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ESTROGEN STIMULATION OF SPECIFIC PROTEIN SYNTHESIS: REGULATION AND PHYSICAL CHARACTERIZATION OF IP

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SUMMARY

The increased synthesis of a specific uterine protein (IP) in response to estrogen has been observed both *in vivo* and *in vitro* in a number of laboratories. The protein has been purified and partially characterized. Studies on the regulation of IP synthesis have implicated RNA synthesis as an essential intermediate step for the estrogen response. Work is underway in several laboratories to find direct evidence for an estrogen-induced mRNA for the IP protein.

INTRODUCTION

A number of years ago several laboratories observed that the sequence of events in the uterus following estrogen administration included a 1–2 h lag prior to a generalized increase in protein synthesis [1, 2]. During this lag in general protein synthesis, estrogen increased the activity of several metabolic pathways such as glucose metabolism, lipid synthesis, phosphate incorporation into nucleic acid, etc. [3]. These increases in metabolic activity due to estrogen were blocked when protein synthesis inhibitors were administered just prior to the hormone. From these observations, the concept arose that estrogens induced the synthesis of specific uterine proteins which, in turn, were essential for the increases in metabolic activity.

Notides and Gorski[4] confirmed this hypothesis, at least in part, by observing that estrogen increased the incorporation of amino acids into a single band of protein when soluble uterine proteins were separated by gel electrophoresis. This single band came to be referred to as the "induced protein" or IP.

The most exciting discovery concerning IP was the demonstration by Mayol and Thayer[5], Katzenellenbogen and Gorski[6, 7] and Baulieu *et al.*[8] that the synthesis of IP can be induced *in vitro* by estrogens added to incubations of whole immature rat uteri.

In our laboratory recent investigations of IP have focused on three main approaches: (1) purification and characterization, (2) regulation of synthesis and (3) search for IP messenger RNA.

PURIFICATION AND CHARACTERIZATION OF IP

Purification of small amounts of IP has been reported by Katzenellenbogen *et al.*[9] who found that IP was a protein very similar in characteristics to ovalbumin. It has a size of approximately 42,000 mol. wt. and migrates like ovalbumin on both 6% polyacrylamide gels in Tris–Borate buffer-pH 8·6 and on SDS gels. In some elegant immunological experiments, Katzenellenbogen[10] has shown that IP does not cross-react with ovalbumin antiserum. It would appear that the similarity in both charge and size are fortuitous and not due to some evolutionary relationship between these proteins. Iacobelli *et al.*[11] have isolated IP from immature rat uteri with a two-step procedure. They have reported size and isoelectric point data similar to that reported above, in addition to an amino acid analysis of their purified protein. However, very little evidence was provided as to the purity of their preparation.

In our laboratory, Denari *et al.*[12], using a three-step procedure, have obtained a preparation homogeneous by electrophoretic analysis. The protein obtained has both size and charge characteristics similar to those previously reported by others. Preparation of larger quantities of this material will permit further investigation of its homogeneity and physical characteristics.

REGULATION OF IP SYNTHESIS

IP is induced by all estrogenic compounds in proportion to the ability of those compounds to bind to the uterine estrogen receptor proteins. Ruh *et al.*[13] reported data which revealed proportional binding and IP induction with estrone, estriol and estradiol-17β under *in vitro* conditions. These estrogens differ in their rates of dissociation from the receptor and this is closely correlated with their ability to induce IP under steady state conditions.

IP is a protein with a half life of approximately 9 h [14]. The *in vitro* induction of IP does not appear to involve adenyl cyclase (cAMP). Katzenellenbogen and Gorski[7] showed that neither cAMP nor dibutyryl-cAMP could induce IP or affect the response to *in vitro* estrogen.

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IP—MESSENGER RNA

The induction of IP is blocked by administration of large doses of actinomycin D (4 mg/kg) [15, 5]. Under *in vitro* conditions actinomycin-D at 20 μg/ml of medium blocks IP induction but does not affect IP synthesis after it has been induced [7].

There is a 30–45 min lag period between estrogen administration and the first detectable IP synthesis whereas the actinomycin-sensitive step appears to start within minutes after estrogen administration, either *in vivo* or *in vitro* [16].

All of the above observations are in agreement with the hypothesis that estrogen causes the induced synthesis of a messenger RNA for IP. The time course suggests that IP-mRNA is being synthesized at the same time as the estrogen–receptor complex moves into the uterine nucleus. The 30–45 min lag before IP synthesis begins is similar to the time required for mRNA to be processed and moved from the nucleus to the cytoplasmic polysomes where protein synthesis takes place.

We and others have made a number of attempts to obtain direct evidence for specific RNA synthesis that could account for IP-RNA synthesis. With the exception of the report of Wira and Baulieu[17] these attempts have not resulted in positive data. Wira and Baulieu[17] have reported that estrogen causes a change in ratio of double labeled RNA from control versus estrogen-treated uteri.

Knowler and Smellie[18] as well as Eilon and Gorski[19] and Frolik and Gorski[20] in our own laboratory have used a variety of techniques and methods for looking at RNA but have not yet been able to detect any changes in the synthesis of specific RNA's. Gross changes in classes of RNA such as rRNA or hnRNA have been reported [18] but they are probably related to the later increases in general synthesis that occur 2–4 h after estrogen administration. The theoretical problems of detecting a single new mRNA are vast. If a cell is manufacturing 1000 or more mRNA's at any given time, one new mRNA or an increased synthesis of one mRNA represents only 0·1% of new mRNA synthesis. As physical methods of separation are presently limited to SDS gel electrophoresis which separates species of RNA on the basis of size, little resolution can be expected of similar sized RNA's.

Cells or tissues such as the chick oviduct which produce large amounts of a single protein in response to hormone present unique opportunities for mRNA isolation. The mRNA for a regulatory protein, however, is probably present in much smaller amounts and will represent a different type of isolation problem. The use of heterologous cell-free protein synthesizing systems represents an approach with a greater probability of success. Following the leadership of workers using hemoglobin mRNA and more recently ovalbumin and avidin mRNA, it seems probable that mRNA for IP can be identified and studied, albeit in an indirect manner [21, 22]. With such systems we can then hope to elucidate more clearly the mechanisms by which steroid hormones regulate expression of specific genes in target tissues.

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