase mRNA and identification of β-glucuronidase cDNA clones. The cloned DNA has been used to characterize β-glucuronidase mRNA by "Northern" blot hybridization and to detect the initiation of mRNA accumulation in response to androgen treatment.

Experimental Procedures

Animals and Hormone Treatment. Female A/J mice were obtained from Jackson Laboratories at 8–10 weeks of age. Androgen-induced animals were implanted for 5–21 days with Silastic rods (2 cm), which were composed of 25% 5α-dihydrotestosterone (DHT) and 75% Silastic. The release rate

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of these subdermal implants was approximately 120 μg of DHT per day.

Male and female NCS mice [randomly bred strain Rku: NCS (S) SPF] were obtained from the Laboratory Animal Research Center of the Rockefeller University. Androgen-insensitive (Tfm/Y) mice were supplied by Dr. C. D. Toran-Allerand of Columbia University. Single doses of testosterone (10 mg) were administered by intraperitoneal injection in 0.2 mL of 10% (v/v) ethanol in sesame oil.

Purification of β-Glucuronidase Antibody. Antiserum to β-glucuronidase purified from mouse urine was prepared by immunizing New Zealand white rabbits with the denatured enzyme according to Pajunen et al. (1982) and was provided by Dr. O. A. Jänne. Purification of the antiserum was accomplished by protein A–Sepharose (Pharmacia) chromatography. The protein A–Sepharose column (5 mL; 0.9 × 7 cm) was equilibrated with 0.1 M KH_2PO_4, pH 7.0. After application of the antiserum (5 mL) the column was washed extensively with the same buffer and eluted with 0.1 M glycine, pH 3.0. Fractions (1 mL) were collected and neutralized with 1 M Tris. The β-glucuronidase immunoglobulin fraction (designated β-G Ab) was adjusted to 330 μg/mL with heparin and dialyzed against 0.1 M phosphate-buffered saline (PBS; 10 mM NaH_2PO_4, pH 7.2, 140 mM NaCl) containing 130 μg/mL heparin.

Purification of β-Glucuronidase-Synthesizing Polysomes. Kidneys (35 g) from female A/J mice induced for 21 days with DHT were homogenized in 20 mM Tris, pH 7.6, 10 mM $Mg(C_2H_3O_2)_2$, 75 mM KCl, 7 mM 2-mercaptoethanol, 250 mM sucrose, 1 mg/mL heparin, and 5 μg/mL cycloheximide. Cell debris was removed by centrifugation at 10000*g*, and the pellet was subjected to a second homogenization step. After removal of cell debris, the supernatant fractions were combined and adjusted to 1% (w/v) deoxycholate and 1% (v/v) Triton X-100. Released membrane fragments were removed by centrifugation at 46000*g* for 20 min at 4 °C. The supernatant containing total polysomes was layered onto sucrose layers of 24% over 60% (w/w) and subjected to centrifugation at 100000*g* at 2 °C for 16 h. The polysome pellets were combined and adjusted to 1 mg of RNA/mL with polysome buffer [25 mM Tris, pH 7.6, 150 mM NaCl, 5 mM $MgCl_2$, 0.1% (v/v) Nonidet P-40, 1 μg/mL cycloheximide, 1 mg/mL heparin].

β-Glucuronidase-specific polysomes were isolated by the addition of 125 μg of β-G Ab/mg of polysomal RNA and heparin to 5 mg/mL, dithiothreitol (DTT) to 1 mM, and RNasin (Biotec) to 50 units/mL. The mixture was incubated at 4 °C for 2 h, and the β-G Ab bound polysomes were adsorbed to the protein A–Sepharose column equlibrated with polysome buffer. After washing, the bound RNA was eluted with 20 mM Na_2EDTA in 25 mM Tris, pH 7.5, containing 150 μg/mL heparin. This enriched polysomal RNA was adjusted to 0.5 M KCl and 0.1% SDS[1] and passed over an oligo(dT)–cellulose column (Aviv & Leder, 1972). Poly(A) mRNA was eluted from the column in 10 mM Tris, pH 7.5, and the oligo(dT)–cellulose steps were repeated. The purified mRNA was stored in sterile plastic tubes at −20 °C.

Translation of mRNA in vitro was carried out with a rabbit reticulocyte lysate IVT kit from Bethesda Research Laboratories. L-[^{35}S]Methionine-labeled translation products were immunoprecipitated by successive treatment with normal rabbit serum and β-G Ab in the presence of formalin-fixed *Staphylococcus aureus* strain Cowan cells (Pansorbin, Calbiochem) (Kessler, 1976).

Synthesis of Complementary DNA. β-Glucuronidase-enriched mRNA (1 μg) was denatured by incubation for 5 min at room temperature in 0.9 mM CH_3HgOH. Complementary DNA synthesis was performed as previously described (Stein et al., 1978). Second-strand synthesis was carried out in 50 μL of 0.1 M Hepes, pH 6.9, 10 mM $MgCl_2$, 2.5 mM dithiothreitol, 70 mM KCl, and 0.5 mM each of the four dNTP in the presence of DNA polymerase I Klenow fragment (New England Nuclear, 50 units/μg of cDNA) at 15 °C for 20 h. The reaction mixture was extracted with chloroform–isoamyl alcohol (24:1), precipitated with ethanol, and dissolved in 100 mM Tris, pH 8.3, 140 mM KCl, 10 mM $MgCl_2$, 28 mM 2-mercaptoethanol, 0.5 mM dNTP, and 800 units/mL AMV reverse transcriptase (Life Sciences, Inc.) in a 50-μL reaction volume. The reaction was incubated for 60 min at 42 °C and stopped by the addition of Na_2EDTA to 20 mM. Cleavage of the hairpin loop was accomplished as previously described (McReynolds et al., 1977) with the addition of 10 μg of poly(A) (P-L Biochemicals).

Tailing of the double-stranded cDNA (dC) and *Pst*I digested pBR322 vector (dG) was done essentially as described by Deng & Wu (1981). Annealing of the tailed cDNA and vector was carried out as described previously (Stein et al., 1978). Transformation of *Escherichia coli* K strain LE392 was performed according to the method of Dagert & Ehrlich (1979).

Differential Colony Hybridization. Complementary DNA (^{32}P-labeled) was prepared from β-glucuronidase-enriched mRNA and from mRNA isolated from the flowthrough fraction of the protein A–Sepharose column to which β-glucuronidase-specific polysomes were bound. Colony hybridization was carried out by a modification of the method of Grunstein & Wallis (1979) using these cDNAs as hybridization probes on duplicate filters. Colonies containing plasmids that hybridized to the former (positive probe) and not the latter (negative probe) were selected for further screening.

Hybridization Selection of β-Glucuronidase mRNA. Individual clones containing plasmids that were positive in the differential hybridization assay were cultured in 80 mL of L broth containing 10 μg/mL tetracycline and amplified by addition of chloramphenicol (200 μg/mL). Plasmid DNA was isolated by the method of Ish-Horowicz & Burke (1981) and linearized with *Bam*HI.

Isolated plasmid DNA was resuspended in TE buffer (20 mM Tris, pH 7.6, 1 mM Na_2EDTA) and heat denatured. The DNA was incubated 20 min at room temperature in 0.5 M NaOH, neutralized, and bound to nitrocellulose filters (BA85; Schleicher & Scheull) by filtration (1 mL/min). After extensive washing in 6 × SSC (SSC = 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) the filters were air-dried and baked in vacuo for 2 h at 80 °C. Hybridization with androgen-induced mouse kidney mRNA was carried out essentially as described by Parnes et al. (1981).

Gel Electrophoresis. Peptide products of cell-free translation were separated by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) on gels containing 10% total acrylamide concentration (T) and 2.7% bis(acrylamide) (C). Stacking gel was composed of 4% T and 2.7% C. [^{35}S]Methionine-labeled peptides were visualized after fixation by impregnating

[1] Abbreviations: SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; dNTP, deoxyribonucleotide triphosphate; testosterone, 17β-hydroxy-4-androsten-3-one; 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; KAP, kidney androgen-regulated protein; poly(A), poly(adenylic acid) containing; kb, kilobase; Tris, tris(hydroxymethyl)aminomethane; Na_2EDTA, disodium ethylenediaminetetraacetate.

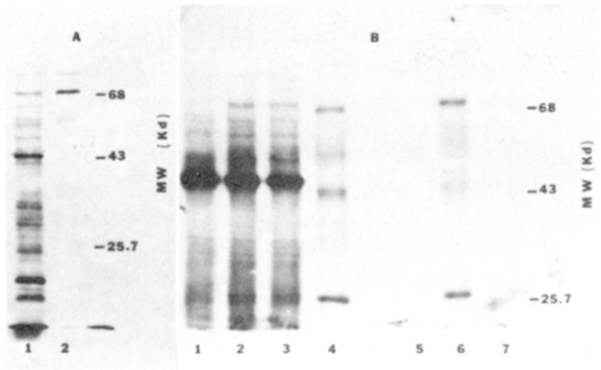

FIGURE 1: (A) Electrophoresis of products of cell-free translation of kidney mRNA on 10% polyacrylamide gels containing 0.1% SDS: lane 1, affinity-purified mRNA (0.03 μg) translated in vitro with rabbit reticulocyte lysate and loaded directly onto the gel after denaturation in 2% SDS; lane 2, immunoprecipitation of translation products of the same RNA (0.2 μg). (B) Immunoprecipitation of the cell-free translation products of hybrid-selected mRNA: lanes 1–3, translation products of hybrid-selected mRNA from plasmids pBR322 (lane 1) and pGUS7 (lanes 2 and 3); lane 4, ^{14}C-labeled molecular weight standards (bovine serum albumin, ovalbumin, and α-chymotrypsinogen); lanes 5 and 6, immunoprecipitation of material shown in lanes 1 and 2, respectively; lane 7, immunoprecipitation of material shown in lane 3 after incubation with excess β-glucuronidase (10 μg) purified from mouse urine.

the gel with En^{3}hance (New England Nuclear), followed by fluorography for various times ranging from 2 h to 7 days.

Agarose gel electrophoresis of glyoxal-denatured mRNA and analysis by Northern blotting and hybridization were performed according to the procedure of Thomas (1980).

Results

Purification of β-Glucuronidase mRNA. β-Glucuronidase mRNA ($mRNA_{Gus}$) was purified from 30 mg of total polysomal RNA by the addition of 3.75 mg of protein A–Sepharose purified β-glucuronidase antibody (β-G Ab), followed by chromatography on protein A–Sepharose (Kraus & Rosenberg, 1982). By this procedure, 0.4% of the original polysomal RNA was recovered. The RNA was further purified by two successive oligo(dT)–cellulose chromatography steps to yield approximately 6 μg of polyadenylated mRNA.

Translation of purified mRNA in vitro followed by immunoprecipitation showed that 2% of the labeled translation products were precipitable with β-G Ab. Analysis of the labeled peptides by SDS–polyacrylamide gel electrophoresis revealed a 69 000-Da peptide (Figure 1A, lane 1) that was not present after translation of total poly(A) mRNA isolated from kidneys of androgen-induced mice (not shown). The 69 000-Da peptide was immunoprecipitated by β-G Ab (Figure 1A, lane 2).

Cloning and Identification of DNA Complementary to $mRNA_{Gus}$. Complementary DNA clones were prepared as described under Experimental Procedures. Since the amount of starting material was small and we expected $mRNA_{Gus}$ to be fairly long, two standard procedures were modified. First, during second-strand synthesis both DNA polymerase I Klenow fragment and AMV reverse transcriptase steps were used. Second, 10 μg of carrier poly(A) was included in the nuclease S_1 reaction to prevent overdigestion of double-stranded cDNA.

Recombinant clones were initially screened by differential colony hybridization. Of 850 clones screened, 23 hybridized to [^{32}P]cDNA from purified $mRNA_{Gus}$ but not to that prepared from mRNA in the flowthrough fraction of the protein A–Sepharose column used in the purification of $mRNA_{Gus}$. Plasmid DNA prepared from these clones was bound to nitrocellulose from which up to 10 pairs of 20-mm^2 discs, each pair representing an individual clone, were cut and hybridized to 40 μg of mouse kidney poly(A) mRNA. Filter-bound mRNA was eluted and translated in vitro. One of the clones assayed by this procedure selected a mRNA which produced a 69 000-Da peptide after translation in vitro (Figure 1B, lanes 2 and 3) that was immunoprecipitated by β-G Ab (lane 6). Furthermore, the immunoprecipitation of this peptide was blocked by the addition of excess cold β-glucuronidase purified from mouse urine (Figure 1B, lane 7). A band at M_r 26 000 was also precipitated by β-G Ab (Figure 1B, lane 6), and its precipitation was abolished by addition of excess β-glucuronidase (lane 7). This peptide was not seen after immunoprecipitation of the translation products of purified $mRNA_{Gus}$ (see Figure 1A) even after long autoradiographic exposure. The origin of this peptide is unknown, but its presence appears to depend upon hybridization–selection of mRNA by the β-glucuronidase cDNA plasmid.

The β-glucuronidase cDNA plasmid, designated pGUS7, was used to screen the remaining clones in our cDNA library. Two additional clones were detected and designated pGUS5 and pGUS48. A restriction map of the region of $cDNA_{Gus}$ contained in these three overlapping clones is presented in Figure 2. Combined these plasmids include 970 bp, which represent over one-third of the $mRNA_{Gus}$ sequence (see below).

Analysis of $mRNA_{Gus}$ by Hybridization to Cloned cDNA. Figure 3 shows that $mRNA_{Gus}$ is 2.6 kilobases in length. Also shown are the relative concentrations of $mRNA_{Gus}$ after 5 and 21 days of induction by DHT. The increase (~3-fold) in mRNA concentration between day 5 and day 21 indicates a slow induction response to the hormone. This is further evidence in support of the identification of the β-glucuronidase cDNA clones as the enzyme exhibits unusually slow induction kinetics, reaching its peak activity after 21 days of chronic androgen treatment (Swank et al., 1978). The overall induction from day 0 (untreated female mice) could not be determined in this experiment as no band was detected with this sample. However, subsequent experiments have shown that the relative $mRNA_{Gus}$ concentration increases approximately 6-fold in the first 5 days of testosterone treatment.[2]

An increase in the accumulation of $mRNA_{Gus}$ can first be detected at 24 h after a single dose of testosterone (Figure 4A). This response precedes the increase in enzyme activity after a similar dose of hormone by approximately 24 h. The increase in $mRNA_{Gus}$ concentration is relatively slow when compared to other androgen-regulated kidney mRNAs in the same mouse strain. Ornithine decarboxylase mRNA starts to increase within 2–6 h of a single dose of testosterone (Kontula et al., 1983), and the mRNA for kidney androgen regulated protein (Toole et al., 1979) increases within 6 h.[2] In similar experiments, androgen-inducible 908 RNA concentration begins to increase after 4 h of chronic testosterone treatment (Berger et al., 1981). It should be noted that the apparent decrease in mRNA concentration at 2 h is probably an artifact. We have inferred this by analyzing KAP[1] and ornithine decarboxylase (Kontula et al., 1983) mRNA on the same nitrocellulose filter after washing to remove the pGUS7 probe. In each case, the 2-h sample gave a reduced signal. The other time points, however, varied independently, following patterns characteristic of the individual hybridization probe used. For instance, KAP mRNA reached maximal level at 6 h and then remained constant.[2] In addition, upon repetition of these experiments we did not observe a decrease in $mRNA_{Gus}$ concentration at 2 h.

[2] C. S. Watson and J. F. Catterall, unpublished results.

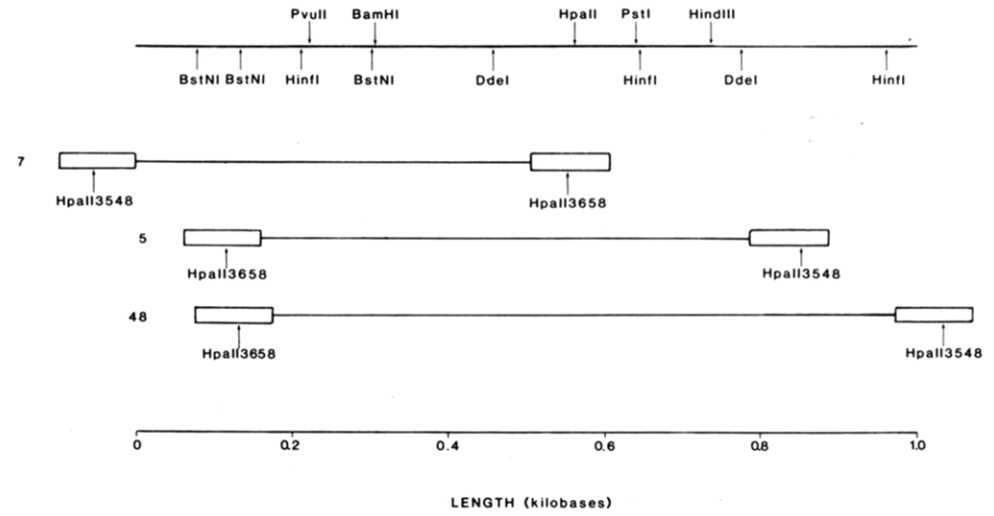

FIGURE 2: Composite restriction map of the cloned region of the $mRNA_{Gus}$ as determined from three overlapping cDNA clones: (—) mRNA strand of cDNA plasmids; (□) pBR322. The numbered *Hpa*II sites refer to those adjacent to the *Pst*I cloning site and are used to define the orientation of each insert in the plasmid. Enzymes tested which do not cleave this region include *Eco*RI, *Hha*I, *Bgl*II, *Hin*cII, *Kpn*I, and *Xba*I.

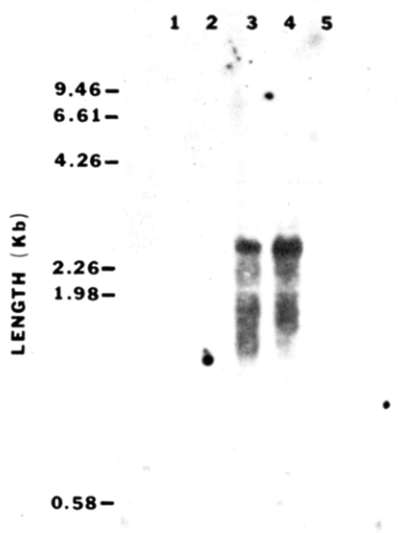

FIGURE 3: Northern blot hybridization analysis of $mRNA_{Gus}$. Polyadenylated RNA from mouse kidney was denatured with glyoxal, fractionated by agarose gel electrophoresis, and transferred to a sheet of nitrocellulose according to Thomas (1980). The nitrocellulose sheet was hybridized to [^{32}P]pGUS7 (5.0×10^7 cpm/μg) which had been linearized with *Hin*dIII. Lane 1 contained no RNA. Lanes 2–5 contained 10 μg of poly(A) mRNA from the following sources: lane 2, polysomal fraction from which β-G Ab-bound polysomes had been removed; lane 3, female mouse kidney after 5 days of induction with DHT; lane 4, female mouse kidney after 21 days of treatment with DHT; lane 5, rabbit reticulocytes. Autoradiographic exposure was 44 h at −70 °C in the presence of a $CaWO_4$ intensifying screen.

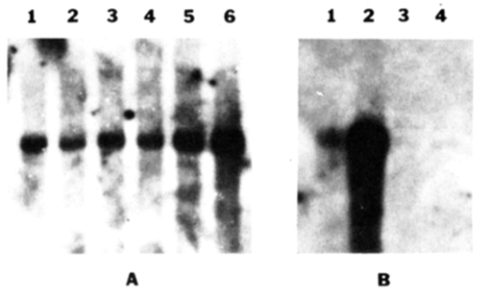

FIGURE 4: Northern blot hybridization analysis of $mRNA_{Gus}$ after acute induction with a single dose (10 mg) of testosterone. RNA denaturation and transfer were as described in Figure 3 except that 8-μg samples were used. (A) Lanes 1–6, poly(A) RNA from kidneys of mice killed prior to or 2, 6, 12, 24, and 48 h after testosterone (10 mg) treatment, respectively. The mobility of the $mRNA_{Gus}$ band was identical with that shown in Figure 3. (B) Lane 1, poly(A) RNA from kidneys of castrated male mice; lane 2, same as lane 1 after chronic testosterone stimulation (4 days); lane 3, untreated Tfm/Y mice; lane 4, Tfm/Y mice treated with testosterone for 4 days.

Finally, $mRNA_{Gus}$ was not induced by testosterone in androgen receptor deficient Tfm/Y mice (Figure 4B, lanes 3 and 4). β-Glucuronidase mRNA was barely detectable in these animals after a 2-week autoradiographic exposure while similarly treated wild-type castrated males exhibited normal induction by testosterone (Figure 4B, lanes 1 and 2). The basal levels of $mRNA_{Gus}$ concentration of wild-type castrates were considerably higher than that in Tfm/Y animals.

Discussion

We have cloned complementary DNA from purified mouse kidney β-glucuronidase mRNA. As amino acid sequence data are unavailable, direct proof that our cDNA clones contain $mRNA_{Gus}$ sequences is not possible. In the absence of these data, we have shown that pGUS7 hybridizes to a mRNA that is translated into a 69 000-Da peptide, which is immunoprecipitated by β-glucuronidase antibody. Immunoprecipitation is abolished by addition of excess β-glucuronidase purified from mouse urine. In addition, the pGUS7 plasmid hybridizes to mRNA of a molecular size (2.6 kb), which is sufficient to encode a 69 000-Da protein and contain expected noncoding regions. Finally, the mRNA detected by hybridization to pGUS7 is induced by androgens in a receptor-dependent fashion and displays slow induction kinetics. All of these characteristics are consistent with the identification of pGUS7 as a β-glucuronidase cDNA clone.

The cloning of rat β-glucuronidase cDNA has been reported (Hieber, 1982). However, the very high concentration of β-glucuronidase mRNA in poly(A)-containing RNA extracts of rat preputial gland used by Hieber (11–15%) is difficult to explain. While rat preputial gland is a rich source of β-glucuronidase, which comprises as much as 7% of total cellular protein, Rosenfeld et al. (1982) found that less than 0.1% of preputial gland mRNA extracted from membrane-bound polysomes was specific for β-glucuronidase. In addition, no restriction enzyme analysis of the rat clone or characterization of rat β-glucuronidase mRNA was performed. A meaningful comparison of the mouse and rat cDNA clones is therefore not possible.

The abundance of mouse kidney $mRNA_{Gus}$ in chronically testosterone-stimulated animals appears to be lower than

previously reported (Labarca & Paigen, 1977). If one assumes quantitative recovery of $mRNA_{Gus}$ during the purification procedure, $mRNA_{Gus}$ represents approximately 0.04% of total poly(A) mRNA based on the estimate of purity by cell-free translation (2%). However, β-glucuronidase-specific clones represented only 0.24% of the colonies screened, which suggests even lower abundance for $mRNA_{Gus}$. Overestimation of mRNA purity by translation has been previously documented (Durnam et al., 1980). Thus, $mRNA_{Gus}$ purification and cloning of its cDNA indicate that $mRNA_{Gus}$ represents less than 0.04% of poly(A)-containing RNA in androgen-induced mouse kidney.

β-Glucuronidase induction by androgens has been extensively studied by using the parameters of enzyme activity and enzyme synthesis. Watson et al. (1981) used a catalytic assay of mRNA activity to study the kinetics of accumulation of biologically active $mRNA_{Gus}$. This assay was based on the injection of mouse kidney mRNA into *Xenopus* oocytes, followed by determination of mouse β-glucuronidase activity during inactivation of the endogenous enzyme at 65 °C. However, the direct measurement of $mRNA_{Gus}$ concentration independent of its biological activity has not been achieved. In addition, basal levels and early changes in mRNA concentration (at less than 2–3 days) cannot be measured in the catalytic assay of $mRNA_{Gus}$ activity. We have administered a single dose of testosterone and measured relative mRNA concentration by Northern blot hybridization over a time course from 0 to 48 h. Induction of β-glucuronidase mRNA under these conditions begins between 12 and 24 h. These represent the first measurements of relative $mRNA_{Gus}$ concentration prior to the induction of enzyme activity which occurs at approximately 48 h after testosterone administration (Pajunen et al., 1982). These data strongly suggest that testosterone acts to increase transcription of the β-glucuronidase gene. However, further experiments are required to measure the actual rate of synthesis of $mRNA_{Gus}$ in response to androgen.

The mouse renal β-glucuronidase model system of steroid hormone induction is unique in its control by alleles of a cis-acting regulatory locus designated Gus-r. The ability to directly measure $mRNA_{Gus}$ concentration over the entire induction period will allow a complete kinetic analysis of the control of $mRNA_{Gus}$ induction by Gus-r. Experiments comparing the induction of mRNA in genetically inbred strains of mice carrying different Gus-r alleles are in progress.

In conclusion, we have cloned a cDNA sequence complementary to mouse β-glucuronidase mRNA. This is the first step toward a molecular characterization of one of the best defined genetic loci in mammals (Paigen, 1979). While many genes of higher eukaryotes have been well characterized at the molecular level, β-glucuronidase is among few that have been well studied genetically (Bulfield, 1980). The combination of these approaches will allow direct correlation of primary gene structure with the phenotypic expression of various mutant strains.

Added in Proof

DNA sequencing studies of the cDNA clones have revealed an encoded amino acid sequence that is identical with one of six cysteine-containing peptides in rat β-glucuronidase (Leighton et al., 1980).

Acknowledgments

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Registry No. β-Glucuronidase, 9001-45-0; testosterone, 58-22-0.

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