Progesterone-Dependent Messenger RNA: Heterospecific Activity in vivo

Abstract. *The action of progesterone in mediating the synthesis of avidin by the chick oviduct can be simulated by the intraoviductal instillation of nitrocellulose-trapped RNA from hormonally prepared chick or pigeon oviduct. Similarly, the pigeon oviduct synthesizes avidin in response to chick oviduct RNA. Thus, a heterospecific transfer of hormonal stimulation, through the transfer of progesterone-induced RNA, is demonstrated. The biological activity is lost after digestion by pancreatic ribonuclease. The 50-fold purification achieved by nitrocellulose chromatography of the total RNA preparation suggests that the activity resides in a messenger RNA fraction.*

Treatment of estrogen-primed chicks with progesterone increases the rate of synthesis by the oviduct of nuclear RNA, induces the appearance of new species of RNA (*1*), and, consequently, evokes the synthesis by the oviduct of the hormone-dependent protein avidin (*2*). The concept that the effect of progesterone is mediated through RNA is supported by the observation that the total RNA extracted from progesterone-stimulated chick oviducts can induce avidin synthesis in chicks treated with estrogen (*3*). RNA from other organs or oviductal RNA hydrolyzed enzymatically is incapable of eliciting this biological response. The shell gland, a specialized region of the chick's Mullerian duct system, does not synthesize avidin in response to progesterone, but does produce avidin when treated with RNA extracted from the oviduct of progesterone-stimulated chicks (*3*). Tissue minces of chick oviduct synthesize avidin when progesterone is added in vitro to the incubation medium; a total RNA extract of progesterone-stimulated oviducts can duplicate this in vitro effect of the hormone (*3*). Considerable purification of RNA can be achieved by nitrocellulose trapping (*4*). This polyadenylate [poly(A)] rich RNA from the oviduct of hormone-treated chicks codes for the synthesis of avidin in a system of lysed rabbit reticulocytes (*5*).

Thus, these are examples of homologous in vivo transfer of the message for avidin synthesis coded in response to progesterone, as well as both homologous and heterologous in vitro systems in which RNA coded for avidin induces the synthesis of this progesterone-dependent protein, even though the hormone is absent. Our study demonstrates that a highly purified, poly(A) RNA synthesized in the oviduct of one avian species, under progesterone stimulation, can initiate avidin synthesis in another avian species.

Female chicks (*6*) were received in the laboratory from a commercial hatchery on day 1 after hatching. They were injected subcutaneously with 0.5 mg of stilbestrol in 0.05 ml of sesame oil daily for 5 days (days 2 to 6 after hatching). On day 7, the chicks were given 5 mg of progesterone in 0.25 ml propylene glycol by subcutaneous injection. Chicks thus treated, usually in batches of 100, were killed 12 hours after the single injection of progesterone, since this is the approximate time of maximum RNA synthesis in the estrogen-primed progesterone-treated chicks (*1*). The oviducts were dissected out, separated from the larger, muscular shell gland, and frozen in glass vessels over Dry Ice. Total RNA was extracted by a cold phenol procedure and purified by nitrocellulose trapping.

The extraction of poly(A) RNA was based on the method of Braverman *et al.* (*4*). Chick or pigeon oviducts were homogenized in 10 to 15 ml of buffer per gram of tissue. The buffer consisted of 100 m*M* tris-HCl, *p*H 7.6; 0.5 percent sodium dodecyl sulfate (SDS); 3 m*M* $MgCl_2$; 0.32*M* sucrose. The homogenate was filtered through cheesecloth and centrifuged at 15,000*g* for 20 minutes. One volume of water-saturated phenol was added to the supernatant. This mixture was stirred at 4°C for 30 minutes, and then centrifuged at 4000*g* for 30 minutes. One volume of buffer (100 m*M* tris-HCl, *p*H 9.0, containing 0.5 percent SDS) was added to the nonaqueous residue, and stirring and centrifuging were repeated. The aqueous phases were combined, and the RNA was precipitated by the addition of 0.1 volume of 1*M* NaCl and 2.5 volumes of ethanol. The alcoholic solution was stored at −20°C overnight. After centrifugation, the precipitate was washed three times with 0.1*M* NaCl in 66 percent ethanol solution. The RNA was dissolved in distilled H_2O and washed five to six times with equal volumes of cold ether. The last traces of ether were removed with an airstream. The total RNA fraction, diluted 20-fold, was passed through a nitrocellulose column (40 mm by 15 mm) equilibrated with the diluent buffer (500 m*M* KCl, 1 m*M* $MgCl_2$, 10 m*M* tris-HCl, *p*H 7.6). The nitrocellulose was transferred into a centrifuge tube, and the RNA was eluted with buffer of high *p*H (100 m*M* tris-HCl, *p*H 9.0, 0.5 percent SDS). The RNA solution was repeatedly chilled to 4°C and centrifuged to remove traces of SDS.

The same procedure was used to prepare purified RNA from the oviducts of stilbestrol-primed, progester-

Table 1. Avidin synthesis by the chick oviduct in response to homologous and heterologous RNA.

Cases (No.)	Final 24-hour treatment*			Avidin (μg/g)
	Material	Amount (μg)	Route	
6	None			0
6	Saline		i.o.†	0
6	Progesterone	5000	s.c.	1.86 ± 0.76
7	Chick RNA	10	i.o.	0.83 ± 0.42
6	Chick RNA, ribonuclease-incubated‡	10	i.o.	0
10	Pigeon RNA	10	i.o.	0.5 ± 0.2

* All chicks were first treated with 0.5 mg of stilbestrol daily for 5 days † Abbreviations are: s.c., subcutaneous; i.o., intraoviductal. ‡ The RNA was treated with bovine pancreatic ribonuclease for 10 minutes at 37°C, and the enzyme was then inactivated by heat (65°C).

Table 2. Avidin synthesis by the pigeon oviduct in response to homologous and heterologous RNA.

Cases (No.)	Final 24-hour treatment*			Avidin (μg/g)
	Material	Amount (μg)	Route	
10	None			0
12	Progesterone	5000	s.c.†	0.9 ± 0.4
6	Pigeon RNA	10	i.o.	0.12 ± 0.03
10	Chick RNA	50	i.o.	0.53 ± 0.25
6	Chick RNA, ribonuclease-incubated‡	50	i.o.	0

* All pigeons were first treated with 0.5 mg of stilbestrol daily for 5 days. † Abbreviations are: s.c., subcutaneous; i.o., intraoviductal. ‡ The RNA was treated with bovine pancreatic ribonuclease for 10 minutes as 37°C, and the enzyme was then inactivated by heat (65°C).

one-treated pigeons (*7*) that were 6 weeks old when hormone treatment was begun.

The nitrocellulose-trapped RNA, dissolved in saline solution, was placed into the oviducts of ether-anesthetized chicks or pigeons by means of a 27-gauge needle and a Hamilton syringe. The volume was 100 μl. The recipient birds were of the same age, and had the same schedule of stilbestrol priming as described above. The birds were killed 24 hours later, and the avidin content of each treated oviduct, as well as that of various controls, was determined by a sensitive and specific assay based on binding of avidin to [^{14}C-carbonyl]biotin (*8*). Tissue was homogenized in 6 ml of 0.02*M* phosphate buffer, *p*H 7.1, containing 0.07*M* KCl, 0.004*M* $MgCl_2$, and 0.07*M* NaCl, and centrifuged at 5000*g* for 30 minutes. To 2 ml of supernatant was added 0.5 ml of [^{14}C]biotin (0.01 μc, 20 mc/mmole; Radiochemical Centre, Amersham) in 0.2*M* ammonium carbonate solution. The mixture was stirred and held at room temperature for 15 minutes. One milliliter of bentonite solution (10 mg of bentonite per milliliter of 0.2*M* ammonium carbonate) was added. The mixture was centrifuged at 2000*g* for 10 minutes. The precipitate was dissolved in 2 ml of 0.2*M* ammonium carbonate, and the centrifugation and purification procedure was repeated until the supernatant showed no significant radioactivity above the background when dissolved in Aquasol. The precipitate was then dissolved in 10 ml of Aquasol and the radioactivity was counted. Computation of the number of micrograms of avidin per gram of oviduct was based on a standard curve, with the use of avidin obtained from Sigma Chemical Company.

The response of the chick oviduct is shown in Table 1. Earlier results revealed that an intraoviductal dose of 500 μg of total RNA from stilbestrol- and progesterone-treated chick oviducts was required to stimulate avidin synthesis in estrogen-primed chicks (*3*). Nitrocellulose trapping effects a purification of approximately 50-fold. The RNA recovered after nitrocellulose chromatography stimulates avidin synthesis at a dose of 10 μg. This activity is lost when the poly(A) RNA is first treated with pancreatic ribonuclease. The oviduct of the stilbestrol-primed chick responds to poly(A) RNA extracted from the oviducts of pigeons treated with stilbestrol and progesterone. There is no avidin present in the oviducts of stilbestrol-primed chicks that served as controls (intraoviductal instillation of saline).

Avidin synthesis by the pigeon oviduct is shown in Table 2. Like the chick, and several other species reported (*9*), the oviduct of the estrogen-primed pigeon synthesizes avidin in response to progesterone. This response is brought about also by the intraoviductal administration of homologous oviductal RNA, prepared from hormone-treated pigeons. A dose of 10 μg of poly(A) RNA elicits a significant level of avidin synthesis. The pigeon oviduct responds, also, to poly(A) RNA recovered from hormone-treated chicks. Prior treatment of the chick RNA preparation with pancreatic ribonuclease eliminates the activity. A similar hydrolysis experiment with pigeon RNA has not been done.

The heterospecific transfer of hormonal stimulation, through the mediation of hormone-induced RNA in vivo, has not been described previously, although in vitro systems have been described (*10*). This finding is of interest with respect to the specificity of RNA coding in the vertebrate classes, as well as in relation to the evolution of hormonal control mechanisms. Evidently, the progesterone-receptor complex (*11*) interacts with highly similar or identical chromatin acceptor sites in the nuclei of both the chick and the pigeon, and does not discriminate between the two. Thus, once the hormone stimulates the production of RNA coded for avidin synthesis in the oviductal nuclei of either species, the hormone molecule itself is no longer required; and the newly synthesized RNA can complete the steps leading to avidin synthesis, interchangeably between the oviductal cells of the two species. This may be the physiological basis for the interspecies cross-reaction of many hormones. Most likely, the active component is a form of messenger RNA, since it is highly purified by nitrocellulose trapping (*4*). It has been concluded (*3*) that a contaminating residue of progesterone cannot account for the avidin synthesis observed after RNA administration. This conclusion is further strengthened by our observation of avidin induction with the purified RNA preparation that is active at a dose as low as 10 μg. As in earlier experiments with either progesterone-induced RNA or estrogen-induced RNA (*12*), enzymatic degradation with pancreatic ribonuclease eliminates the activity.

Our experiments do not reveal whether the exogenous RNA—homologous or heterologous—is translated directly or acts in some manner at the nuclear level to influence transcription of avidin-coded RNA. A lack of absolute antigenic identity between the avidin molecules produced by the two species, if found to exist, would provide an excellent experimental basis to resolve this question.

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Male Specific Antigen: Homology in Mice and Rats

Abstract. *Male lymphoid cells from some strains of rats can sensitize C57BL/6 female mice against C57BL/6 male skin grafts; this indicates that the male specific antigen of these two species is homologous.*

In both mice (*1*) and rats (*2, 3*) there is one exception to the rule that skin grafts are uniformly accepted between members of an isogenic strain. This occurs when females are challenged with grafts from male donors. Since the only genetic difference between male and female members of an isogenic strain is that males have the Y chromosome, of which there is no homolog in females, the simplest explanation for this incompatibility is the