Journal of Steroid Biochemistry, Vol. 9, pp. 697–702

0022-4731/78/0801-0697$02.00/0

CHROMATIN-ASSOCIATED RIBONUCLEASES ARE ACTIVATED BY ESTRADIOL IN CHICK OVIDUCT

RONALD F. COX
Department of Molecular Medicine, Mayo Clinic, Rochester, Minnesota 55901, U.S.A.

(*Received* 21 *November* 1977)

SUMMARY

To gain some insight into the mechanism of regulation of gene expression by estradiol in chick oviduct, the effect of steroid administration *in vivo* on the size distribution of nascent RNA transcripts synthesized in isolated 'chromatin' *in vitro* was analyzed. In 'chromatin' from withdrawn (control) animals, the majority of form B-directed RNA products were large (> 18S) and degradation of these chains was minimal. After treating chicks with estradiol *in vivo* for 1–6 h, a marked shift to a smaller size distribution was observed for the nascent RNA population, suggesting that 'chromatin' contained tightly-bound ribonucleases whose activity was enhanced by estradiol. This was confirmed by measuring the ability of 'chromatin' preparations to increase the number of 3'-ends in isolated endogenous RNA. The results indicate that the steroid-induced accumulation of mRNAs coding for egg white proteins in chick oviduct may be mediated by an activation of nuclear ribonucleases.

INTRODUCTION

There are still large gaps in our comprehension of the mechanism of action of the steroid hormone estradiol in chick oviduct tissue. The sequence of events which occurs between the binding of the steroid to receptor proteins in the nucleus [1, 2] and the onset of accumulation of specific mRNA molecules coding for egg white proteins in the cytoplasm 2–3 h later is poorly understood [3–5]. Recent studies have attempted to analyze steps occurring between the apparent *de novo* synthesis of the gene product and the appearance of mRNA in the cytoplasm for a single specific egg white protein gene, namely that coding for ovalbumin [5–7]. However, ovalbumin mRNA sequences are detected at very low levels in the nuclei both before and after steroid treatment, and nuclear precursors containing this mRNA have been difficult to identify, since 95% of the nuclear RNA sequences containing this message are identical in size with sequences found in cytoplasmic polysomes [5, 8]. Consequently it is not clear yet whether ovalbumin gene transcription is the primary site of steroid action, or whether alternative regulatory sites are also affected.

Given our present ignorance of the sequence of events occurring between transcription and translation in eukaryotic cells, several mechanisms, which are not mutually exclusive, could feasibly account for the estradiol-mediated induction of ovalbumin mRNA: (i) *de novo* transcription of previously inactive ovalbumin genes or an enhancement of the rate of transcription of active genes; (ii) an activation of processing mechanisms which precede the passage of mRNA to the cytoplasm; (iii) selective stabilization of ovalbumin mRNA in the cytoplasm. In this context, processing mechanisms would include the association of nascent transcripts with proteins [9], correct cleavage of precursor mRNA by specific ribonucleases (if indeed, a high molecular weight precursor for ovalbumin mRNA exists), link up of the correct primer sites with poly-A polymerases [10], methylation [11], and interaction of the 'completed' translatable mRNA with components governing transport to the cytoplasm. It is conceivable that if any of these processes become severely rate limiting, or occur out of sequence, transcripts containing ovalbumin mRNA sequences may never reach their intended destination, the cytoplasm, but eventually contribute to the pool of rapidly-turning-over nuclear RNA [12].

As part of this investigation, I have measured the size of the nascent RNA chains attached to endogenous RNA polymerases in 'chromatin' isolated before and up to 6 h after estradiol treatment *in vivo*, and the ability of chromatin-associated ribonucleases to degrade endogenously-synthesized RNA. This report indicates that treatment of chicks with estradiol activates nuclear ribonucleases capable of degrading the RNA products synthesized by form B RNA polymerases, suggesting a possible action of the steroid on mechanisms which contribute to the efficiency of conversion of RNA transcript to completed mRNA.

EXPERIMENTAL

Chicks and injections

One-week old, female, White Leghorn chicks were given 14 days of primary stimulation with estradiol benzoate or diethylstilbestrol (1 mg per day) and then withdrawn from steroid treatment for at least three weeks prior to a short-term secondary stimulation with estradiol-17β (2 mg in sesame oil injected intramuscularly).

Chromatin isolation

All solutions were adjusted to 0.5% diethylpyrocarbonate (Eastman) and boiled for 15–30 min before use. Excised oviducts were frozen immediately to −10°C in 25% glycerol, 0.5% triton X-100, 3 mM $CaCl_2$, 0.25 M sucrose and 10 mM Tris-HCl, pH 7.5 prior to tissue homogenization. Nuclei were then prepared by centrifugation through 2.1 M sucrose medium as described [1], resuspended and stirred in 10 mM Tris–HCl, pH 8.0 for 30 min at 4°C, and the resulting 'chromatin' collected by centrifugation. 'Chromatin' observed under the light microscope appears as clumped nuclei, and is consequently used only as an operational definition.

Synthesis, isolation and analysis of RNA

Standard reaction mixtures for RNA synthesis (1 ml) contained 25 mM Tris-HCl, pH 8.0, 1 mM $MnCl_2$, 0.5 mM DTT, 0.3 mM each of GTP and CTP, 250 mM $(NH_4)_2SO_4$, 1 mg heparin and 600–800 μg 'chromatin' DNA. Mixtures incubated for 5–30 min at 37°C also contained 0.3 mM ATP and 50 μM [^{3}H]-UTP (1950 d.p.m./pmol). Zero time mixtures were only supplemented with 50 μM [^{3}H]-ATP (2000 d.p.m./pmol) and incubated 2 min at 37°C. All tubes were then rapidly frozen in salt/ice and labeled RNA was isolated using cetyltrimethylammonium bromide (CTAB) and analyzed on 2.2% polyacrylamide gels containing SDS and 0.5% agarose [13].

Preparation and analysis of RNA hydrolysates

After addition of 10% trichloro-acetic acid to reaction mixtures, precipitates were washed extensively, hydrolyzed with 0.3 M KOH for 16 h at 37°C and the resulting nucleotides and nucleosides absorbed onto charcoal and eluted with pyridine [14]. Aliquots were chromatographed on PEI-cellulose thin layer plates, and radioactivity in UMP and uridine estimated as described [14], except that relevant sections or plastic-backed cellulose plates were cut out and counted directly.

RESULTS

Isolated 'chromatin' contains tightly-bound RNA polymerases [15] which are predominantly form B (that is, sensitive to α-amanitin at a concentration of 0.5 μg/ml), which already bear nascent RNA chains [13] (presumably synthesized *in vivo*), and which can be elongated *in vitro* under high salt conditions. Incubations were carried out in the presence of heparin, an inhibitor of initiation of form B RNA polymerase, to restrict RNA synthesis to elongation of pre-existing chains and to minimize breakdown of RNA during the isolation procedure [13, 16]. The increase in size of these nascent chains *in vitro* is then monitored by isolating and separating labeled RNA on polyacrylamide gels (Fig. 1). The size of the nascent RNA prior to elongation *in vitro* was determined by a brief end-labelling with [^{3}H]-ATP in the presence of GTP and

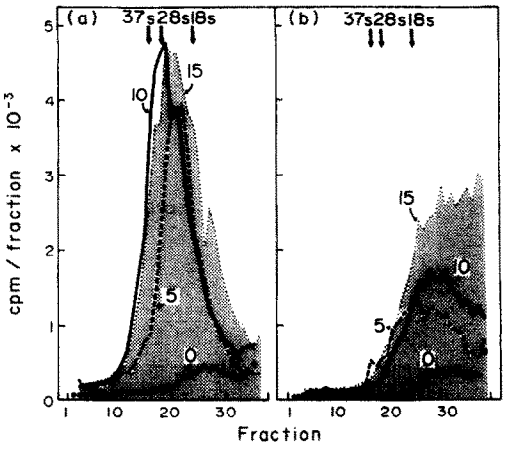

Fig. 1. Polyacrylamide gel electrophoresis of labeled RNA chains synthesized by endogenous RNA polymerases associated with chromatins isolated from withdrawn chick oviduct (panel A), or after treatment with estradiol for 1 h *in vivo* (panel B). RNA chains were elongated *in vitro* for 0–15 min (as indicated), then isolated and analyzed on 2.2% polyacrylamide gels containing SDS and 0.5% agarose. The positions to which chick embryo rRNAs (18S; 28S) and encephalomyocarditis virus RNA (37S) migrate under identical conditions are indicated. Gels were fractionated and radioactivity was estimated [13]. For the 10 min sample in panel B, the magnitude of the labeled RNA profile has been reduced by 50% for the sake of clarity. Direction of migration is from left to right.

CTP (designated time 0). Using 'chromatin' isolated from withdrawn (control) chicks (Fig. 1A), nascent RNA chains with an average peak size of 10S are extended in ten minutes to chains which migrate at the same rate as 28S rRNA, but a further incubation for 5 min causes an overall decrease in chain size, indicative of a shift of the balance between synthesis and degradation in favor of the latter.

When an identical analysis was performed with oviduct 'chromatin' isolated from chicks treated with estradiol *in vivo* for 1 h (Fig. 1B), the distribution of RNA chains differed markedly. The size of RNA chains prior to elongation is similar to that in control 'chromatin,' but the rate of increase in chain size with time during elongation is diminished. Clearly, degradation of nascent RNA chains is occurring in both 'chromatin' samples, but after steroid treatment, the rate of degradation appears to be increased. In the latter case, a significant proportion of the smaller chains are lost from the bottom of the gel. These effects are seen under conditions where the rate of incorporation of [^{3}H]-UTP into RNA per unit DNA is comparable for both control and steroid-treated tissues [16].

Similar profiles of degraded RNA were obtained after incubating 'chromatin' isolated up to 6 h after estradiol injection, as shown in Fig. 2 (upper set of profiles). After 15 min of RNA synthesis, chain sizes in control 'chromatin' resemble those obtained in Fig. 1, but after an extended period of incubation (30 min), a significant fraction of labeled chains are

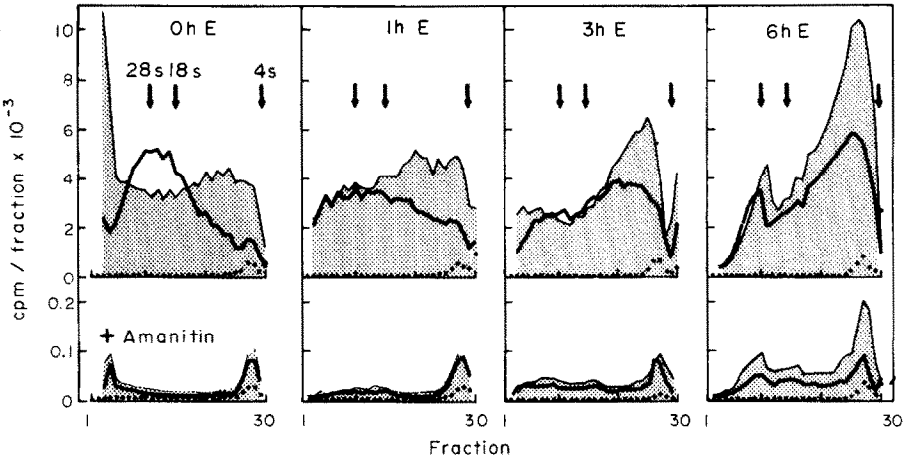

Fig. 2. Polyacrylamide gel electrophoresis of labelled RNA chains synthesized by endogenous RNA polymerases associated with oviduct chromatins isolated before and after estradiol treatment *in vivo*. Chromatin was isolated from withdrawn oviduct (0 h E), or after treatment with estradiol *in vivo* for 1 h. 3 h or 6 h (as indicated). RNA chains were then elongated *in vitro* at 37°C for 0 min (dotted line), 15 min (heavy line) or 30 min (shaded profile) either in the absence (upper set of profiles) or presence (lower set of profiles) of α-amanitin (0.45 μg/ml). The position to which *E. coli* tRNA (4S) or chick embryo rRNAs (18S; 28S) migrate under identical conditions is indicated. Direction of migration is from left to right.

too large to enter the gel. In contrast, RNA chains synthesized on steroid-treated 'chromatin' are, on average, smaller after incubation for 30 min, and have a peak distribution which diminishes approximately in proportion to the time of estradiol treatment *in vivo*; after prolonged RNA synthesis, the majority of RNA species in 6 h-treated 'chromatin' migrate close to the tRNA marker. End-labelling of RNA chains with [^{3}H]-ATP prior to elongation showed that for all 'chromatins', 'zero time' RNA chains had a peak distribution at approximately 4–6S. The significance of a second peak of RNA chains, migrating at approximately 21S in 6 h steroid-treated 'chromatin', is not clear. These effects were not seen when chicks were injected with sesame oil only; however, results similar to those shown in Figs 1 and 2 have been obtained on each of four separate occasions when RNA transcripts from control and steroid-treated oviduct tissue were analyzed.

The effect of estradiol on the RNA product sizes synthesized by endogenous form A and C RNA polymerases was also analyzed (Fig. 2, lower set of profiles) under conditions in which form B RNA polymerase was selectively inhibited (0.45 μg α-amanitin/ml). In withdrawn oviduct, two major RNA peaks were observed, one of which migrated at 4–5S and the other which remained at the top of the gel. This pattern is consistent with the idea that form A and C enzymes are responsible for the synthesis of 45S rRNA precursor, and tRNA and 5S rRNA respectively [17, 18]. At present, direct evidence for the identity of these RNAs is lacking, but when RNA synthesis is carried out in the presence of high α-amanitin levels (200 μg/ml), the radioactive profile remains unchanged except that the 4–5S peak disappears (not shown), thus strengthening the possibility that this peak represents tRNA and 5S rRNA synthesized by form C RNA polymerase. After estradiol treatment, there appears to be some degradation of the high molecular weight peak. However, the rate of migration of the original 4–5S RNA peak does not change, suggesting that these RNA species are comparatively stable.

The effect of estradiol *in vivo* on the absolute levels of RNA synthesis in isolated oviduct nuclei has been reported [16] and in these experiments the results obtained were identical; that is, endogenous form B RNA polymerase activity (80% of total RNA synthesis) was essentially unchanged after steroid treatment for 6 h, but forms A and C activity (6–20% of the total RNA synthesis) was activated 2–3 fold after 6 h. This explains the marked increase in the area of the radioactive RNA profiles in Fig. 2.

In view of the above results, it seemed likely that estradiol was enhancing the level of ribonuclease activity in isolated 'chromatin' fractions. To test this directly, labelled RNA chains synthesized *in vitro* from chicks receiving 0–6 h steroid treatment were isolated and added back to a second preparation of 'chromatin'. Each mixture of 'chromatin' and endogenous RNA was incubated at 37°C in the absence of RNA synthesis. To monitor the rate of degradation of the added RNA, each reaction mixture was acid-precipitated and hydrolyzed with alkali, thus releasing internal RNA chain units as ribonucleoside monophosphates, and units from 3′ ends as ribonucleosides [19]. A given RNA labeled with [^{3}H]-UTP has a constant ratio of UMP:U, but degradation by endonucleases or exonucleases will increase the number of 3′-ends relative to internal residues, thus diminishing the UMP:U ratio [16].

Table 1 displays data obtained from two indepen-

Table 1. Effect of estradiol treatment *in vivo* on the degradation of labelled nascent RNA by ribonucleases associated with oviduct chromatin

Experiment number	Time after estradiol (h)	Time of incubation of RNA with chromatin (min)	Radioactivity associated with uridine (U) (c.p.m.)	UMP (c.p.m.)	Ratio UMP/U	Average ratio
1	0	0	393	46813	119.1	119.2
			412	49217	119.4	
		15	410	47427	115.6	116.6
			416	48988	117.7	
		30	332	38947	117.3	117.9
			345	40924	118.6	
	1	0	256	27648	108.0	106.1
			307	32032	104.3	
		15	336	29316	87.2	87.4
			449	39414	87.7	
		30	287	24241	84.4	84.2
			320	26921	84.1	
	3	0	283	26768	94.5	90.4
			294	25400	86.3	
		15	325	26341	81.0	82.1
			290	24139	83.2	
		30	293	23369	79.7	78.4
			299	23055	77.1	
2	0	0	340	67533	198.6	
		15	351	68523	195.2	
		30	373	73592	197.3	
	3	0	356	70854	199.0	
		15	360	66743	185.3	
		30	385	67958	176.5	
	6	0	321	65218	203.1	
		15	355	61913	174.4	
		30	337	56803	168.5	

Experiment 1: RNA was synthesized using chromatin isolated from withdrawn (control) chicks, or after treatment for 1 or 3 h *in vivo* with estradiol. Standard reaction mixtures (except that [^{3}H]UTP was present at a specific activity of 26×10^3 d.p.m./pmole) were incubated at 37°C for 10 min, and RNA was isolated using CTAB. Aliquots (containing 2.5×10^5 c.p.m.) were then added back to fresh preparations of oviduct chromatin (containing 160–180 μg DNA) from the same source, incubated for 15 or 30 min at 37°C, and then precipitated with 10% trichloroacetic acid. Incubations were carried out in duplicate using the same preparations of chromatin and RNA. For zero time incubations, labeled RNA was present, but chromatin was added after the addition of acid. Precipitated RNA was then washed and hydrolyzed, hydrolysis products were charcoal-extracted, and aliquots were analyzed by chromatography on thin layer plates as described [14] to determine the radioactivity in UMP and uridine (U). The ratio of labeled UMP:U, which indicates the concentration of 3′-nucleoside ends in a given RNA preparation, was then calculated. Experiment 2: Chromatins isolated from control, 3 h- or 6 h-estradiol-treated chicks were incubated with RNA synthesized from control chromatin, and the UMP:U ratios then analyzed as above.

dent experiments. In experiment 1, 'chromatins' from control, 1 h- and 3 h-estradiol-treated chicks were mixed with labelled RNA synthesized from the same 'chromatin' source, and in experiment 2, 'chromatins' from control, 3 h- and 6 h-estradiol-treated chicks were mixed with RNA synthesized from control 'chromatin'. After incubation for 0, 15 and 30 min the UMP:U ratio of the RNA was determined. Values were then expressed as a percentage of those measured at zero time and plotted in Fig. 3 (experiment 1 in panel A; experiment 2 in panel B). The results indicate that control 'chromatin' has little effect on the degradation of control RNA, but 'chromatins' isolated after 1–6 h of estradiol treatment *in vivo* markedly decreased the UMP:U ratio, irrespective of the source of the added labelled RNA.

It is important to note that this assay does not detect all ribonuclease activity, which may partly explain why relatively small changes in the ratio UMP:U are seen compared to the dramatic changes in RNA size visualized on polyacrylamide gels. Endonucleases cleaving to form 5′-monophosphate ends will be detected [20], but those which form 3′-monophosphate ends will not, since the terminal unit will hydrolyze to a nucleoside monophosphate. Secondly, removal of protein from, or structural modification to, RNA transcripts (during their isolation) may modify the specificity of the chromatin-associated nucleases. Considerations of this nature could also explain why 1 h-estradiol-treated 'chromatin' appears to have a higher level of ribonuclease activity than 3 h- and 6 h-steroid-treated preparations,

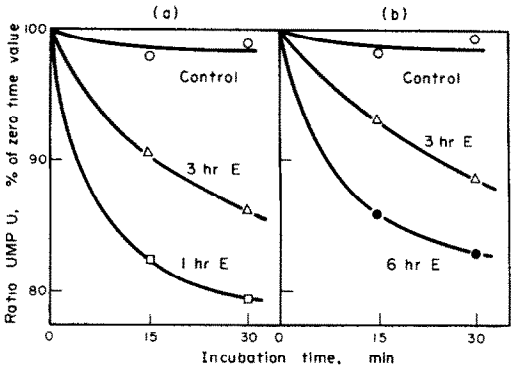

Fig. 3. Effect of estradiol treatment *in vivo* on the degradation of labeled nascent RNA by ribonucleases associated with oviduct chromatin. The UMP:U ratios obtained in Table 1 were expressed as a percentage of the ratio obtained at zero time of incubation of RNA with chromatin. Panels A and B depict the results obtained for experiments 1 and 2 respectively. Captions indicate the source of chromatin in each incubation. In panel A, labelled RNA was synthesized by chromatin from the same source. In panel B, labelled RNA was synthesized from 'control' chromatin.

in contrast to the situation suggested by the data in Fig. 2.

To check that the differential ability of 'chromatin' to alter the ratio of UMP:U during incubation of labeled RNA is not caused by differential addition of poly-A to the 3'-ends, levels of poly-A polymerase activity were tested in isolated 'chromatin' using a method described previously [21]. Results obtained using 0–6 h-estradiol treated 'chromatin' (not shown) indicate that in all cases the amount of poly-A synthesized, as determined by ribonuclease-resistant acid-precipitable radioactivity, represents 2–3% of the total ATP incorporated into RNA. This result, coupled with the fact that RNA chains are physically degraded *in vitro* (Fig. 1), makes it unlikely that poly-A addition accounts for the change in UMP:U ratios observed in Fig. 3.

DISCUSSION

Eukaryotic nuclei undoubtedly contain high levels of ribonuclease activity. Available evidence [22] indicates that, of the total RNA transcript synthesized, approximately 10% reaches the cytoplasm and the remainder is degraded in the nucleoplasm. Both exo- and endonucleases have been implicated in the processing of ribosomal RNA precursors [23, 24], and endonucleases specific for RNA have been identified in rat liver nuclei [25] as well as in association with 30S ribonucleoprotein particles [26]. In keeping with this evidence, the size patterns of nascent RNA chains observed in these experiments clearly reflect a balance between rates of synthesis and degradation. These RNA chains are being degraded in all control and hormone-treated 'chromatin' preparations studied, and apparently by ribonucleases which are tightly bound, since the latter survive several washes in solutions containing Triton X-100, salt and concentrated sucrose. In addition, they are capable of degrading RNA products even in the presence of heparin, which probably inhibits some classes of nuclease [13].

Since the rates of RNA synthesis, as well as the number of actively-elongating form B RNA polymerase molecules, are similar in both control and steroid-treated 'chromatins' [16], two principal effects might feasibly account for the reduction in the size spectrum of nascent RNA chains seen after estradiol administration. Firstly, the steroid may activate chromatin-associated ribonucleases. My analysis of the effects of 'chromatins' on the UMP:U ratios in labelled nascent RNA strongly indicates that this mechanism is operative, and that ribonuclease activity is enhanced within the first hour of steroid treatment. Secondly, estradiol might alter the kinds of RNA transcripts copied from the chick genome, resulting in the production of RNA molecules which are smaller, on average, than those in control 'chromatin'. Currently, this remains an open question, and an analysis of isolated RNA transcripts using competition-hybridization techniques would be necessary to clarify this point.

Form B RNA polymerase is responsible for transcribing structural genes [27], but it is not clear from this work whether estrogen-induced ribonucleases are specific for form B-directed RNA products. A concise interpretation is complicated by the fact that several nucleases with different specificities may be present in 'chromatin', some of which may be steroid-sensitive. It seems unlikely that the present results can be explained on the basis of differential contamination of chromatin by nonspecific nucleases, since 4–5S RNA products synthesized on 'chromatin' isolated before or after estrogen treatment remain relatively stable, but such a conclusion must be considered tentative. Secondly, non-specific degradation might be expected to reduce RNA transcripts to sizes which are smaller than those required of putative mRNAs. The average molecular weight of oviduct proteins is less than 0.04×10^6 [28], suggesting that mRNAs of 1000–2000 nucleotides in length would be sufficient to code for these molecules. The heavy side of the pattern of degraded RNA products synthesized in steroid-treated 'chromatin' (Fig. 2) could certainly include putative mRNAs of this size. Direct proof that the estradiol activates specific processing enzymes for form B-directed products will ultimately require an analysis of the RNA species involved in the transition from nascent transcript to cytoplasmic RNA for one particular gene, and of the enzymes responsible for each conversion step.

The activation by estradiol of chromatin-associated ribonucleases observed here, and the phenomenon of induction of egg white protein mRNAs [4, 5], occur simultaneously in chick oviduct. The rate of transcription of egg white protein genes is probably enhanced by estrogen [3], but it is conceivable that the appearance of completed mRNAs in the cytoplasm is also dependent on the activation of RNA processing or

transport mechanisms. One can envisage rate-limiting steps in the nucleus through which all potential mRNAs must pass and which involve cleavage by specific ribonucleases. A low level of chromatin-associated ribonuclease activity may result in an accumulation of nascent RNA transcripts in the vicinity of a given gene, resulting in an inhibition of RNA synthesis. Another possibility is that cleavage of RNA transcripts at the correct site may be a prerequisite for subsequent processing events.

An alternative to the idea that estradiol activates chromatin-associated nucleases *per se* is that transport to the nucleus is enhanced, resulting in a greater association of these enzymes with isolated 'chromatin'. Indeed, it has been noted in rat vaginal cells that lysosome-like bodies are found in close association with nuclear membranes after estradiol treatment, suggesting that degradative lysosomal enzymes invade the nucleus in response to steroid [29]. The concept that in withdrawn chick oviduct processing mechanisms operate with low efficiency and that steroid stimulates these activities in order to achieve maximal rates of mRNA processing remains an intriguing possibility.

Acknowledgements—This investigation was supported by the National Institutes of Health grant no. HD-9140-C. I would like to thank Nancy A. Tallman for excellent technical assistance.

REFERENCES

1. Cox R. F., Catlin G. H. and Carey N. H.: Studies on the mode of action of oestradiol on chicken oviduct. Characterisation of oestradiol receptor proteins. *Eur. J. Biochem.* **22** (1971) 46–56.
2. Palmiter R. D., Catlin G. H. and Cox R. F.: Chromatin-associated receptors for estrogen, progesterone, and dihydrotestosterone and the induction of egg white protein synthesis in chick magnum. *Cell Diff.* **2** (1973) 163–170.
3. O'Malley B. W. and Means A. R.: Female steroid hormones and target cell nuclei. The effects of steroid hormones on target cell nuclei are of major importance in the induction of new cell function. *Science N.Y.* **183** (1974) 610–620.
4. Palmiter R. D.: Rate of ovalbumin messenger ribonucleic acid synthesis in the oviduct of estrogen-primed chicks. *J. biol. Chem.* **248** (1973) 8260–8270.
5. Cox R. F., Haines M. E. and Emtage J. S.: Quantitation of ovalbumin mRNA in hen and chick oviduct by hybridization to complementary DNA. *Eur. J. Biochem.* **49** (1974) 225–236.
6. Palmiter R. D., Moore P. D. and Mulvihill E. R.: A significant lag in the induction of ovalbumin messenger RNA by steroid hormones: a receptor translocation hypothesis. *Cell* **8** (1976) 557–572.
7. Harris S. E., Rosen J. M., Means A. R. and O'Malley B. W.: Use of a specific probe for ovalbumin messenger RNA to quantitate estrogen-induced gene transcripts. *Biochemistry* **14** (1975) 2072–2081.
8. McKnight G. S. and Schimke R. T.: Ovalbumin messenger RNA: Evidence that the initial product of transcription is the same size as polysomal ovalbumin messenger. *Proc. Natn. Acad. Sci. U.S.A.* **71** (1974) 4327–4331.
9. Georgiev G. P. and Samarina O. P.: D-RNA containing ribonucleoprotein particles. *Adv. Cell Biol.* **2** (1971) 47–83.
10. Edmonds M. and Caramela M. G.: The isolation and characterization of adenosine monophosphate-rich polynucleotides synthesized by Ehrlich ascites cells. *J. biol. Chem.* **244** (1969) 1314–1324.
11. Perry R. P., Kelley D. E., Friderici K. and Rottman F.: The methylated constituents of L cell messenger RNA: Evidence for an unusual cluster at the 5′ terminus. *Cell* **4** (1975) 387–394.
12. Scherrer K.: Messenger RNA in eukaryotic cells: The life history of duck globin messenger RNA. In *Protein Synthesis in Reproductive Tissue* (Edited by E. Diczfalusy). Karolinska Institutet, Stockholm (1973) pp. 95–129.
13. Cox R. F.: Transcription of high-molecular-weight RNA from hen-oviduct chromatin by bacterial and endogenous form-B RNA polymerases. *Eur. J. Biochem.* **39** (1973) 49–61.
14. Cox R. F., Haines M. E. and Carey N. H.: Modification of the template capacity of chick-oviduct chromatin for form-B RNA polymerase by estradiol. *Eur. J. Biochem.* **32** (1973) 513–524.
15. Butterworth P. H. W., Cox R. F. and Chesterton C. J.: Transcription of mammalian chromatin by mammalian DNA-dependent RNA polymerases. *Eur. J. Biochem.* **23** (1971) 229–241.
16. Cox R. F.: Quantitation of elongating form A and B RNA polymerases in chick oviduct nuclei and effects of estradiol. *Cell* **7** (1976) 455–465.
17. Weinmann R. and Roeder R. G.: Role of DNA-dependent RNA polymerase III in the transcription of the tRNA and 5S RNA genes. *Proc. natn. Acad. Sci. U.S.A.* **71** (1974) 1790–1794.
18. Reeder R. H. and Roeder R. G.: Ribosomal RNA synthesis in isolated nuclei. *J. molec. Biol.* **67** (1972) 433–441.
19. Richardson J. P.: RNA polymerase and the control of RNA synthesis. *Prog. nucl. Acad. Res. molec. Biol.* **9** (1969) 75–116.
20. Razzell W. E.: Phosphodiesterases. In *Methods in Enzymology* (Edited by S. P. Colowick and N. O. Kaplan). Academic Press, New York, Vol. 6 (1963) pp. 249–251.
21. Jelinek W. R.: Poly adenylic acid synthesis *in vitro* in isolated HeLa cell nuclei and whole cell homogenates. *Cell* **2** (1974) 197–204.
22. Weinberg R. A.: Nuclear RNA metabolism. *Ann. Rev. Biochem.* **42** (1973) 329–354.
23. Liau M. C., Craig N. C. and Perry R. P.: The production of ribosomal RNA from high molecular weight precursors. I. Factors which influence the ability of isolated nucleoli to process 45-S RNA. *Biochim. biophys. Acta* **169** (1968) 196–205.
24. Kelley D. E. and Perry R. P.: The production of ribosomal RNA from high molecular weight precursors. II. Demonstration of an exonuclease in isolated nucleoli. *Biochim. biophys. Acta.* **238** (1971) 357–362.
25. Cordis G. A., Goldblatt P. J. and Deutscher M. P.: Purification and characterization of a major endonuclease from rat liver nuclei. *Biochemistry* **14** (1975) 2596–2603.
26. Niessing J. and Sekeris C. E.: Cleavage of high-molecular-weight DNA-like RNA by a nuclease present in 30-S ribonucleoprotein particles of rat liver nuclei. *Biochim. biophys. Acta* **209** (1970) 484–492.
27. Zylber E. A. and Penman S.: Products of RNA polymerases in HeLa cell nuclei. *Proc. Natn. Acad. Sci. U.S.A.* **68** (1971) 2861–2865.
28. Palmiter R. D.: Regulation of protein synthesis in chick oviduct. II. Modulation of polypeptide elongation and initiation rates by estrogen and progesterone. *J. biol. Chem.* **247** (1972) 6770–6780.
29. Szego C. M.: The lysosome as a mediator of hormone action. *Recent Prog. Horm. Res.* **30** (1974) 171–233.