Regulation by Estrogen of Organ-Specific Synthesis of a Nuclear Acidic Protein

by Ching-Sung Teng* and Terrell H. Hamilton

Department of Zoology
The University of Texas
Austin, Texas 78712

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Summary

Estradiol-17β acting in vivo in the uterus of the ovariectomized adult rat stimulates about 75% the incorporation of ^{3}H-tryptophan into the fraction of nuclear acidic protein extracted in 0.05 N NaOH. The electrophoretic distribution of this nuclear protein fraction in gels containing 10% acrylamide, 5 M urea, and 0.1% sodium dodecyl sulfate indicates that this stimulation of incorporation is restricted almost entirely to a single band. No appreciable stimulation of incorporation of ^{3}H-tryptophan by the hormone was observed in the acidic protein of hepatic nuclei. It is concluded that induction of organ-specific synthesis of a nuclear acidic protein is an important aspect of the regulation of gene activation by estrogen in the uterus.

It is a current concept that acidic as well as basic proteins are important participants in the regulation of nuclear genetic transcription in eucaryotic cells (for review, see ref. 1). Earlier attempts to explain selective gene activation by alterations in the structure and function of histones alone have proven for the most part unsuccessful (1, 2). Histones are now known to be relatively few in number and to exhibit a notable homogeneity of composition among phylogenetically separated eucaryotes (3). For these and other reasons, the acidic proteins of the nucleus have become likely candidates for the control of the organ-specific and selective transcription of the DNA contained in the chromosomes of higher cells (1-9).

That regulation of organ-specific nuclear transcription is an early

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* Present address: The Rockefeller University, New York, N. Y. 10021.

and major feature in the subcellular action of many growth-promoting and developmental hormones is well known (for reviews, see refs. 10-12). We previously demonstrated that the rate of synthesis of both nuclear and chromosomal acidic proteins is controlled by estradiol-17β acting in the uterus of the ovariectomized rat (13). The accelerated rate of synthesis of uterine acidic proteins and the resulting increase in their concentration in the interphase chromosomes induced by the hormone were correlated with a temporal rise in the template activity of the chromosomes, assayed in vitro using bacterial RNA polymerase (9, 13).

We now report the results of a comparative investigation of the effects of administration to the ovariectomized rat of estradiol-17β on the incorporation of ^{3}H-tryptophan in vivo into several protein fractions of both uterine and hepatic nuclei. The effect of the hormone on the electrophoretic distribution of labeled uterine and hepatic nuclear acidic proteins in polyacrylamide gel containing 5 M urea and 0.1% sodium dodecyl sulfate is also described.

Briefly stated, we exploited the fact that histones do not contain tryptophan, and used ^{3}H-tryptophan to label in vivo the more acidic proteins of the nucleus. Also, there is now good evidence that the majority of the deoxyribonucleoprotein of nuclei is extracted by 0.5 - 2 M NaCl, and that the residue, soluble in 0.05 N NaOH, contains the majority of the nuclear acidic protein along with the nucleoli, nuclear envelopes, and some residual chromatin (14-17). Isolation of nuclei was as previously described (18). The details of the fractionation of the nuclear proteins and of the other experimental procedures employed are given in the relevant figures.

Fig. 1 compares the effects of a single injection of estradiol-17β on the incorporation of ^{3}H-tryptophan in vivo into four fractions of nuclear protein in both the uterus and the liver. In both uterine and hepatic nuclei, there

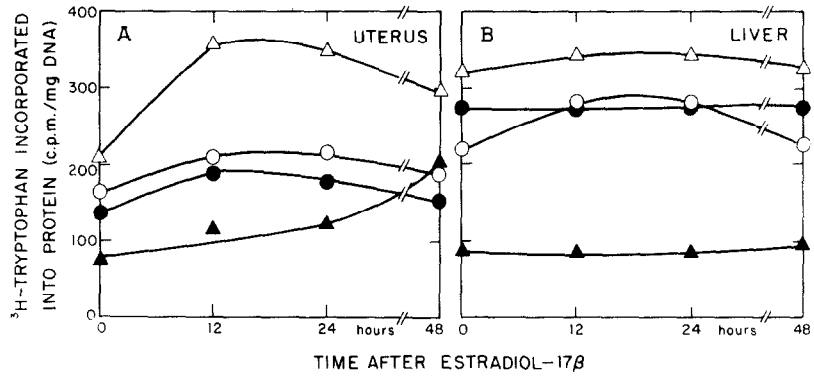

Fig. 1. Effect of administration of estradiol-17β on the incorporation in vivo of ^{3}H-tryptophan into several fractions of nuclear protein in the uterus (A) and liver (B) of the ovariectomized adult rat. All animals received intraperitoneally 100 μC (6.6 C/mmole) of ^{3}H-tryptophan (Schwarz) at 24 hours before killing. At the time indicated after administration intraperitoneally of 10 μg of estradiol-17β, 8-10 animals per group were killed and the uterine and hepatic nuclei were isolated as previously described (18). Nuclei were washed (17), suspended in 0.2 ml of $MgCl_2$-Tris buffer, pH 7.6, and extracted as follows: (a) A fraction of soluble proteins was obtained by three extractions, each by addition of 15 ml of 0.14 M NaCl (containing 5 mM $MgCl_2$ and 0.05 M Tris buffer, pH 7.6), followed by centrifugation at 25,000 X g for 20 minutes. (b) The sediment remaining was twice extracted for 2 hours with additions of 5 ml of the $MgCl_2$-Tris buffer containing 1.0 M NaCl. The resulting suspension was then homogenized using three strokes in the Dounce homogenizer. The residual material was pelleted by centrifugation at 25,000 X g for 20 minutes. The supernatant fraction was diluted to low salt concentration by dialysis against the $MgCl_2$-Tris buffer containing 0.14 M NaCl at 4° C for 24 hours. DNA and histone were sedimented by centrifugation at 25,000 X g for 20 minutes. The acidic proteins remaining in solution were precipitated by addition of 10% trichloroacetic acid, and then successively washed with alcohol and ether. (c) After the preceding salt extractions, the alkali-soluble residual protein was extracted in 0.05 N NaOH during 2 hours. (d) The alkali-insoluble protein was isolated from the alkali-soluble protein by centrifugation at 25,000 X g for 30 minutes. The pellet was solubilized by heating at 80° C for 15 minutes in 1.0 N NaOH. The radioactivity incorporated into each of the four fractions of nuclear protein was measured and the results expressed as counts per minute (c.p.m.) per mg of DNA contained in each nuclear preparation, as described elsewhere (13). O——O, 0.14 M NaCl-soluble protein; ●——●, 1.0 M NaCl-soluble protein; △——△, 0.05 N NaOH-soluble protein; ▲——▲, 0.05 N NaOH-insoluble protein.

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was a stimulation of 25-35% in the incorporation of the labeled amino acid into the soluble protein extracted in 0.14 M NaCl, at 12-24 hours after hormone treatment. At 48 hours after treatment, the incorporation into this

nuclear protein fraction was decreased. In the uterus, all four fractions of nuclear protein showed increased incorporation of ^{3}H-tryptophan at 12 hours after hormone treatment, and with one exception, showed decreased incorporation at 24 and 48 hours (Fig. 1A). The specific activity of the alkali-soluble or acidic protein fraction extracted in 0.05 N NaOH exhibited the greatest response to the hormone. The alkali-insoluble protein fraction, however, exhibited a delayed rise in specific activity, with the highest incorporation occurring at 48 hours after hormone treatment.

In both uterine and hepatic nuclei, the incorporation of ^{3}H-tryptophan was higher in the alkali-soluble or acidic protein fraction than in the other protein fractions, and was at a maximum at 12 hours after hormone treatment (Fig. 1). The incorporation into the alkali-soluble protein fraction of uterine nuclei was stimulated about 75% at 12 hours after hormone treatment (Fig. 1A), whereas in hepatic nuclei the stimulation did not exceed 6% (Fig. 1B).

Preliminary experiments had indicated that estradiol-17β acting in the uterus *in vivo* causes no qualitative effects on the histones discernible by their electrophoretic distribution in polyacrylamide gel. The nuclear fraction of alkali-soluble or acidic protein, into which the incorporation of ^{3}H-tryptophan was stimulated about 75% by the hormone acting in the uterus (Fig. 1A), was therefore selected for distribution in polyacrylamide gels. The alkali-soluble or acidic nuclear proteins extracted in 0.05 N NaOH are highly aggregated, and the electrophoretic separation of these proteins was achieved in gels containing 10% acrylamide, 5 M urea, and 0.1% sodium dodecyl sulfate.

Fig. 2 shows the effects of a single injection of estradiol-17β on the electrophoretic distribution and labeling patterns of the acidic proteins labeled *in vivo* with ^{3}H-tryptophan, and extracted from nuclei of the uterus or the liver. Approximately 6 major gel bands were recognizable by the staining and label-

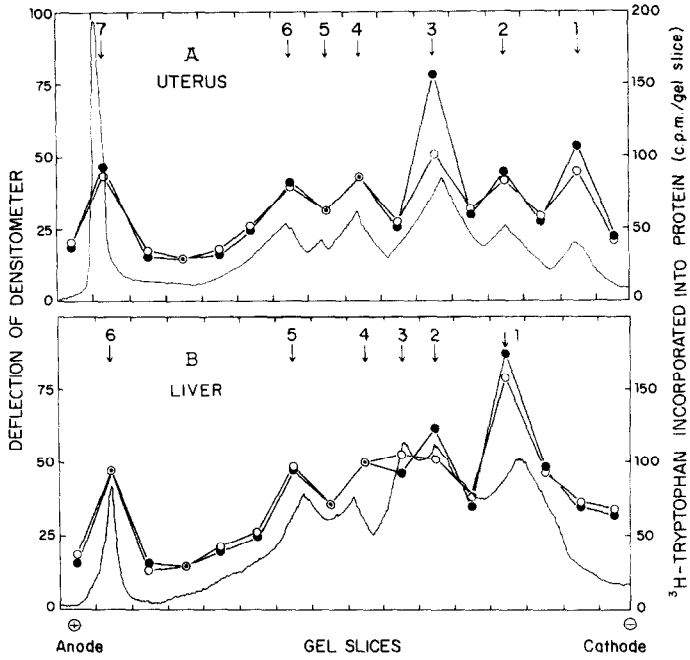

Fig. 2. Effect of administration of estradiol-17β on the electrophoretic distribution and labeling patterns of acidic protein extracted from the nuclei of the uterus (A) and liver (B) of the ovariectomized rat. All animals received 100 μC of ^{3}H-tryptophan at 12 hours before killing. The experimental animals received 10 μg of hormone at the same time. The alkali-soluble or acidic proteins of uterine and hepatic nuclei, isolated according to step c of Fig. 1, were distributed electrophoretically in gels containing 10% acrylamide, 5 M urea, and 0.1% sodium dodecyl sulfate. Each gel was 7 mm in diameter and 64 mm in length. Samples of uterine and hepatic acidic protein were distributed in triplicate gels. After staining, each gel was destained and sliced at intervals of 4 mm. Corresponding slices of the gels were pooled respectively, and digested at 50° C for 2 hours in 0.5 ml of NCS Solubilizer (Amersham-Searle). To each digest, 15 ml of Bray's solution was added for measurement of the radioactivity as previously described (13). The other experimental details were as described in Fig. 1. O———O, Acidic protein from control animals; ●———●, acidic protein from hormone-treated animals; ———, densitometer recording (essentially identical for the acidic protein isolated from the nuclei of either control or hormone-treated animals).

ing patterns of the acidic proteins extracted from the nuclei of either organ. With one major exception, no effect of estradiol-17β in vivo on the labeling pattern of the acidic proteins extracted from uterine nuclei was discernible throughout the gel (Fig. 2A). Band 3, however, showed repeatedly an in-

creased incorporation of about 70-75%. Band 1 showed a minor and perhaps insignificant stimulation of incorporation of about 9% or less.

The acidic proteins extracted from hepatic nuclei and similarly separated electrophoretically showed no stimulation of incorporation due to the hormone, except for a small and perhaps insignificant increase of 8% or less in bands 1-3 (Fig. 2B).

Discussion

The experiments described here provide the first clear evidence that the synthesis of a specific nuclear acidic protein is involved in gene activation induced by estradiol-17β acting in its characteristic target organ, and further, that this effect of the hormone is organ-specific. The temporal variations in the rate of incorporation of ^{3}H-tryptophan into the nuclear acidic proteins of the uterus indicate that this hormone-induced synthesis is most rapid at about 12 hours after treatment (cf. Figs. 1A and 2A). We emphasize that during this period of hormone action gene activation in the uterus is at a maximum, as measured by the template activity *in vitro* of the chromosomes isolated from the organ (9). Also during this period of hormone action, the amount of acidic protein contained in the chromosomes increases markedly (13), in correlation with the rapid appearance of newly formed ribosomes in the cytoplasm (12).

Whether the acidic protein of uterine nuclei stimulated by estrogen as described in the present report is truly a DNA-associated protein remains to be determined. However, the electrophoretic distribution of the acidic proteins of uterine nuclei described in Fig. 2 is similar to that of the proteins extracted from the interphase chromosomes isolated from the uterus, and the alkali-soluble fraction of nuclear proteins extracted for electrophoretic distribution as described in step *c* of Fig. 1 is rich in the acidic amino acids

(work in progress by C. -S. Teng).

Finally, we acknowledge that Shelton and Allfrey (19) have recently demonstrated that hydrocortisone acting in rat liver in vivo causes the specific synthesis of an acidic nuclear protein. Their findings and now ours point clearly to an important role for the synthesis of specific nuclear acidic proteins in gene activation regulated by steroid hormones. Strictly speaking, it is unknown whether the synthesis of specific nuclear acidic protein is a cause or a consequence of the specificity of the response of an organ to hormone. Nevertheless, the remarkable congruence between their investigation and ours at least hints that specificity for organ-specific gene activation may in part reside in the acidic proteins of the chromosomes.

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