Biochemical Actions of Hormones

Edited by GERALD LITWACK

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Preface

Two important areas have had impact on biochemical endocrinology recently, one of which is the application of recombinant DNA technology to genes for hormones. New information on the genetic regulation of these genes can be expected. Another exciting development is that the nuclear matrix may represent a preferred acceptor site for certain steroid hormone receptor complexes. These two advances are highlighted in two contributions to this volume, the first as approached by the Feigelson group for the α_{2u} globulin gene and its regulation by several hormones, and the second by the Coffey laboratory reviewing their observations on the nuclear matrix. An excellent model for determining the roles of various receptors operating at the genetic level has embodied the use of cells in culture derived from the anterior pituitary. These cells are capable of secreting hormones in response to the stimuli of releasing factors as well as other hormones and have retained capacity for feedback inhibition. Progress along these lines is reported in a contribution from the Samuels laboratory. Future volumes can be expected to concentrate on similar models. Since certain polypeptide hormones apparently share ancestral, closely related genes and consequently various aspects of their structures, Bradshaw summarizes this conceptual advance by reviewing the nerve growth factor and related hormones. Also, many of the polypeptide hormones have come to be recognized as growth factors for cells in culture. Kano-Sueoka reviews her work in this area and related studies on factors affecting the growth of mammary cells in culture. Some new insights into the pineal hormone, melatonin, have appeared. These are summarized in a timely article from Wurtman's laboratory.

The remainder of the topics covered here deal with more specific subjects. Recent studies in the Nebert laboratory on the *Ah* receptor have produced a great deal of information on a specific carcinogen receptor which seems to be analogous in many respects to a steroid receptor. This interesting subject appears here for the benefit of endocrinologists who may not be fully aware of this fascinating system. The remaining five chapters center around various aspects of steroid receptors. Current interest has been expressed in specific acceptor sites in genes and their flanking sequences.

Therefore, the work taking place in Dickerman's laboratory on synthetic oligonucleotide acceptors for steroid receptor complexes is reviewed. With this interesting introduction it is to be hoped that future treatises in this series will review the developing work on identifying gene sequences involved in high-affinity binding sites for steroid receptor complexes. Hollander's laboratory reviews mammary tumor growth and response to ovariectomy, which is of particular interest with respect to specific alterations in the estradiol receptor. Leavitt's laboratory reviews the hormonal regulation of estrogen and progesterone receptors. The genesis of cleft palate provides an experimental model which may lead to an understanding of the genetic regulation of factors involved in the expression of activities of the glucocorticoid receptor and possibly to specific chromosomal assignment of these functions. This subject is emphasized in a contribution from Gasser and Goldman. Finally, the Stevens group brings us up to date on the mechanisms of glucocorticoid resistance in leukemia cells and discusses the possibility of two apparently different steroid receptors which could result from the occurrence of separate gene products.

Modern endocrinology is developing very rapidly at the experimental level and is overlapping other disciplines, particularly molecular biology. I will attempt to keep abreast of these developments in future volumes of this publication.

Gerald Litwack

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Tamiko Kano-Sueoka

CHAPTER 1

The Application of Recombinant Techniques to the Study of the Control of α_{2u} Globulin Gene Expression

Kevin P. Dolan, Rosemary Reinke, Kevin R. Lynch, Margaret McLaughlin, Ronald Unterman, Hira L. Nakhasi, Yannick Laperche, and Philip Feigelson

I. INTRODUCTION

α_{2u} Globulin is a small (M_r 18,700, p*I* 5.1–5.5) protein synthesized predominantly in the liver and excreted in the urine of male rats (Roy and Neuhaus, 1966a; Roy *et al.*, 1966). It was first described by Roy *et al.*, who found it in the urine of male but not female rats and demonstrated that in order to excrete normal levels of α_{2u} globulin an adult male rat requires androgen, glucocorticoid, growth hormone, thyroxine, and insulin

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(Roy and Neuhaus, 1967; Roy, 1973a,b; Roy and Leonard, 1973). Female rats will excrete α_{2u} globulin in response to androgen only if they are first ovariectomized (Sippel *et al.*, 1975); α_{2u} globulin excretion is drastically reduced in male animals by estrogen treatment (Roy *et al.*, 1975).

Glucocorticoids have been shown to act directly on hepatocyte suspensions *in vitro* to elevate α_{2u} globulin synthesis and secretion and this induction was shown to be α-amanitin sensitive (Chen and Feigelson, 1978, 1980). It should be noted, however, that the stimulatory effects of androgen, thyroid hormone, growth hormone, and insulin, as well as the suppressive effect of estrogen, have only been convincingly demonstrated *in vivo*. The possibility exists that certain of these latter hormones may elicit their hepatic α_{2u} globulin responses indirectly, e.g., estrogen via prolactin, thyroid hormone via growth hormone, and insulin and growth hormone via somatomedin. Thus, α_{2u} globulin synthesizing hepatocytes might be directly responding to a smaller set of hormones.

In addition to the complex hormonal control of α_{2u} globulin synthesis in adult male rats, its synthesis also follows a distinct developmental pattern. α_{2u} globulin is undetectable in the urine of male rats until the onset of puberty (about 31 days of age) (Roy, 1973b). From this point until adulthood the amount of α_{2u} globulin excreted increases reaching the mature level of approximately 20 mg/day at about 50 days of age. α_{2u} Globulin is excreted in this quantity throughout the life of the adult male rat, decreasing again to nearly undetectable levels in senescence (Roy and Neuhaus, 1966b; Roy *et al.*, 1974). Prior to the onset of puberty, administration of androgen will not induce α_{2u} globulin excretion (Roy, 1973b). Rats castrated before puberty, however, respond to androgen administration after 50 days of age by excreting α_{2u} globulin at near normal levels. It therefore appears that androgen is not required for the development of the competence to synthesize this protein. Conversely, hypophysectomy does prevent the development of this competence. Male rats hypophysectomized after puberty respond to injections of androgen, growth hormone, glucocorticoid, and thyroxine by excreting approximately normal levels of α_{2u} globulin, while animals hypophysectomized before puberty and treated similarly with these hormones at 65 days of age excrete only about 10% the normal levels (Lynch *et al.*, 1982) (Fig. 1). These results suggest that a hormone provided or directed by the pituitary is responsible for the development of the competence of the mature male liver to respond normally to androgen.

Further interest in the control of α_{2u} globulin synthesis is based on the finding that several transplantable hepatomas have lost the ability to synthesize this protein although the livers of the host rats continue to do so normally (Sippel *et al.*, 1976; Feigelson and DeLap, 1977). Thus, it is evident that understanding the control of α_{2u} globulin synthesis has the

potential to provide useful insights into basic biological questions including the mechanisms responsible for tissue specific differentiation, the mode of actions of polypeptide, steroid, and other hormones, and the basis for altered gene expression consequent to neoplastic transformation. Considerable effort has, therefore, been focused on the elucidation of the biochemical processes underlying this complex hormonal and developmental control.

In order to determine whether the altered rates of α_{2u} globulin synthesis were due to fluctuations in the level of its mRNA, the hepatic levels of functional α_{2u} globulin mRNA were measured. These experiments involved the translation, in a heterologous cell-free system, of the mRNA isolated from the livers or tumors of rats in different hormonal states. The α_{2u} globulin synthesized *in vitro* under the direction of this mRNA was precipitated with monospecific antibody to α_{2u} globulin, separated on sodium dodecyl sulfate (SDS)–polyacrylamide gels, and quantified. In every case of endocrine (Kurtz *et al.*, 1976a,b; Roy and Dowbenko, 1977; Lynch *et al.*, 1982; Roy, 1973a,b; Roy and Leonard, 1973; Roy *et al.*, 1976a,b; Feigelson and Kurtz, 1978; Chen and Feigelson, 1980), developmental (Chatterjee and Roy, 1980), and neoplastic control (Sippel *et al.*, 1976; Feigelson and DeLap, 1977; Nakhasi *et al.*, 1982), the enrichment of the hepatic mRNA, measured by *in vitro* translation, paralleled exactly its *in vivo* rate of synthesis and the level of α_{2u} globulin excreted by the animal. A representative experiment of this type is shown in Figs. 1B and 5C. The coordinate rise in α_{2u} globulin synthesis and in its hepatic mRNA makes it likely that the site of developmental, neoplastic, and endocrine action is prior to the appearance of functional cytoplasmic α_{2u} globulin mRNA. For reviews of this work see Kurtz and Feigelson (1978) and Roy (1979). While transcription is a likely site of control (Chan *et al.*, 1978), processing of the gene transcript, transport of the mRNA to the cytoplasm, and the stability of each of the processing intermediates are all potential sites of regulation. To distinguish between these alternatives a specific probe capable of hybridizing with α_{2u} globulin sequences was required.

II. CLONING

We prepared and cloned α_{2u} globulin cDNA using standard technology (Unterman *et al.*, 1981). A cDNA copy was made from oligo(dT)-primed mature male liver RNA using reverse transcriptase to synthesize the first strand (Kacian and Myers, 1976) and DNA polymerase I for the second strand (Seeburg *et al.*, 1977). The double-stranded cDNA was inserted in the *Pst*I site of pBR322 by the G–C tailing method and transformed into

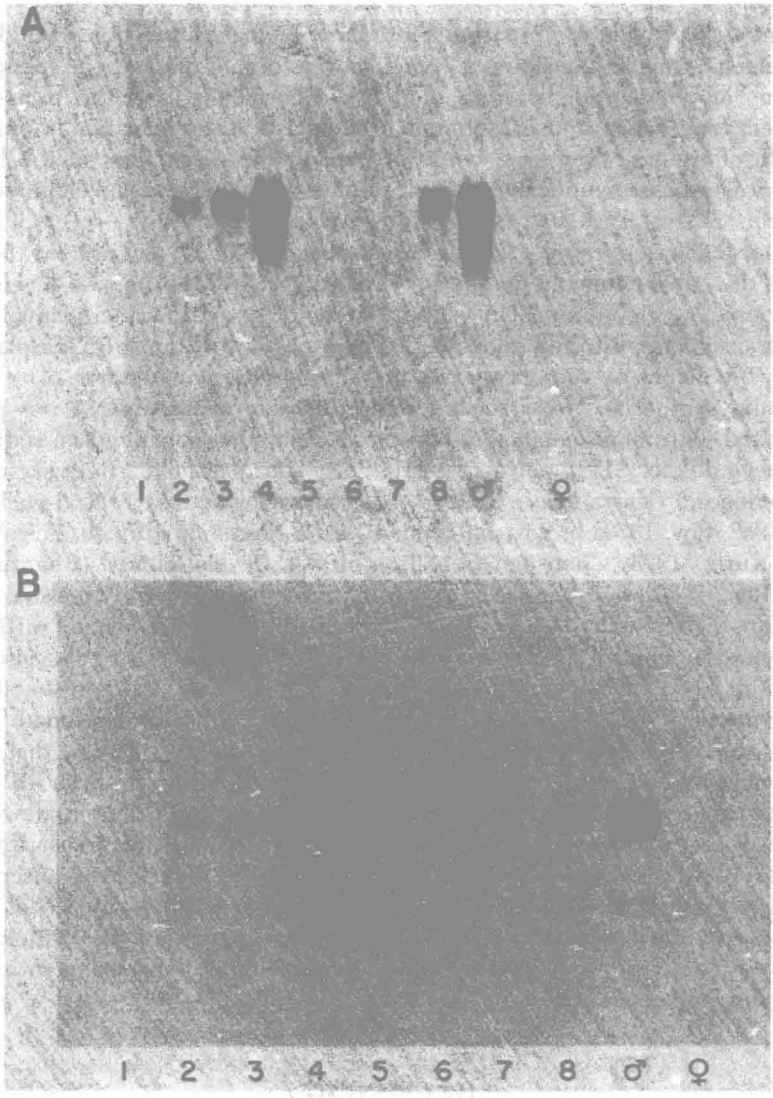

FIG. 1 Amount of α_{2u} globulin RNA present in the livers of hormone-treated hypophysectomized rats. (A) The autoradiograph depicted was generated by electrophoresis of 30 μg of total RNA extracted from the livers of prepubescently hypophysectomized (lanes 5, 6, 7, and 8) or postpubescently hypophysectomized (lanes 1, 2, 3, and 4) rats through a 1.75% denaturing agarose gel, after which the RNA was blotted onto nitrocellulose paper and hybridized to ^{32}P-labeled α_{2u} globulin cDNA. The rats were treated with one of the following hormonal regimens for 12 days: no hormones (lanes 1 and 5); dihydrotestosterone, L-thyroxine, and hydrocortisone