A Mechanism of Estrogen Action on Gene Expression at the Level of Translation¹

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Summary

One of the earliest events following the administration of estradiol to ovariectomized rats is an increase in transfer RNA (tRNA) methyltransferase activity of the uterus. Ovariectomy diminishes by about one-half the capacity of extracts of uterus to introduce methyl groups in vitro into heterogeneous tRNA. Administration of estradiol to ovariectomized animals restores the tRNA methyltransferase capacity to normal. Alterations in the population of uterine serine tRNA were also observed following ovariectomy. These findings represent reversible qualitative changes produced by a hormone in the target tissue.

Whether the tRNA’s are involved in regulating hormone-induced protein synthesis was investigated by studying the estrogen-induced ovalbumin synthesis in immature chick oviducts. tRNA’s from oviducts of estrogen-stimulated chicks or from oviducts of laying hens produced an enhanced stimulation of ovalbumin synthesis in vitro compared with tRNA’s from oviducts of immature chicks that were withdrawn from estrogen stimulation (these chicks do not synthesize ovalbumin). Some of these hormone-induced alterations may be of importance in understanding the molecular mechanisms of estrogen action on gene expression.

During these studies, we have observed a novel effect of the administration of the carcinogen, ethionine, in the immature chicks. Ethionine induces ovalbumin and conalbumin synthesis in immature chick oviducts that were withdrawn from estrogen stimulation for 3 to 4 weeks following primary estrogen stimulation.

Introduction

Steroid hormones regulate gene expression in the target tissues. A model for estrogen action, based on the concept of the binding of estrogen in the cytoplasm of the target cell and subsequent translocation of a receptor protein-estrogen complex to the nucleus and its effect on altered gene expression, was independently proposed by Gorski et al. (14) and Jensen et al. (18). The current status of evidence for this model has been recently reviewed (12, 20).

Some of the early effects of estrogen in the target tissue are: rapid uptake of RNA precursors (31), increase in RNA polymerase activity (4, 16) and tRNA methyltransferases (3), synthesis of “induced protein” (19), stimulation of ornithine decarboxylase activity (7), increased synthesis of phospholipid (1, 13), RNA (5), and protein (15), and increased production of cyclic guanosine 3′:5′-monophosphate (25, 33). In the chick oviduct, estrogens induce specific mRNA (37, 42). In the rat prostate, there is, within 10 min after androgen administration, an increase in formylmethionyl-tRNA-binding protein (26). The significance of these sequelae of hormone administration on gene expression is obscure.

We will describe here the effects of hormones, especially estrogens, on tRNA’s and tRNA methyltransferases and their significance in the modulation of the synthesis of hormone-induced proteins.

tRNA’s are the most complex of macromolecules and have varied functions (6). After the transcription the macromolecule undergoes a series of modifications, some of which are very complex. A complete understanding of the role of all specific modifications of tRNA’s has not been reached yet, however, some of the modifications are known to be required for proper functioning of tRNA’s (6). The tRNA methyltransferases are some of the enzymes that modify the structure of macromolecules and are species, organ, site and base specific (22). These enzymes show profound alterations, both qualitative and quantitative in systems undergoing shifts in regulatory processes (22, 50). It has been suggested that tRNA’s and the enzymes, tRNA methyltransferases which modify their structure, serve some regulatory functions (for a review see Ref. 22).

Effect of Hormones on tRNA’s and Their Methylation

Hormonal stimulation by estrogen, testosterone, or hydrocortisone results in a disproportionate increase in tRNA’s in immature chick oviduct (36) and immature rat uterus (5), seminal vesicles (59), and rat liver (5). A relative increase in tRNA content is mediated by prolactin in explants of midpregnancy mouse mammary gland incubated in the presence of insulin and hydrocortisone (56). Increase in tRNA is not due to an increase in epithelial cell population, since this does not occur during insulin-induced division of epithelial cells in vitro.

Munns and Katzman (32) have observed increased methylation of uterine tRNA and rRNA within 2 hr following the administration of estradiol to immature rats; the increase mounts to 300% in 6 hr. The rate of methylation of tRNA declines after this time, although the rate of methylation of rRNA becomes maximum around 12 hr. However, methylation of both tRNA and rRNA is still above control levels 24 hr after estradiol administration.

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Hormonal Control of tRNA Methyltransferases

Earlier studies from our laboratory showed that ovariectomy invariably diminishes by about one-half the capacity of extracts of rat uteri to introduce methyl groups in vitro into heterologous tRNA. The reduction in tRNA methyltransferase capacity was not uniform for various base-specific enzymes (50). Administration of estradiol to the ovariectomized animals restored the methyltransferase capacity to normal. One of the earliest events 2 hr following injection of estradiol to ovariec-tomized rats is an increase in tRNA methyltransferase activity (3). Due to the limited amounts of uterine tissue in the rat, our studies were extended to the pig uterus (46). The effect of ovariectomy on the capacity of tRNA methyltransferases extracted from the uterus was confirmed on ovariectomized pigs, and the administration of physiological doses of estradiol restored the tRNA methyltransferase capacity to that of normal uteri (Chart 1). (For the explanation of the mechanism of this phenomenon see Refs. 23 and 46).

Alterations in tRNA’s under the Influence of Hormones

It has been suggested earlier that protein synthesis can be regulated at the translational level by the availability of certain species of tRNA (46). If such mechanisms are operative, alterations in tRNA populations would be expected in systems undergoing shifts in regulatory processes. Changes in the populations of tRNA’s have been observed during embryonic development, differentiation, and neoplasia (6, 27, 55). In all cases examined, the changes were restricted to a few tRNA species.

Alterations in tRNA populations have also been observed in systems under the influence of hormones (48). Chromatographic comparisons of 10 different tRNA’s from normal and ovariectomized pig uteri aminocylated with rat liver aminocyl-tRNA synthetases showed a difference only in the elution profiles of the tRNAser 3 (46), this eluted at a lower salt concentration than the tRNA’s from the normal uterus (Chart 2). However, the elution profile of tRNAser from an ovariectomized animal following the administration of estradiol coincided with that of normal animals (Chart 3). No change in the population of tRNAser was observed in the liver following ovariectomy. It is not known whether the altered elution profile of tRNAser from ovariectomized uteri stems from altered patterns of methylation or from a changed primary sequence. Whatever the source of the alteration, this finding is significant because it represents a reversible qualitative change produced by a hormone in the target tissue, i.e., there is not more or less tRNA but a “different” tRNA.

Enhancement of the Synthesis of a Hormone-induced Protein by tRNA’s

It is evident from the earlier discussion that the tRNA’s and tRNA methyltransferases are under hormonal control. Whether the tRNA’s are involved in regulating hormone-induced, specific protein synthesis has remained obscure. We decided to investigate estrogen-induced ovalbumin synthesis in immature chick oviducts and to explore whether the limiting participant in translation may be specific populations of tRNA’s. Administration of estrogen to immature chicks causes cytodifferentiation and growth of the primitive oviduct (24, 34, 35, 40). The tubular gland cells synthesize ovalbumin, conalbumin, ovomucoid, and lysozyme which comprise 85 to 90% of the egg white proteins (38). The continuous presence of estrogen is required for sustained synthesis of these proteins in immature chicks; withdrawal of estrogen is accompanied by a gradual decline in cell-specific protein synthesis as well as in the weight of the oviduct and the RNA content of the tissue (35). But the tubular gland cells in the oviduct magnum are retained during withdrawal, although they do not synthesize cell-specific secretory proteins (35, 39). Readmission of hormone to chickens after withdrawal (secondary stimulation) results in restoration of cell-specific protein synthesis without concomitant need for DNA synthesis (35).

We decided to study the effect of the addition of exogenous tRNA’s on ovalbumin synthesis in chick oviduct magnum explants. We were encouraged to do this because Herrera et al. (17) have shown by tracer studies the uptake of Escherichia coli tRNA by mammalian cells in culture, and Yamamoto et al. (60) have reported that exogenous suppressor tRNA can function in a permeable mutant of E. coli. Addition of oviduct tRNA’s from laying hen to the magnum explants stimulated significantly ovalbumin synthesis after both primary (Chart 4) and secondary estrogen stimulation (Chart 5). The enhancement of ovalbumin synthesis by exogenous oviduct tRNA’s was pronounced during the earlier stages of estrogen stimulation. After this the indigenous population of tRNA is apparently no longer limiting and, therefore, no stimulation was observed (47).

Control experiments were performed by incubation with tRNA’s extracted from rooster liver and rat liver. These tRNA’s had no stimulatory effect on ovalbumin synthesis. The enhancement of ovalbumin synthesis in the presence of oviduct tRNA is eliminated by exposure of the exogenous tRNA to pancreatic RNase.

In these studies, the mechanism of stimulation of ovalbumin synthesis was not clear. However, a stimulatory role of tRNA’s in ovalbumin synthesis was established. These experiments imply that some functional tRNA’s can penetrate avian cells. The ease of penetration of a regulatory molecule into metazoan cells has implications on immigration of such molecules during differentiation and neoplastic transformation.

In order to study the mechanism of potentiation of ovalbumin synthesis by oviduct tRNA’s, we have developed a tRNA-dependent cell-free system from Ehrlich ascites cells (51). Such a system was essential for studying the contribution of tRNA’s from different sources to the efficiency and fidelity of translation of specific mRNA’s, otherwise the residual endogenous tRNA can mask any specific effect (45). The Krebs II ascites cell-free system has been reported to be tRNA dependent for the translation of encephalomyocarditis viral RNA (2) and globin mRNA (28). However, it was found in our laboratory and others that addition of exoge-
Chart 1. Methylation of E. coli B tRNA by 100,000 x g supernatant fractions of pig uteri of normal, ovariectomized, and ovariectomized estradiol-treated (46).

Chart 2. Methylated albumin-Kieselguhr column chromatography of tRNA from normal and ovariectomized pig uteri (46).

Chart 3. Elution profile on methylated albumin Kieselguhr column of tRNA from normal and ovariectomized estradiol-treated pig uteri (46).

ous tRNA is not required for protein synthesis in this system. Our results along with others indicated that the preincubation conditions should be altered to make the system partially tRNA dependent. A tRNA-dependent globin synthesizing system from rabbit reticulocytes has been reported by Gilbert and Anderson (11). This system contains large amounts of endogenous globin mRNA. Our attempts to use the Gilbert and Anderson procedure for preparing the
tRNA-dependent protein-synthesizing system from ascites cells resulted in inactivation of the system. We have made the Ehrlich ascites cell-free system tRNA dependent by passing both the ribosomes and the ammonium sulfate fractions through DEAE-cellulose in 0.3 M KCl (Chart 6). A number of investigators have reported the requirement of a factor extracted with 0.5 M KCl from rabbit reticulocyte ribosomes for efficient translation of mRNA's in Krebs II ascites cell extracts (28, 43). However, the fractionated tRNA-dependent Ehrlich ascites system efficiently translated exogenous mRNA's in the absence of 0.5 M KCl ribosomal wash. The homology of the system is an added advantage in studying the rate and fidelity of translation of different mRNA's (51).

In Chart 7, the effect of tRNA from 3 different sources on amino acid incorporation is presented. It is evident that tRNA from the oviducts of estrogen-stimulated chicks or from oviducts of laying hens produced an enhanced stimu-
was confirmed by gel coelectrophoresis with authentic markers (Chart 9). The faster moving components are probably nascent, incomplete ovalbumin precursors. The experiments were reproducibly repeated with 3 different preparations of tRNA, isolated from different batches of chicks, using separate preparations of a protein-synthesizing system from Ehrlich ascites cells. There were some variations in the activity of protein-synthesizing systems. However, in different experiments, ovalbumin mRNA translation was 50 to 75% greater by oviduct tRNA from estrogen-stimulated chicks than from withdrawn chicks. For reproducible results, it is essential to use protein-synthesizing systems that are completely dependent on the presence of exogenous tRNA.

To rule out the possibility that the relative inefficiency of oviduct tRNA from unstimulated chicks in the synthesis of ovalbumin stems from some unknown artifact of the isolation, its effectiveness in the translation of another mRNA was tested. In the efficiency of translation of rabbit globin mRNA, no differences were found in the tRNA's from the 2 sources (data not shown). The functional integrity of the tRNA's from the 2 sources in the translation of mRNA's other than that of ovalbumin is also implied, although to a quantitatively lesser degree by the equivalent translation of the endogenous mRNA's from the ascites cells (Chart 7, lower curves).

The efficiency of translation of oviduct mRNA was also assayed by the extent of synthesis of ovalbumin and the results are presented in Chart 8. Ovalbumin, precipitable with specific antisera, was synthesized to a 70% greater extent in the presence of tRNA from estrogen-stimulated chicks than in the presence of tRNA from chicks that had not received secondary estrogen stimulation.

The identity of the polypeptides precipitated by antisera...
The findings presented here imply that the relative inefficiency of oviduct tRNA from withdrawn chicks in ovalbumin synthesis may be due to a deficiency in the population of tRNA's. The validity of such an assumption can be probed experimentally because the oviduct of the laying hen offers an abundant source of tRNA's with a high efficiency of translation.

**Induction of Ovalbumin and Conalbumin Synthesis in Immature Chick Oviducts by Ethionine**

Prolonged feeding of rats with ethionine results in a high frequency of hepatic carcinoma. Ethionine induces other pathological manifestations in other organs and tissues of experimental animals (8), and some of its effects can be reversed rapidly by the administration of methionine. At the molecular level, ethionine induces a rapid decrease in hepatic ATP concentration (10, 53, 57), followed by inhibition of RNA (58) and protein synthesis (9). The ATP pool is depleted by the formation of Sadenosylmethionine (54), an inhibitor of tRNA methyltransferases (29, 30); consequently, tRNA isolated from the liver of rats given injections of adenine and ethionine is hypomethylated (21, 41). Several viral and eukaryotic mRNA's are methylated on the 5' terminus and such modification is required for efficient translation (62). To study the mechanism of methylation we attempted to produce a specific methyl-deficient mRNA by the administration of ethionine. To monitor the effects of the deprivation of methyl groups, the synthesis of hormone-induced ovalbumin in immature chick oviducts was assayed in hormone-stimulated chicks with and without ethionine.

A totally unexpected observation emerged during these studies: ethionine simulated the effects of the injection of estrogen for secondary stimulation (49). When, 4 weeks after primary estrogen stimulation, at which time oviducts do not synthesize ovalbumin, ethionine and adenine were injected for 3 days followed by secondary estrogen stimulation with estradiol for 18 hr, the outcome was the reverse of the expected. Ovalbumin synthesis in these chicks was greater than in those that received estradiol alone (data not shown). Therefore, the effect of the administration of ethionine without secondary estrogen stimulation was studied in chicks 4 weeks after primary stimulation.

Ethionine itself induced ovalbumin synthesis, although a combination of ethionine and adenine was more effective. Adenine alone and in combination with methionine did not induce ovalbumin synthesis. The induction of ovalbumin synthesis by ethionine was slower than by estrogen (Chart 10). Conalbumin synthesis was also induced by ethionine as measured by precipitation with monospecific antiserum to conalbumin (data not shown). Induction of ovalbumin and conalbumin synthesis was confirmed by sodium dodecyl sulfate-acrylamide gel electrophoresis (Chart 11).

Electron microscopy of the oviduct revealed in the control tissues well-developed tubular glands but the cytoplasm of the glandular cells was devoid of the organelles associated with specific intracellular synthesis (Fig. 1a). In the tissues of ethionine-treated chicks, the nuclei of the glandular cells were located basally, the rough endoplasmic reticulum and Golgi complex were well developed, electron-dense secretory granules were prominent, and the cells had membrane specialization resembling tight junctions, desmosomes, and microvilli (Fig. 1b). In the cells of estradiol-treated oviduct, the nuclei were basal and the rough endoplasmic reticulum and Golgi complexes were even better developed than in the tissue of ethionine-treated animals. The secretory granules were more prominent, membrane differentiation was obvious, and the cytoplasm contained abundant ribosomes (Fig. 1c). A process similar to that in the glands was obvious in the surface epithelium. The surface cells in the controls had few organelles, only occasional cilia, and sparse membrane specialization. In the tissues of the animals exposed to estradiol or ethionine, organelles, membrane specialization, and cilia became abundant (Fig. 2).

Induction of ovalbumin and conalbumin synthesis by ethionine was not observed in the 'primitive oviduct' of chicks (5 to 6 weeks old) that had not received primary stimulation with estrogen. This suggests that ethionine alone cannot cause cytodifferentiation and growth of the primitive oviduct, but that in tissues already differentiated by the action of estradiol, it can induce the further cellular differentiation needed for specific protein synthesis. The mechanism of induction of protein synthesis by ethionine remains obscure, it may or may not stem from interference with the methylation of either mRNA or tRNA or both.
Whether the findings reported here have any relevance to carcinogenesis by ethionine is obscure. However, since ethionine is known to be synthesized by E. coli and other microorganisms, and therefore it may be a ubiquitous carcinogen in the gut, studies of its various attributes are of importance.

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References


Fig. 1. Electron micrographs of chick oviduct magna (49). A, tubular gland from control chicks. The nuclei are randomly distributed and the cytoplasm has a minimal number of organelles. × 5400. B, tubular gland from ethionine-treated chicks. The nuclei are basal and the cytoplasm contains abundant rough endoplasmic reticulum, Golgi complex, and apical secretory granules. × 5600. C, tubular gland from estradiol-treated chicks. The cytoplasmic organelles are even more prominent and there are abundant ribosomes. × 5800.

Fig. 2. Electron micrographs of epithelia of chick oviduct magna (49). Surface epithelium from control chicks (A). The epithelial cells contain sparse cytoplasmic organelles and few cilia are present on the surface. × 7500. B, surface epithelium from ethionine-treated chicks. The cells contain abundant cytoplasmic organelles and cilia are prominent in the oviduct cavity. × 9500.
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