IN VITRO REGULATION OF RNA POLYMERASE IN ESTROGEN-TREATED UTERI[1]

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In vivo studies with antibiotics inhibiting RNA or protein synthesis support the conclusion that estrogenic hormones accelerate genetic expression in the rat uterus (Mueller, Gorski, and Aizawa, 1961; Ui and Mueller, 1963; Hamilton, 1963, 1964). Underlying the stimulated expression of genetic information and the augmented rate of RNA synthesis resulting from hormone treatment is the increased activity of DNA-dependent RNA polymerase (Gorski, 1964). In an attempt to explain the sensitivity of this enzyme to estrogens and to gain insight into the molecular mechanism of estrogen action, factors regulating RNA polymerase activity in the rat uterus were studied. The present report demonstrates the striking dependence of the estrogen-induced polymerase of surviving uteri on the continued synthesis of new protein and reveals a highly temperature-sensitive process which also functions in the regulation of the enzyme. Possible roles of estrogens in controlling the protein metabolism necessary for polymerase activity are discussed.

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Experimental. Four hours before killing, 22-day old female Holtzman rats were injected intraperitoneally with 5 μg of estradiol-17β in 0.5 ml of 0.9% NaCl-1% ethanol or the same vehicle without the hormone. After sacrifice the uteri were removed and either assayed immediately for RNA polymerase activity or placed into 25-ml Erlenmeyer flasks containing 2 ml modified Eagle's tissue culture medium (Mueller et al., 1962). Flasks containing two uteri each were incubated in a shaking water bath either at 23°C or 37°C in an atmosphere of 95% O_2 and 5% CO_2. Where indicated, cycloheximide (25 μg/ml) was added to the incubation medium.

The uteri were homogenized, the nuclear fraction isolated, and the RNA polymerase assayed according to the methods described by Gorski (1964). This assay was always carried out at 37°C. Duplicate determinations were made by dividing equally the pooled homogenates from the two uteri of each group. Cytidine triphosphate-H^3 (CTP-H^3, 1.25 μC/ml, 1.2 C/mmole) was the usual precursor used, although uridine triphosphate-H^3 and guanosine triphosphate-H^3 gave similar results. The amount of radioactivity in a product insoluble in 10% perchloric acid and hydrolyzable by RNAase or 0.3 N KOH was determined in a Packard liquid scintillation counter.

Results. As previously found by Gorski (1964), the activity of DNA-dependent RNA polymerase of the rat uterus rises rapidly after treatment in vivo with estradiol. The difference in the level of polymerase activity between the estrogen-treated and control uteri was maintained over a 2-hour period when the surviving uteri were incubated at 37°C in tissue culture medium (Fig. 1). The estrogen-induced portion of RNA polymerase activity, however, was unusually sensitive to the action of cycloheximide; 25 μg/ml of this agent in the tissue culture medium reduced the RNA polymerase activity of surviving uteri from estrogen-treated rats to the level of the control uteri within 1 hour. In contrast, this concentration of cycloheximide, which prevented 95% of the in vitro incorporation of leucine-C^{14} into the rat uterus, had almost no effect on the polymerase activity of control uteri. To counteract

the estrogen stimulation, cycloheximide had to be present during the in vitro incubation of the surviving uteri, as it had no effect when added directly to the nuclear system used to assay RNA polymerase. These results, along with similar findings with puromycin, suggest that the maintenance of the estrogen-induced fraction of uterine polymerase activity requires the concomitant synthesis of protein.

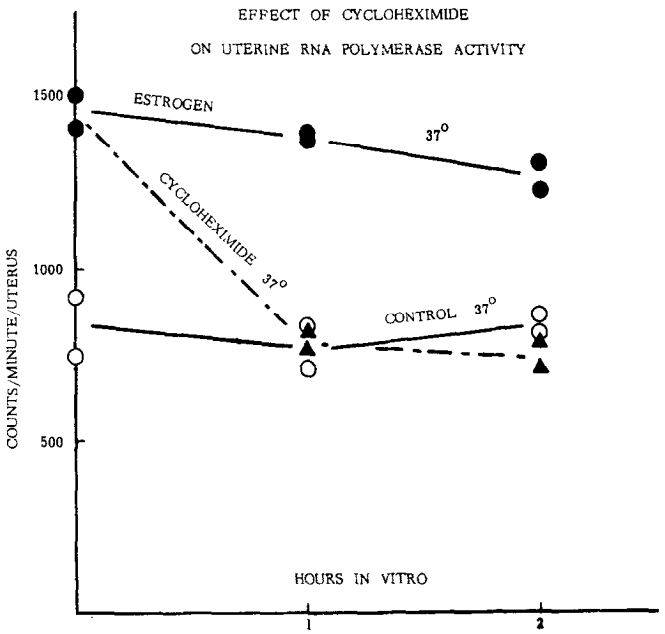

Figure 1. The stimulation of uterine RNA polymerase activity by estrogen (●—●) and the effect of cycloheximide (25 μg/ml) (▲---▲) on this activity. Estradiol (5 μg) given in vivo 4 hours before in vitro incubation at 37°C; controls (○—○) received no estradiol. RNA polymerase assayed by the method of Gorski (1964).

The unusual sensitivity of the estrogen-induced RNA polymerase activity to a temperature-dependent process is shown in Fig. 2. Incubation of intact uteri from hormone-treated animals at 23°C rather than at 37°C rapidly reduced the polymerase activity to that of control uteri also incubated at 23°C, while the control's activity showed comparatively little sensitivity to the lowered temperature. The polymerase activity lost during incubation of the uteri at 23°C could, however, be restored by incubating at 37°C

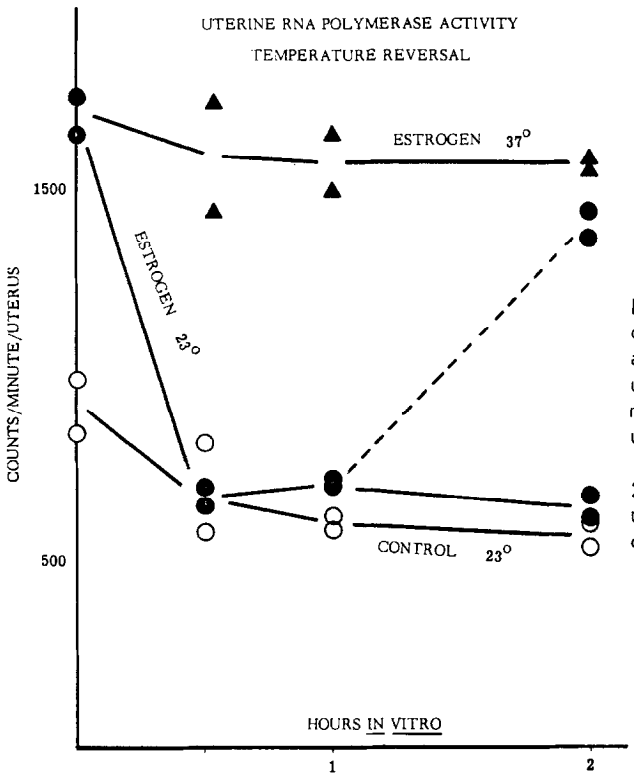

Figure 2. The effect of in vitro incubation of estrogen-treated (● — ●) and control (○ — ○) uteri at 23°C on uterine RNA polymerase activity. Polymerase activity of estrogen-treated uteri incubated continuously at 37°C (▲ — ▲), or at 37°C after 1 hour at 23°C (● - - ●) were also determined using the procedures and conditions described in the text.

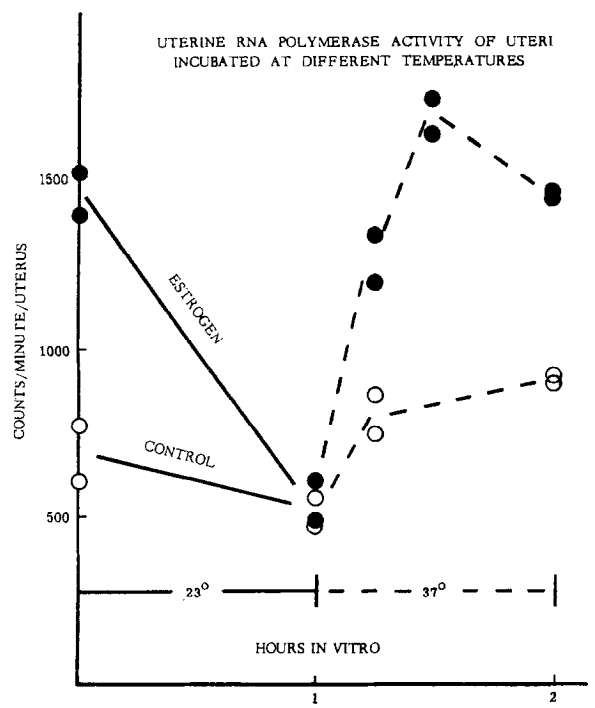

Figure 3. The alteration of the uterine RNA polymerase activity in uteri incubated in vitro at different temperatures. Uteri from control (○) or estrogen-treated (●) were incubated initially at 23°C for 1 hour and then transferred to 37°C for 15, 30 or 60 minutes.

(Fig. 2). Fig. 3 shows how quickly this recovery takes place. The level of enzyme activity characteristic of estrogen stimulation was completely regained during the first hour at 37°C, even if the uteri had been incubated at 23°C for as long as 3 hours. An interesting aspect of the recovery was the tendency for the polymerase activity to overshoot the values observed immediately after removal of the uteri from the animal (Fig. 3 and 4A). While the polymerase activity of control uteri tended to rise on the shift of the incubation temperature back to 37°C, the response was variable and less dramatic. Taken together, these findings show that lowering the temperature did not abolish the mechanisms underlying the estrogen activation of polymerase, but only prevented the manifestation of this activation by some temperature-sensitive process.

Although the restoration of enzyme activity occurred very rapidly, the increase was dependent upon protein synthesis (Fig. 4A). The addition of cycloheximide to the in vitro incubation medium 10 min before the return to

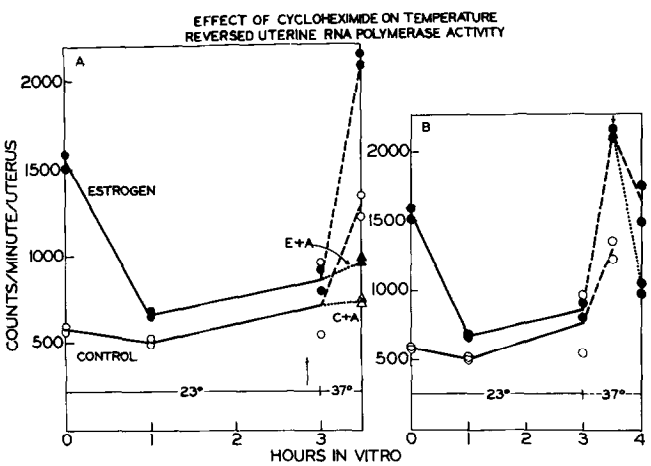

Figure 4. The effect of cycloheximide on the temperature-induced reversal of uterine RNA polymerase activity. A: Uteri from estrogen-treated (●—●) or control (○—○) animals incubated at 23°C for 3 hours, then transferred to 37°C (●--●) (○--○). Ten minutes before transfer to 37°C, 25 μg/ml cycloheximide added to appropriate flasks with estrogen-treated (●···▲) (E + A) and control (○···△) (C + A) uteri. Conditions and procedures as described in text. B: Same experiment and symbols as A; cycloheximide added 30 minutes after transfer to 37°C (●···●).

37°C completely prevented any increase in polymerase activity in either estrogen-treated or control uteri. Similarly, that polymerase activity regained by reincubation of the uteri for 30 min at 37°C was rapidly lost when cycloheximide was added to the tissue culture medium (Fig. 4B).

Discussion. These experiments show that the estrogen-stimulated level of uterine RNA polymerase activity can be maintained for lengthy periods while the surviving uteri are incubated in tissue culture medium. The increased activity, however, is highly dependent upon the continued synthesis of protein, a finding previously observed in experiments with puromycin in intact animals (Noteboom and Gorski, 1963; Gorski, Noteboom, and Nicolette, 1965). Thus the present results complement earlier findings on the control of hormone-induced, uterine RNA polymerase activity, all indicating that the estrogen stimulation of this enzymatic activity is regulated through a process requiring protein synthesis. The in vivo action of the hormone appears to involve a mechanism for utilizing or providing this essential protein. Once the mechanism is established, it survives an interval in which the polymerase activity is limited by a newly found, temperature-sensitive process in the regulation of RNA polymerase.

The dependence of that portion of RNA polymerase activity associated with rapid growth on the continued synthesis of protein is not limited to the estrogen response, since a similar phenomenon has been found in the nuclei of HeLa cells. Limiting the protein synthetic capacity of HeLa cells growing logarithmically by puromycin, cycloheximide (Summers, Noteboom, and Mueller, 1966) or levallorphan (Noteboom and Mueller) markedly depressed the activity of nuclear RNA polymerase. While the requirement for newly synthesized protein in both the HeLa cell and uterine systems might be related to a rapid turnover of a fraction of the RNA polymerase, it appears even more probable that it relates to a need for protein synthesis in the operation of polymerase at certain gene sites. This concept accords with the earlier demonstration that recently synthesized RNA is associated with newly synthe-

sized protein in the nucleus of the HeLa cell, and that this association is obligatory for the accumulation of ribosomal precursor RNA (Tamaoki and Mueller, 1965a, 1965b).

The newly found, temperature-sensitive step contributing to the regulation of RNA polymerase may involve intracellular transport of protein rather than synthesis; this subject is currently being investigated.

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