CHANGES IN HYBRIDIZABLE NUCLEAR RNA DURING PROGESTERONE INDUCTION OF A SPECIFIC OVIDUCT PROTEIN

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It is well established that steroid hormones dramatically affect RNA synthesis in many model systems (Tata, 1966). Cortisol has been reported to stimulate the synthesis of messenger RNA (mRNA) activity, ribosomal precursor RNA, and new species of hybridizable mRNA in liver nuclei (Drews & Brawerman, 1967). We have previously reported that progesterone (P) induces synthesis of the specific chick oviduct protein, avidin (O'Malley, 1967; O'Malley & McGuire, 1968). Unlike the response to steroids seen in most target tissues, new avidin synthesis is not accompanied by increased synthesis of other major oviduct proteins. We have demonstrated changes in oviduct nuclear rapidly-labeled RNA (O'Malley & McGuire, 1968), RNA polymerase (McGuire & O'Malley, 1968), DNA-chromatin template activity and dinucleotide composition of RNA (McGuire & O'Malley, unpublished observations) following administration of progesterone but prior to induction of avidin. The question arose whether these changes in nuclear RNA synthesis were accompanied by the formation of new species of nuclear RNA. Therefore, we have chosen to employ molecular hybridization techniques to estimate changes in the population of nuclear hybridizable RNA's during progesterone mediated induction of avidin synthesis in the chick oviduct.

EXPERIMENTAL PROCEDURE

Three-day old female Rhode Island Red chicks were injected subcutaneously with 5 mg diethylstilbestrol (DES) in sesame oil daily for 20 days.

Half of the group (DES+P) were given a single injection of progesterone (5 mg) in sesame oil 18 hours prior to sacrifice. The oviducts from the chicks receiving only DES were removed and incubated in sterile Medium 199 with 25 µc/ml of ^{3}H-uridine (SA=20 c/mM) and 12 µc/ml of ^{3}H-adenine (8 c/mM) for 20 minutes at 37°C. The oviducts from DES+P chicks were incubated similarly with 0.25 mc of phosphoric acid -^{32}P. The incubations were terminated by freezing. The two separately labeled tissues were combined, homogenized and carried through the remainder of the procedure as a unit. All nuclei were isolated as previously described (O'Malley & McGuire, 1968). The nuclei were suspended in a bentonite buffer (0.01 M sodium acetate, 0.01 M EDTA, 0.05% bentonite, pH 5.1) and sodium dodecyl sulfate (SDS) added to 0.1 per cent. The mixture was extracted with 90% phenol for 5 minutes at 23° and then for 4 minutes at 75°. Sodium chloride was added to the aqueous residue to a concentration of 3% and the nucleic acid was precipitated twice with 2 volumes of 95% ethanol. The precipitate was washed twice with 3 M sodium acetate, once with 95% ethanol, digested with DNase, subtilisin, and pronase. The nucleic acid was re-extracted with hot (70°) phenol, precipitated from 95% ethanol and stored in 2 x SSC, (1 x SSC = 0.15 M NaCl, 0.015 M Na_3citrate, pH 7) at -30°C. DNA was preapred according to the method of Marmur (1961) and stored in 0.1 x SSC. Hybridization was carried out using a modification of a liquid-liquid annealing system using membrane filter separation techniques (Nygaard and Hall, 1964; Torelli et al, 1968). All hybrids were digested with RNase to improve specificity.

A relative saturation plateau was obtained in our system at a ratio of RNA/DNA of 2:1 to 3:1. All competition studies were done at an RNA/DNA ratio of 2:1 or less. Bacterial RNA did not compete with chick oviduct RNA for sites on chick liver DNA in the system. Rabbit liver and rat liver nuclear RNA contained some sequences complementary to the chick DNA (50% maximum) but human lymphocyte RNA competed poorly. In each of these heterologous species, the competition quickly reached a plateau where further displacement of homologous chick RNA was not possible.

RESULTS AND DISCUSSION

When unlabeled nuclear RNA from DES-stimulated oviducts was used to compete for complementary DNA sites with labeled RNA from DES or DES+P oviducts, greater competition occurred with the ^{3}H-RNA from animals which received only DES (Fig. 1A). We would interpret these results to mean that a new specie(s) of nuclear hybridizable RNA was produced by the chick oviduct following progesterone administration. The differences were not large but we would not expect great differences between the DES and DES+P competition curves because of the specificity of the progesterone response. Since the RNA's were handled simultaneously under a double-label protocol, technical error should be eliminated. When unlabeled nuclear RNA from DES+P oviducts was used as the competing RNA, no differences were noted between the labeled DES and DES+P competition curves (Fig. 1B). This would be expected

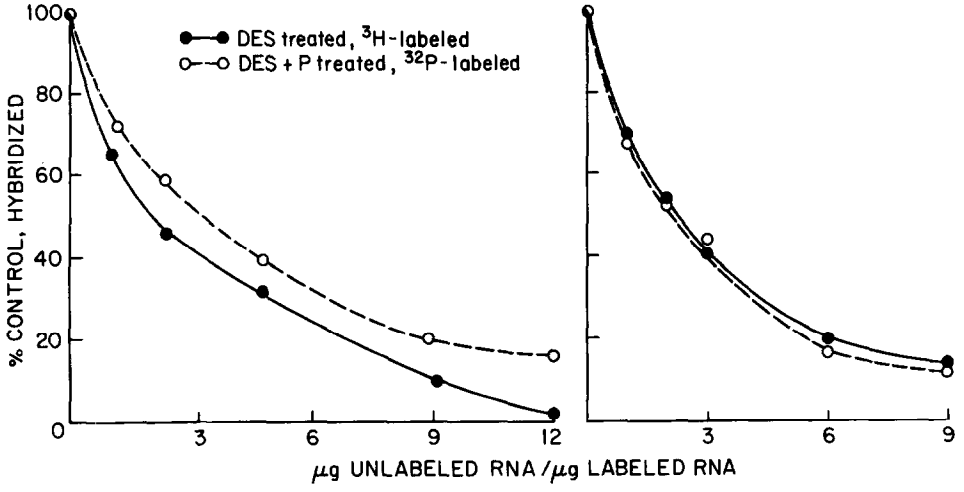

Fig. 1A (left) and 1B (right). Effect of progesterone on species of nuclear RNA. Chick oviduct DNA (40 μg) was incubated with labeled nuclear RNA (80 μg) from DES or DES + P treated oviducts for 12 hours at 67°C. Increasing amounts of unlabeled nuclear RNA from DES (1A) or DES + P (1B) treated oviducts were added to the initial reaction mixture to competitively hybridize to the DNA. The total labeled RNA hybridizing to DNA with no unlabeled competitor RNA present equals 100% control, hybridized, and represents 1% hybridization of the total input RNA at relative saturation. The specific activity of the labeled RNA ranged from 2000 to 8000 cpm/μg in 6 separate experiments.

if the unlabeled DES+P RNA should contain the same species present in both ^{32}P - DES+P and ^{3}H - DES nuclear RNA's and suggests that progesterone does not qualitatively inhibit production of estrogen-induced species of nuclear RNA.

It is tempting to postulate that these new species of nuclear RNA represent new mRNAs transcribed in response to progesterone and involved in directing new avidin synthesis. The fact that mRNA hybridizes with homologous DNA is indisputable, but one cannot assume all hybridizable RNA to be mRNA. Similarly we cannot prove that this new specie(s) of nuclear RNA represents the template for avidin. However, these results further substantiate the hypothesis that in our model system, progesterone causes new nuclear transcriptions prior to and during the initiation of avidin synthesis.

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