and chondroitin sulfates A and C, but not chondroitin sulfate B, heparin, or keratosulfates (*8*). The uterine factor is also sensitive to digestion with chondroitin ABC lyase (E.C. 4.2.2.4) from *Proteus vulgaris*. These results indicate that the critical conversion of proacrosin to acrosin is stimulated by a uterine glycosaminoglycan, possibly through the interactions between the specialized proteinase zymogen (proacrosin) and the anionic surfaces of the glycosaminoglycan.

The presence of glycosaminoglycans in porcine uterine fluid is not surprising. Partial characterization of murine uterine secretions reveals a high carbohydrate content and the presence of a heparinlike substance (*9*), which is most likely a glycosaminoglycan. Furthermore, metachromic staining with basic dyes such as toluidine blue demonstrates the presence of glycosaminoglycans in rabbit endometrial stroma (*10*).

Female reproductive tract secretions are obligatory for fertilization since mammalian spermatozoa must reside in the female reproductive tract before they acquire the capacity to penetrate the zona pellucida of the ovum (*11*). During the incubation of boar spermatozoa in vivo for the time period established for boar spermatozoa to acquire zona pellucida-penetrating ability (*12*), 70 to 80 percent of the proacrosin is converted to acrosin. No conversion is observed in control spermatozoa incubated for the same time period in buffer (*13*), thus indicating that a female reproductive tract factor effects the physiological conversion of proacrosin to acrosin.

The present study documents the interaction of sperm proacrosin with a factor present in uterine flushings and indicates that this factor is a glycosaminoglycan. This interaction between proacrosin and the glycosaminoglycan may be one reason why spermatozoa must reside in the female reproductive tract prior to acquiring fertilization potential.

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A Regulatory Locus for Mouse β-Glucuronidase Induction, *Gur*, Controls Messenger RNA Activity

Abstract. *A regulatory locus in a higher organism has been shown to control a specific messenger RNA activity. The* Gur *locus in mice regulates the production of kidney β-glucuronidase messenger RNA activity after induction of the β-glucuronidase structural gene,* Gus, *by testosterone. β-Glucuronidase messenger RNA was assayed by its ability to direct the synthesis of catalytically active murine β-glucuronidase in* Xenopus *oocytes.*

In higher organisms, changes in messenger RNA (mRNA) activity underlie the alterations in protein synthesis that accompany hormonal induction (*1*) and the appearance of specialized proteins during differentiation. It has thus been an article of faith that a set of regulatory genes must exist in eukaryotes whose function is to modulate mRNA concentrations, but it has been frustrating to demonstrate this experimentally. This difficulty reflects on the one hand the lack of appropriate regulatory mutants for genes whose mRNA products can be readily assayed, and, on the other, the inability to assay the mRNA of genes that have characterized regulatory mutations. The recent development of a sensitive catalytic assay for mammalian β-glucuronidase mRNA (*2*) has made it possible to test for the presence of mRNA regulatory genes in a system with several characterized regulatory mutations (*3, 4*). In so doing we have found that the *Gur* regulatory locus (*5*) that controls rates of β-glucuronidase synthesis in kidneys in response to hormonal induction does, in fact, determine the response of mRNA activity.

Mouse kidney β-glucuronidase (E.C. 3.2.1.31) is induced by androgens such as testosterone (*4, 6*) when normal pituitary function is available (*7*). The response occurs in the epithelial cells of the proximal part of the convoluted tubule (*8*), and results from a dramatic increase in the rate of enzyme synthesis (*4*). The extent of induction is under the control of a regulatory locus, *Gur*, that is located in close proximity to or within the structural gene *Gus* (*4*). No recombination between *Gur* and *Gus* has been observed in conventional genetic crosses. The increase in rate of enzyme synthesis in mice of strain A/J carrying the Gur^a allele is about three times greater than in B/6 [see (*9*)] strain mice carrying the Gur^b allele. *Gur* acts in a cis manner (that is, it affects only the *Gus* gene on the same chromosome), and heterozygotes carrying Gur^a Gus^a on one chromosome and Gur^b Gus^b on the other synthesize a preponderance of GUS-A subunits (*4*).

β-Glucuronidase mRNA activity was assayed in mice of different genotypes to test whether the relative rates of enzyme synthesis seen in Gur^a and Gur^b mice actually reflect differences in mRNA activity. Assays were conducted on three strains of mice. Two of these were the strains originally used to define the *Gur* locus: A/J, which is homozygous for the Gur^a regulatory allele and Gus^a structural allele, and B/6, which is homozygous for the Gur^b and Gus^b alleles. The third strain, B/6 · Gur^a, carries a chromosome segment from strain A/J containing the associated Gur^a and Gus^a alleles transferred onto a B/6 genetic background by repeated backcrossing. This congenic line was developed by V. Chapman, advantage being taken of the close linkage between *Gur* and *Gus* and the ease of scoring *Gus* alleles in crosses. It is the result of six generations of backcrossing A/J onto a B/6 background with continued selection of $Gus^{a/b}$, $Gur^{a/b}$ heterozygotes, followed by an intercross between heterozygotes to produce $Gus^{a/a}$,

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$Gur^{a/a}$ homozygotes. These were then inbred for 12 generations to produce a population of homozygous $Gus^{a/a}$, $Gur^{a/a}$ mice with a B/6 genetic background. Strain B/6 · Gur^a was used to ensure that any differences found between A/J and B/6 in fact reflect the *Gur* alleles they carry, and not other genetic differences between the two strains.

For specific mRNA assay we utilized the fact that catalytically active mouse glucuronidase is formed in *Xenopus* oocytes that have been injected with mouse total RNA preparations. The murine activity is readily distinguished from the endogenous frog β-glucuronidase by virtue of its much greater thermal stability, and the assay of thermostable β-glucuronidase formed after RNA injections provides a quantitative assay for β-glucuronidase mRNA (*2*). In order to minimize the effects of nucleases, we used guanidine hydrochloride to extract RNA from tissues (*2, 10*). This method yielded consistent and stable preparations of both total RNA and β-glucuronidase mRNA activity. The assay for glucuronidase mRNA is extremely sensitive and can readily detect the messenger activity contained in 5 ng of total RNA from induced mouse kidney injected into a single oocyte. From the relative rate of glucuronidase protein synthesis in induced mouse kidney, and the proportion of total RNA that is mRNA, we estimate that the test is sensitive to about 0.1 pg of β-glucuronidase mRNA. The fidelity of translation of message injected into oocytes is very good (*2*). The efficiency of translation of injected RNA is also very high. The amount of β-glucuronidase synthesized per day per nanogram of RNA is 5 to 10 percent of that obtained in the intact mouse. If the comparison is adjusted for the considerable competition provided by endogenous frog mRNA (*11*) and the fact that oocyte synthesis proceeds at 25° rather than 37°C, we estimate that injected message is nearly (50 to 100 percent) as efficient as in the mouse. This confirms that the yield of intact mRNA during extraction must also be very high.

The change in enzyme synthesis after hormonal induction of A/J mice is presented in Fig. 1. Groups of mice were treated with [^{3}H]leucine at various times of induction, and 1 hour later kidney β-glucuronidase was purified and the radioactivity incorporated into enzyme was compared with the radioactivity incorporated into total protein. The results confirm very well the previous data of Swank *et al.* (*4*).

The β-glucuronidase mRNA activity present in total kidney RNA was then assayed over the same time span after induction of A/J and B/6 · Gur^a mice. The results (Fig. 1) indicate that β-glucuronidase induction, like other steroid inductions (*1*), is the consequence of an increase in mRNA activity. Under the conditions of the assay we were unable to detect glucuronidase mRNA activity in RNA from noninduced kidney.

Table 1. Glucuronidase mRNA activity in three strains of mice. Eight preparations of total kidney RNA from fully induced mice were made over a 3-month period. The RNA was stored at −70°C as a solution (5 mg/ml) in water without loss of activity. The RNA extraction and assay was done as described in Fig. 1. In experiment 1, injected RNA was at a concentration of 2 mg per milliliter of water (total amount injected was 56 ng per oocyte). Experiment 2 is a partial repeat of experiment 1 in which oocytes from a different frog were used and the RNA was at a concentration of 1 mg/ml (28 ng per oocyte). Data are mean activities ± standard errors for individual RNA preparations. The data are expressed as microunits of mouse β-glucuronidase per oocyte per nanogram of total RNA injected.

B/6 $Gur^{b/b}$	A/J $Gur^{a/a}$	B/6 · Gur^a $Gur^{a/a}$
	Experiment 1	
1.13 ± 0.15	4.96 ± 0.89	3.85 ± 0.67
0.57 ± 0.13	3.97 ± 0.55	3.35 ± 0.65
0.64 ± 0.09	3.80 ± 0.39	
	Experiment 2	
0.77 ± 0.21	4.59 ± 0.55	4.35 ± 1.22
0.56 ± 0.14	4.47 ± 0.09	

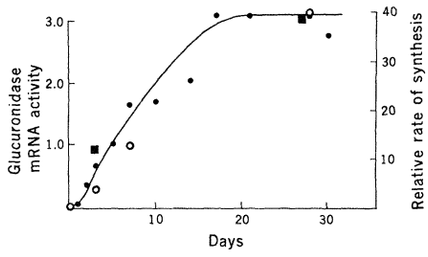

Fig. 1. Induction of β-glucuronidase synthesis and mRNA activity. Female mice were induced by subcutaneous implantation of testosterone pellets (30 mg). The rate of enzyme synthesis at various times after induction was determined by injecting groups of three to six mice with [^{3}H]leucine (100 μCi per mouse). One hour after injection kidneys were removed and β-glucuronidase was purified by heating followed by antibody affinity chromatography (*16*). The purity of the product was monitored by SDS gel electrophoresis. The relative rate of enzyme synthesis (●) in A/J is expressed as the ratio (× 10^4) of radioactivity incorporated into β-glucuronidase to radioactivity incorporated into total kidney protein. Total kidney RNA from A/J (○) and B/6 · Gur^a (■) mice was extracted at various times after testosterone administration with guanidine hydrochloride (*2, 10*). β-Glucuronidase mRNA was assayed by injecting about 50 oocytes with 28 nl each of total RNA at a concentration of 2 mg/ml (that is, 56 ng of RNA per oocyte). Oocytes were incubated individually for 24 hours at 25°C, intact oocytes were pooled in groups of six, and thermostable (mouse) β-glucuronidase activity was determined as described (*2*), a 20-hour incubation period at 37°C being used. A unit of enzyme activity is defined as one micromole of product per hour, with 4-methylumbelliferyl-β-D-glucuronide being used as substrate (*17*). The assay is capable of detecting one microunit of enzyme per sample. β-Glucuronidase mRNA activity is expressed as microunits of mouse β-glucuronidase per oocyte per nanogram of total RNA injected.

To compare the effects of Gur^a and Gur^b on messenger activity, we assayed concentrations of glucuronidase mRNA in a series of separate RNA preparations from fully induced A/J, B/6, and B/6 · Gur^a mouse kidneys (Table 1). At the concentrations of RNA injected, glucuronidase mRNA activity was linear with RNA concentration. The mRNA activity was also reproducible from one experiment to another. Thus Gur^a kidneys clearly have more glucuronidase messenger activity than Gur^b kidneys. The magnitude of this difference is similar to the difference in β-glucuronidase activity and rate of enzyme synthesis determined by the *Gur* locus (*4*), and accounts for *Gur* locus control of induction.

In the original report on *Gur* (*4*) several hypotheses were considered that could explain the function of this locus. With the present findings two of these remain—namely, that *Gur* determines the number of mRNA molecules produced after induction or that it determines the quality of the induced mRNA produced. We know that *Gur* alleles do not affect β-glucuronidase concentration or synthesis before induction in kidney or in other organs after administration of androgens (*4, 12*). Thus, if *Gur* affects the structure of the messenger rather than the number of molecules formed, it must do so only after induction. This would require that induction be associated with a qualitative change in mRNA synthesis or processing. It would also require that *Xenopus* oocytes recognize any difference in mRNA structure that is operational in the mouse. It is worth reiterating that, since our mRNA assay is a functional one, our experiments cannot distinguish these two possibilities; this requires an experiment capable of comparing either the relative efficiency or the number of β-glucuronidase mRNA molecules present.

In addition to these questions, the location of *Gur* relative to the enzyme coding sequence, *Gus*, remains to be determined. Conventional genetic crosses indicate that the two are closely linked. The *Gur* locus could precede, follow, or lie within the coding sequence, or it could be located in a noncoding insert.

Mutants have been described previously that are deficient in messenger activity, notably hemoglobin mRNA in some thalassemias [for discussion, see (*13*)]. In some cases the deficiency reflects a deletion of the structural gene (*14*), in another case mRNA is made but no protein is synthesized (*15*), and in still other cases the cause is unknown. *Gur* represents the first eukaryotic locus whose normal function is identified as the regulation of a specific mRNA. As such, it offers an approach to the study of regulatory mechanisms fundamental to gene induction by steroid hormones.

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Thalamic Projections to Layer I of Striate Cortex Shown by Retrograde Transport of Horseradish Peroxidase

Abstract. *The diffusion of horseradish peroxidase was restricted to layers I and II of the striate cortex in* Tupaia glis *and* Galago senegalensis. *In the lateral geniculate body of* Tupaia *labeled cells were found only in layer 3; some labeled cells were also found in the lateral nucleus. In* Galago *labeled cells were found only in layers 4 and 5 of the lateral geniculate body; a band of cells was also found in the pulvinar nucleus. These results support the distinction between two overlapping thalamic systems, a layer I and a layer IV system.*

That some projections from the thalamus may reach layer I of the cortex in mammals has been known for some time and it comes as no surprise that Cajal noted some fibers, presumably of thalamic origin, ascending to the cortical surface (*1*). Lorente de No advanced the inquiry by defining several characteristics that distinguish the projections to layer I from the projections to layer IV. He called the layer I fibers "nonspecific," mainly because they appeared to give off collaterals into different cortical subdivisions (*2*).

Thalamic projections to layer I were not demonstrated experimentally until Nauta had developed a method for tracing degenerating axons and their terminals (*3*). Chiefly by the use of this method it was concluded that there are two overlapping but distinct thalamic systems: the specific projection system, which includes the main sensory relay nuclei and their projections mainly to layer IV, and the nonspecific system, which includes the intralaminar nuclei and their projections mainly to layer I (*4*).

In previous studies we have used the method of anterograde degeneration to trace projections from the various laminae of the lateral geniculate body (GL) (*5*) to the striate cortex in two species, the tree shrew (*Tupaia glis*) and the prosimian bush baby (*Galago senegalensis*) (*6, 7*). After large lesions of all six geniculate layers, cortical layer IV was black with degenerated axons and terminals but a few degenerating axons could also be seen ascending to layer I. The importance of trying to complement the anterograde method with that of retrograde transport of horseradish peroxidase (HRP) is that the cells in the thalamus which project to layer I can be identified.

The results presented here show that only one layer of GL in *Tupaia* and only two layers of GL in *Galago* project to layer I of striate cortex. These findings may contribute to our understanding not only of the relationship between the so-called specific and nonspecific systems, but also of the lamination of the GL.

The special feature of our method was that we did not inject HRP deeply into the cortex. Instead, the pia mater was pricked a number of times with a small glass pipette (less that 1 μm in diameter at the tip) and crystals of HRP (Boehringer Mannheim) were laid on the moist pial surface and allowed to dissolve in the cerebral spinal fluid for 10 to 30 minutes. Optimal results were obtained with the addition of 1 to 2 μl of 5 percent dimethyl sulfoxide into the HRP solution. The animals survived this procedure for 48 hours, when they were perfused and the brains prepared for histological study (*8*).

The effect of applying HRP to the striate cortex in five tree shrews is illustrated by two experiments shown in Fig. 1. The sites of application are shown on a 45° view of the cortex (Fig. 1a); a photomicrograph of one of the two sites is shown in Fig. 1b. In these experiments labeled cells were found concentrated in a restricted part of layer 3 of GL. A number of labeled cells were also found in nuclei medial to GL. Most of these cells were in the lateral nucleus (Li); a few, however, were found scattered among the fibers that pass between the borders of the pulvinar and the lateral nuclei.

The effect of applying HRP to the surface of the striate cortex in five bush babies is illustrated by one experiment shown in Fig. 2. The locus of the application of HRP to layer I is shown in a diagrammatic 45° view of the cerebral hemisphere (Fig. 2a). A photomicrograph of a parasagittal section through the lateral geniculate body (Fig. 2b) shows the labeled cells confined to layers 4 and 5 (*9*). The pattern of labeled cells in the pulvinar nucleus is shown in a micrograph (Fig. 2d) and drawing of a sagittal section (Fig. 2c). The labeled cells form two limbs of an arc, the upper limb in the superior division of the pulvinar nucleus (Pul S) and the lower in the inferior division (Pul I). The orientation of most cells is especially clear because the HRP entered dendritic processes; Fig. 2d shows that the cell orientation is aligned with the tangent to the curved band.

These results complement our earlier studies of the cortical projections of the various geniculate layers in *Tupaia* and *Galago*. In the tree shrew experiments, anterograde degeneration was traced from GL layers 1 and 2 to the superficial

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