Biochem. J. (1975) **150**, 345–355
Printed in Great Britain

Differential Subnuclear Distribution of Polyadenylate-Rich Ribonucleic Acid during Induction of Egg-Yolk Protein Synthesis in Male *Xenopus* Liver by Oestradiol-17β

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(*Received* 17 *March* 1975)

1. A 4–8-fold increase in the rate of hepatic nuclear RNA synthesis occurred within 11 h after a single injection of oestradiol-17β to male *Xenopus* to induce egg-yolk protein synthesis. 2. By using a gentle procedure for fractionating nuclei into their major structurally different components [J. R. Tata & B. Baker (1974) *Exp. Cell Res.* **83**. 111–124], it was found that the hormone-induced increase in the total amount of newly made RNA was associated with a 2–10-fold increase in the poly(A) content of nuclear RNA. 3. When the poly(A) content of nuclear RNA was determined by hybridization to poly[^{3}H](U) or specific binding to oligo(dT)-cellulose, most of the increase (10-fold) in poly(A) content of newly synthesized RNA was associated with the euchromatin fractions, whereas the increase was less marked in the other subnuclear fractions. 4. Resolution of nuclear RNA into poly(A)-poor and poly(A)-rich RNA species by chromatography on oligo(dT)-cellulose, followed by polyacrylamide-gel electrophoresis with sodium dodecyl sulphate or in the presence of 99% formamide, revealed that the hormone caused a preferential enhancement of high-molecular-weight (25S–60S) poly(A)-rich HnRNA (heterogeneous nuclear RNA), much of which was associated with euchromatin and not with the nuclear sap. 5. Induction of vitellogenin in male frogs was in particular characterized by the appearance of a high-molecular-weight polyadenylated component exhibiting a peak at 35–36S, i.e. a molecular weight of approx. $2.05\times10^6\pm0.15\times10^6$. Although there is no evidence as yet that such a polyadenylated high-molecular-weight nuclear RNA species contains sequences corresponding to vitellogenin mRNA, it is possible that a high proportion of the most stable form of the putative nuclear precursor to vitellogenin mRNA induced by oestrogen in male *Xenopus* liver may be only marginally bigger than the cytoplasmic mRNA, and may at any one time be predominantly associated with the euchromatin fraction.

One of the most remarkable actions of oestrogen is the capacity of the hormone to 'switch on' genes for ovalbumin and other egg-white proteins in the oviduct (O'Malley & Means, 1974; Schimke *et al.*, 1973) and of egg-yolk protein (vitellogenin) in the liver of both male and female adult egg-laying animals (Greengard *et al.*, 1964; Grüber, 1967; Wallace & Dumont, 1968; Clemens, 1974; Follett & Redshaw, 1974). In the male frog, *Xenopus laevis*, administration of oestrogen induces the synthesis *de novo* of large quantities of the major primary product vitellogenin which, in the female, is cleaved in the ovary to phosvitin and lipovitellin (Wallace *et al.*, 1972). Although a large number of non-specific growth and secretory processes (increase in number and translational capacity of ribosomes, proliferation of endoplasmic reticulum) are ultimately enhanced, vitellogenin synthesis is preceded by, and dependent on, a greatly enhanced synthesis of all classes of RNA (Witliff & Kenney, 1972*a*,*b*; Clemens *et al.*, 1975; Lewis *et al.*, 1975; Dolphin *et al.*, 1971). Clemens *et al.* (1975) have been able to follow the induction of vitellogenin in tissue culture and have calculated that in the male *Xenopus* as many as 70–90% of polyribosomes may be engaged in the synthesis of vitellogenin at the height of response of the hepatocyte to the female sex hormone. Such a massive demand for a specialization of an already differentiated tissue must involve the mobilization of not only the machinery for transcription, but also for post-transcriptional modifications of RNA within the nucleus.

It is now widely held that the post-transcriptional processing of the primary gene transcript, generally referred to as HnRNA,* to yield the appropriate translatable mRNA is an important process which may aid in understanding gene expression during differentiation (Darnell *et al.*, 1973; Weinberg, 1973). The discovery that polyribosomal mRNA and HnRNA molecules containing such mRNA sequences have, at the 3′-terminus, a 100–200 nucleotide-long

* Abbreviations: HnRNA, heterogeneous nuclear RNA; RNAase, ribonuclease; DNAase, deoxyribonuclease.

tract of polyadenylic acid [poly(A)], added post-transcriptionally, has stimulated the study of selection of primary RNA transcripts for mRNA (Darnell *et al.*, 1971, 1973; Lee *et al.*, 1971; Greenberg & Perry, 1972; Edmonds *et al.*, 1971). At the same time, the unique properties of the poly(A) 'tail' [resistance to nuclease attack at moderate ionic strength, adsorption to and hybridization with poly(U) or oligo(dT)] have also facilitated the isolation of mRNA and its possible large nuclear precursor.

In the intact cell nucleus, different transcriptional and post-transcriptional processes are highly compartmentalized into the various structurally distinct components (euchromatin, heterochromatin, nucleolus, nuclear sap etc.). In our laboratory, we have devised a gentle procedure for resolving nuclei into eight fractions, which also allows the separation of the major transcriptional units (Tata & Baker, 1974*a*,*b*). We have since shown that poly(A)-rich RNA in rat liver nuclei is unequally distributed in the different nuclear compartments and that the major part of newly synthesized HnRNA containing 100–150 nucleotide-long poly(A), as opposed to HnRNA with short intramolecular poly(A) tracts (Nakazato *et al.*, 1974), is largely associated with euchromatin and not in the nuclear sap (Tata & Baker, 1975). One would therefore expect, during rapid developmental changes, a different pattern of distribution of newly labelled RNA and its polyadenylation in the different nuclear compartments.

In view of the massive synthesis *de novo* of vitellogenin mRNA that must be induced by oestradiol in male *Xenopus* liver, we decided to analyse the intranuclear distribution of HnRNA and poly(A)-rich RNA, some of which may contain specific mRNA sequences. We were particularly encouraged by the fact that vitellogenin, and the mRNA coding for it, would be relatively easy to identify because of the large molecular weight of the former (approx. 200000) and the 50–60% serine residues in the phosvitin moiety (see Clemens, 1974). In our laboratory we are simultaneously characterizing the nature and utilization of induced vitellogenin mRNA in cytoplasmic polyribosomes, as well as studying the metabolism of RNA in the different nuclear compartments soon after hormonal stimulation. While awaiting sufficient purification of cytoplasmic mRNA as a template for complementary DNA to be synthesized as a probe for analysing the nature of nuclear RNA formed on hormonal administration, as has been accomplished for ovalbumin mRNA (Schimke *et al.*, 1973; McKnight & Schimke, 1974; Cox *et al.*, 1974), we have made some noteworthy observations on the subnuclear distribution of newly synthesized RNA.

In the present paper we describe the first results on the subnuclear distribution of poly(A)-rich RNA formed in hepatocytes of male *Xenopus* soon after the administration of oestradiol to induce vitellogenin synthesis. It will be shown that although all subnuclear fractions show some change, there is a 10-fold increase in newly synthesized RNA as well as in the poly(A) content of RNA associated with the euchromatin fraction.

Experimental

Animals and materials

Adult male *Xenopus laevis* frogs were collected in South Africa and shipped directly to us by the South African Snake Farm, Fishoek, C.P., S. Africa. They were stored in plastic tanks at 18–20°C and fed twice weekly on sliced liver and heart. Where indicated, 1 mg of oestradiol-17β [5 mg/ml in 50% (v/v) propylene glycol and 0.1 M-NaCl] was injected via the dorsal lymph sac, whereas radioactive precursors to RNA were given intraperitoneally.

All chemicals were of analytical grade and were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., or BDH Chemical Co., Poole, Dorset, U.K. RNAase T_1 was obtained from Sigma, whereas RNAase A and RNAase-free DNAase were from Worthington (Cambrian Chemicals Ltd., Croydon, U.K.). *Escherichia coli* 23S and 16S ribosomal RNA species were purcahsed from Miles Chemical Co., Stoke Poges, Bucks., U.K. Phenol and chloroform for RNA extraction were freshly distilled before use. [5-^{3}H]-Uridine (specific radioactivity 27.0 Ci/mol), [5-^{3}H]-orotic acid (specific radioactivity 26.0 Ci/mol) and [^{32}P]orthophosphate (84 Ci/mg of P) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and poly[^{3}H]uridylic acid (specific radioactivity 58.9 mCi/mmol of P) was from Miles Chemical Co.

Preparation of nuclei and subnuclear fractions

Nuclei were as a routine isolated by the modified (Tata & Baker, 1974*a*) procedure of Blobel & Potter (1966). Livers were washed and homogenized in 4 vol. of a medium containing 0.25 M-sucrose, 50 mM-Tris–HCl (pH 7.6), 5 mM-$MgCl_2$, 25 mM-KCl and 25 mM-NaCl. Subnuclear fractions were prepared from gently sonicated nuclei as described by Tata & Baker (1974*a*). In some experiments for extraction of RNA, nuclei were also prepared in the glycerol–Tris–NaCl–$MgCl_2$ medium at −20°C as described by Schibler & Weber (1974). The nomenclature of subnuclear fractions is as described by Tata & Baker (1974*a*), except that the fraction occasionally termed 'nucleoplasm' refers to nuclear sap. The major components of the different subnuclear fractions derived from chromatin (after removal of nuclear sap) were as follows: fractions 1, 2 and 3, euchromatin and

membranes with no DNA attached; fraction 4, mixture of euchromatin and nucleoli; fractions 5 and 6, nucleoli, membrane-bound heterochromatin and ribonucleoprotein particles; fraction P, heterochromatin and ribonucleoprotein particles.

Extraction of RNA

All glassware was autoclaved before use, and only sterile plasticware was purchased for work on the extraction of RNA. Nuclei or subnuclear fractions were suspended in 10–12ml of 0.05M-sodium acetate buffer, pH6.0, along with 0.5% sodium dodecyl sulphate, 100–200mg of bentonite, 5mg of polyvinyl sulphate and 0.2% diethyl pyrocarbonate. RNA was extracted by the hot (65°C) phenol–sodium dodecyl sulphate–chloroform procedure, exactly as described by Tata & Baker (1975), except that the preparations were treated with RNAase-free DNAase (25μg/ml) for 30min at 30°C. The RNA was re-extracted with hot phenol–sodium dodecyl sulphate and precipitated with 2M-LiCl (which does not precipitate degraded RNA of size below 6–7S as well as a residual native DNA or glycogen). Purified RNA was stored in 0.05M-sodium acetate buffer, pH5.5, at −25°C.

Hybridization of poly[^{3}H](U) with RNA

The poly(A) content of non-radioactive RNA was determined by its capacity to form a RNAase-resistant hybrid with poly[^{3}H](U). The procedure used was that described originally by Gillespie *et al.* (1972) and slightly modified by Tata & Baker (1975).

Adsorption of RNA to oligo(dT)-cellulose

RNA preparations were adsorbed to, and eluted from, small columns (4mm×2mm) of oligo(dT), covalently linked to cellulose and prepared by Gilham's (1964) procedure, as described by Edmonds (1971) and Kates (1973). RNA samples, dissolved in 0.5M-KCl–0.01M-Tris–HCl, pH7.6, were applied to the columns. Poly(A)-free RNA was eluted with the same buffer, whereas poly(A)-rich RNA was eluted in a stepwise fashion with 0.1M-KCl–0.01M-Tris buffer and 0.01M-Tris buffer alone. In some experiments, any residual RNA still bound to the adsorbent after the salt-free elution step was eluted with 90% formamide (Molloy *et al.*, 1974). This step usually released 2–10% of input radioactive RNA. The capacity of oligo(dT)-cellulose columns to discriminate between poly(A)-free and poly(A)-rich RNA species was independently checked with *E. coli* ribosomal RNA and poly[^{3}H](A) (Tata & Baker, 1975).

Determination of length of poly(A) tracts in RNA

^{32}P-labelled RNA was hydrolysed with 2μg of pancreatic RNAase (RNAase A)/ml and 5 units of RNAase T_1/ml by incubation at 37°C for 1h. The undigested RNA was precipitated with 2vol. of ethanol and dissolved in formamide for polyacrylamide-gel electrophoresis.

Chemical and radioactivity determinations

RNA, DNA and protein were chemically assayed as described elsewhere (Tata & Baker, 1974*a*), as was the determination of ^{3}H and ^{32}P in samples precipitated on Whatman GF/C glass-fibre filters (counting efficiency 29% for ^{3}H and >90% for ^{32}P).

Disc-gel electrophoresis of RNA

Because of the high endonuclease activity in isolated amphibian nuclei, RNA for gel electrophoresis was only obtained from nuclei prepared by the glycerol–Triton X-100 procedure at −20°C. RNA samples were loaded on pre-run 0.1% sodium dodecyl sulphate–2.3% (w/v) polyacrylamide gels and electrophoresed at 5mA/disc for 2.5±0.2h (Tata & Baker, 1975). Parallel gels were run with 23S and 16S *E. coli* rRNA and 4S tRNA or 28S, 18S and 5S RNA from rat liver ribosomes as markers. To check on aggregation of RNA when electrophoresed on sodium dodecyl sulphate–polyacrylamide gels, the same samples were also analysed by electrophoresis on 3.5% (w/v) polyacrylamide gels formed in 99% formamide (Duesberg & Vogt, 1973). After electrophoresis, the gels were rapidly scanned for RNA at 254nm in a gel-scanner attachment for a Gilford 240 spectrophotometer. They were then frozen in solid CO_2, 2mm slices were cut, dissolved in 6M-NH_3 and the radioactivity was measured in each slice in 10ml of Triton X-100–toluene-based scintillation fluid, with a counting efficiency of 18% for ^{3}H and 90% for ^{32}P.

Results and Discussion

Fig. 1 shows the rate of incorporation of [^{3}H]-uridine into RNA extracted from different subnuclear fractions of the uninduced male *Xenopus* liver. The gross pattern of incorporation is similar to that for ^{3}H-labelled RNA observed earlier for rat liver subnuclear fractions, except that in *Xenopus* the overall rate was about five times slower than for the mammalian preparations (Tata & Baker, 1974*b*). Thus the most rapid rate of labelling and the highest specific radioactivity is seen for RNA from the nuclear-sap fraction, but this was not sustained beyond the first 45min after injection of the label. On the other hand, the incorporation of [^{3}H]uridine into RNA from the other subnuclear fractions continued to rise until 90min or longer (not shown in

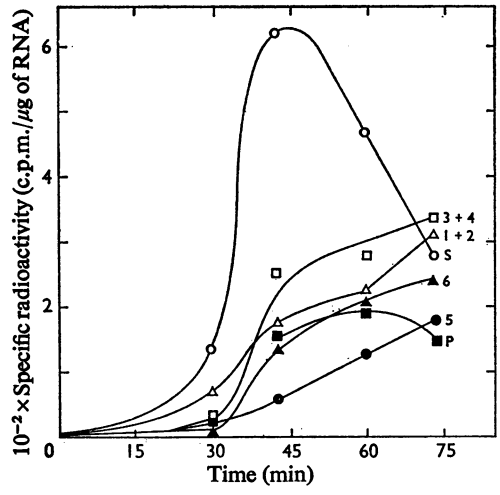

Fig. 1. *Kinetics of labelling of RNA in different subnuclear fractions from male Xenopus liver*

Groups of three frogs were killed at different time-intervals after the injection of 150 μCi of [^{3}H]uridine/animal. Nuclei from 4–6 g of liver were fractionated by the procedure of Tata & Baker (1974*a*) as described for *Xenopus* tissue and the specific radioactivity of the RNA was determined. The nomenclature of the different subnuclear fractions, going from top to bottom of the gradient tube after centrifugation, is as follows: S (○), nuclear sap or 'nucleoplasm'; 1+2 (△) and 3+4 (□), two pooled euchromatin-rich fractions; 5 (●), fraction enriched in nucleoli; 6 (▲) and P (■) are fractions containing heterochromatin, various types of ribonucleoprotein particles and some nucleoli. Each value is the mean of three determinations, with variations of ±10%.

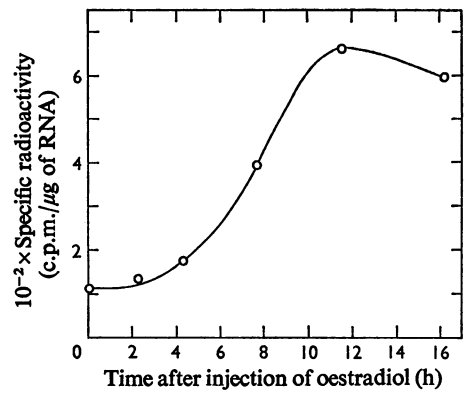

Fig. 2. *Stimulation of rate of nuclear RNA synthesis in male Xenopus after an injection of oestradiol-17β*

Groups of three frogs were killed at the various time-intervals indicated after a single intraperitoneal injection of 1 mg of oestradiol-17β (except for zero time). All animals received 100 μCi of [^{3}H]uridine 50 min before death. Nuclei were isolated by the standard procedure of centrifugation through 2.3 M-sucrose (Blobel & Potter, 1966) and the specific radioactivity of the ^{3}H-labelled RNA was measured. Each value is the mean of three determinations.

Fig. 1). Also, oestradiol administration to male frogs did not cause a major quantitative shift in the kinetics of labelling of RNA in the different subnuclear fractions (results not shown). Of these fractions, the euchromatin fractions (1+2 and 3+4) had the highest specific radioactivity, with the heterochromatin-rich fractions (6 and P) being less heavily labelled, whereas the fraction rich in nucleoli, fraction 5, acquired label slowly, but the incorporation continued to rise for a considerable time. This pattern of distribution of newly synthesized RNA conforms to that expected for the synthesis of non-ribosomal RNA in the nucleus (see Weinberg, 1973). On the basis of the data of Fig. 1, and on preliminary trials on the effect of oestrogen on the labelling of liver RNA, a period of 40–70 min after the intraperitoneal injection of [^{3}H]uridine was chosen as the most suitable time-interval for comparing the rates of synthesis of RNA in different subnuclear fractions in control and oestrogen-treated male *Xenopus* liver.

A most impressive increase in the rate of synthesis of liver nuclear RNA resulted from the injection of oestradiol to male frogs (Fig. 2). After a lag of 3–6 h after hormone administration, the specific radioactivity of the total nuclear RNA reached values 6–10-fold higher than the controls at 10–18 h after the hormone was administered. Measurement of trichloroacetic acid-soluble radioactivity and total uptake of radioactivity suggested that only a relatively minor component of this enhanced labelling of RNA was likely to be due to a hormone-induced increase in precursor specific radioactivities, although the latter were not directly measured. The finding of enhanced RNA synthesis is qualitatively similar to the fourfold increase in specific radioactivity of total cellular RNA labelled with [^{3}H]orotic acid observed by Witliff & Kenney (1972*b*) at 12 h after oestradiol administration. In our laboratory, Clemens *et al.* (1975) also observed a 2–3-fold elevation of total tissue RNA labelled with longer pulses of [^{3}H]-uridine in organ cultures of livers of male frogs injected 24–48 h earlier with oestradiol.

At the peak time-interval of 11 h after hormone administration, the increase in specific radioactivity of newly formed RNA varied according to the subnuclear fraction in which the RNA was present (Table 1). There was an 8–10-fold increase in the specific radioactivity of RNA from the nuclear sap and from a 'pellet' fraction (fraction P) which

Table 1. *Effect of oestradiol administration on the synthesis of rapidly labelled RNA distributed in different subnuclear fractions of male Xenopus liver*

One group of four male frogs was injected with 1 mg of oestradiol-17β 11 h before death (treated animals) and an untreated group served as control. All animals were killed 60 min after an intraperitoneal injection of 100 μCi of [^{3}H]uridine. Nuclei were isolated from 3 g batches of pooled liver and were then fractionated into subnuclear fractions as in Fig. 1. They were, however, not pooled as in the experiment in Fig. 1 because of small variation in the resolution of the subnuclear components as judged by the recovery of DNA, RNA and protein from one run to another. Each value is the average of six determinations on three separate subnuclear fractionations. Other details are as in Fig. 1. Values in parentheses refer to the subnuclear fractions indicated in Fig. 1 and are based on earlier characterization (Tata & Baker, 1974*a*). Values for total ^{3}H-labelled RNA recovered represent RNA recovered from nuclei equivalent to 3 g of liver.

Subnuclear fraction	Total ^{3}H-labelled RNA recovered (c.p.m.)		Specific radioactivity of ^{3}H-labelled RNA (c.p.m./μg)	
	Control	Treated	Control	Treated
Nuclear sap	1830	30865	68	957
Euchromatin (2+3)	5153	69200	36	185
Nucleolar (5+6)	5470	30418	41	122
Pellet (heterochromatin, nucleoli, ribonucleoprotein particles)	3630	46336	45	416

Table 2. *Determination of poly(A) content by hybridization to poly[^3H](U) of unlabelled RNA extracted from different liver subnuclear fractions of male Xenopus treated or not with oestradiol-17β*

RNA was extracted from subnuclear fractions from four pooled livers of control male frogs and from animals treated with 0.8 mg of oestradiol-17β 17 h before killing. The numbers (in parentheses) and nomenclature of subnuclear fractions is based on the work of Tata & Baker (1974*a*). Hybridization was carried out by the procedure of Gillespie *et al.* (1972), by using 100 ng of poly[^{3}H](U) (0.02 μCi) and input RNA varying in amount from 0.5 to 4.0 μg. For other details see Tata & Baker (1975).

Subnuclear fraction	Poly[^{3}H](U) hybridized (c.p.m.)		Poly(A) in RNA (%)	
	Control	Treated	Control	Treated
Nuclear sap (S)	384	746	0.27	0.80
Euchromatin (1)	64	598	0.03	0.73
Euchromatin (2)	77	187	0.04	0.38
Nucleoli and heterochromatin etc. (5, 6, P)	205	486	0.24	0.39

consists of heterochromatin and granular material, perhaps made up of heterogeneous nuclear ribonucleoprotein particles (Tata & Baker, 1974*a*). The specific radioactivity of RNA associated with euchromatin (corresponding to fractions 2+3 in Fig. 1) and nucleolar-enriched fractions (4 and 5+6) increased about 2–4-fold at the same time. Under the mild conditions of preparation of subnuclear fractions used here, the nuclear sap, according to Table 1, contained only a small part of rapidly labelled nuclear RNA, even though the specific radioactivity was high. This is in contrast with the recovery of the major portion of non-nuclear RNA as 'nucleoplasmic' or nuclear-sap RNA if the more brutal conventional methods of disrupting nuclei were used (Tata & Baker, 1974*a*,*b*). Thus Table 1 shows that nearly 75% of the radioactive RNA was associated, in both controls and hormone-induced animals, with fractions enriched in euchromatin, nucleoli and in the pellet fraction. A 5–8-fold higher amount of labelled RNA after oestradiol treatment still represented only about 20% of unlabelled nuclear RNA.

When the poly(A) content of unlabelled RNA derived from subnuclear fractions was determined, a different pattern was obtained in control and induced animals, as compared with the distribution of labelled nuclear RNA. Table 2 and Fig. 3 summarize the results of experiments in which the proportion of RNA in subnuclear fractions present as poly(A) tracts was determined by hybridization to poly[^{3}H](U) in controls, and in animals 17 h after oestradiol administration. A 9–24-fold increase in the proportion of poly(A) was observed in the RNA recovered from the two euchromatin fractions after hormonal induction, whereas it was threefold for the RNA recovered in the nuclear sap. The amount of poly(A) in both these

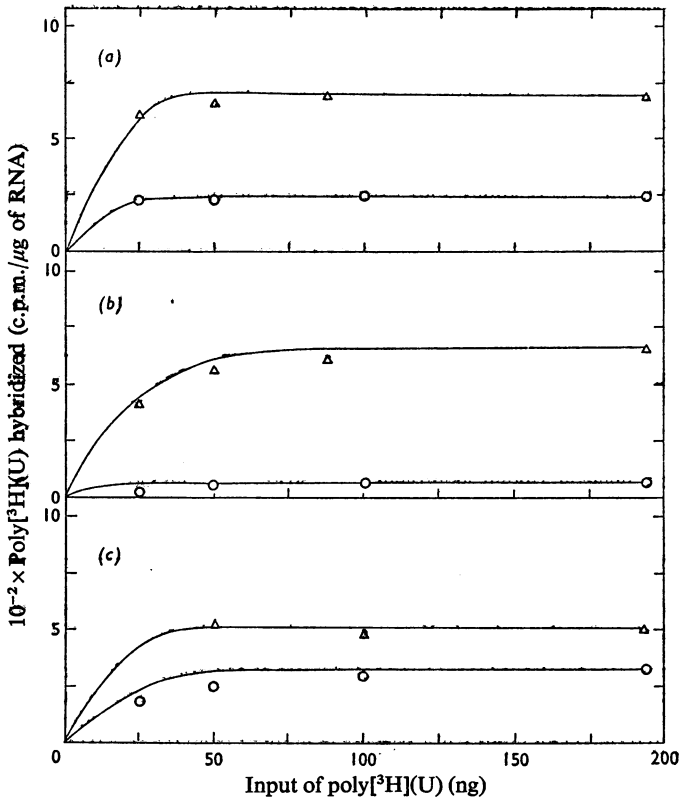

Fig. 3. *Poly(A) content of unlabelled RNA from three major subnuclear fractions of control and oestradiol-treated male Xenopus, as determined by hybridization with poly*[^{3}H](*U*)

Nuclei were prepared from two batches of six male *Xenopus*, one group being injected with 0.9mg of oestradiol-17β 17h before death (△) and the other batch served as controls (○). They were fractionated as in Fig. 1 and the following three fractions were used: (*a*), nuclear sap; (*b*) euchromatin (fractions 1, 2+3); (*c*) nucleoli+heterochromatin+ribonucleoprotein granules (fractions 6+P). RNA was extracted by the hot phenol–sodium dodecyl sulphate–chloroform procedure and used for hybridization with different amounts of poly[^{3}H](U), as indicated. The RNAase-resistant radioactivity per μg of RNA was measured after incubation in 50% (v/v) formamide and 2×SSC buffer (0.2M-NaCl–0.02M-sodium citrate) at 37°C for 18h. The amount of input RNA per incubation was in the range 0.5–4.0μg for preparations from control animals and 0.4–1.4μg for oestradiol-treated animals.

fractions of treated animals was the same, at 0.8% of RNA. This value is about double that reported for total nuclear RNA in mammalian cells and rat liver nuclei (Darnell *et al.*, 1973; Nakazato *et al.*, 1974; Sullivan & Roberts, 1973; Tata & Baker, 1975). In the RNA recovered from the rest of the nucleus, i.e. fractions consisting of nucleoli, nuclear membranes, heterochromatin, interchromatin granules etc. (Tata & Baker, 1974*a*), hormone treatment only led to a 50% enhancement of poly(A) content. In the control animals, the values for poly(A) content of these fractions were six times those associated with RNA from euchromatin fractions. As regards the subnuclear fractionation process or the recovery of RNA (usually 60–80%) from the various fractions, neither of these was markedly affected by hormone treatment.

The most marked enhancement in the amount of poly(A)-rich RNA associated with different subnuclear fractions, as a result of oestrogen administration, was recorded in the euchromatin fraction. This indirectly suggests that the site of polyadenylation of HnRNA may be close to that of its transcription, which is compatible with a similar conclusion drawn by us in an earlier study on the kinetics of polyadenylation and RNA synthesis in similarly derived subnuclear components from rat liver (Tata & Baker, 1974*b*, 1975). Further, the absence of any

Table 3. *Separation of ^{3}H-labelled nuclear RNA from control and oestradiol-treated male Xenopus liver into poly(A)-rich RNA by chromatography on oligo(dT)-cellulose*

Livers were pooled from three frogs; where indicated, 1 mg of oestradiol-17β had been injected 23 h before death. All animals were killed 3 h after receiving 300 μCi of [^{3}H]orotic acid. RNA was extracted from nuclei prepared by the glycerol–Triton X-100 procedure, and subjected to chromatography on oligo(dT)-cellulose by the procedure of Edmonds (1971), except that the residual RNA was eluted with 90% formamide at the end of the water wash. The RNA eluted with 0.5 M-KCl is poor in poly(A) at the 3′-terminus, whereas it is poly(A)-rich in the other fractions (Tata & Baker, 1975).

		RNA recovered	
Treatment	RNA eluted in	(c.p.m.)	(%)
Control	0.5 M-KCl	9410	72.0
	0.1 M-KCl	2644	20.2
	Water	252	1.9
	90% Formamide	1768	5.9
Oestradiol	0.5 M-KCl	31684	82.9
	0.1 M-KCl	2436	6.4
	Water	1680	4.4
	90% Formamide	2438	6.4

accumulation of high-molecular-weight poly(A)-rich RNA in the nuclear sap in rat or *Xenopus* liver, stimulated or not by the hormone, indicates that the processing of HnRNA into ribonucleoprotein particles (which would be largely present in our 'heterochromatin' fractions) and the transport of the latter into the cytoplasm must take place very rapidly.

The above experiments were then extended to determine the population of labelled RNA containing poly(A) by fractionation of RNA on oligo(dT)-cellulose columns. According to this procedure (Edmonds, 1971; Kates, 1973), ribosomal and non-poly(A)-containing RNA species are eluted at high ionic strength, whereas poly(A)-rich RNA is eluted by lowering the salt concentration. Preliminary experiments showed that RNA from *Xenopus* liver nuclei, when labelled for short periods of time, is not polyadenylated to any significant extent. There is also considerable nucleolytic degradation which does not allow analysis of size classes of RNA, so that it became essential to prepare nuclei in a glycerol medium at −20°C (Schibler & Weber, 1974). Therefore, to determine to what extent oestrogen did induce the synthesis of poly(A)-rich RNA, we had to change the procedure for isolating nuclei as well as to resort to longer labelling periods with [^{3}H]orotic acid or [^{3}H]uridine (3–16 h instead of 1 h), and longer exposure to oestradiol (24–40 h). The results thus obtained, and presented in Table 3, show that the synthesis of nuclear RNA rich in poly(A) was much enhanced, as was that of non-poly(A)-containing RNA.

Of the several hormones considered to be 'inducers' of specific proteins, or initiating a differentiative process, there are relatively few examples of true induction *de novo* at the level of the gene (Tata, 1970). Although the initiation of egg-protein synthesis by oestradiol is a good example of such a function, the goal of these studies was not to solve the question of mechanism of hormone action, but to explore the possibilities of obtaining a nuclear precursor for vitellogenin in mRNA male *Xenopus*, after its induction by oestrogen. As *Xenopus* vitellogenin is composed of subunits of mol.wt. about 200000 (see Clemens, 1975), the nuclear precursor to mRNA would be expected to be larger than 28S. Total nuclear RNA and poly(A)-rich nuclear RNA from control and oestrogen-treated male *Xenopus* livers were therefore analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. As shown in Fig. 4, induction of vitellogenin synthesis by oestrogen was characterized by a rapid increase in the rate of synthesis of all size classes of RNA in the hepatic nuclei of male frogs. These included 45S RNA species and molecules larger than 45S (Fig. 4*a*). When the nuclear RNA was adsorbed on oligo(dT)-cellulose columns and eluted at low ionic strength, a large part of the fraction of resulting poly(A)-rich RNA was found to migrate with mobilities corresponding to 25–60S. In both the control and treated animals, the residual radioactive RNA bound to the oligo(dT)-cellulose columns after extraction with water exhibited the same electrophoretic pattern after elution with 90% formamide. Results of other experiments, not shown here, revealed that, although the poly(A) content of RNA in nuclear sap was as high as that associated with euchromatin, most of the nuclear-sap poly(A)-rich RNA was degraded or of low molecular weight. That the electrophoretic peaks of labelled poly(A)-rich RNA, seen in Fig. 4, corresponding to sizes greater than 28S, were not artifacts of substantial aggregation was confirmed by analysing the same samples on 3.5% polyacrylamide gels in 99% formamide (Duesberg & Vogt, 1973). Electrophoresis in 99% formamide (Fig. 5) did not resolve high-molecular-weight RNA species into more peaks than seen in Fig. 4 on sodium dodecyl sulphate–polyacrylamide gels, nor cause a significant lowering in the range of size of the molecules. Since, in the experiment illustrated in Fig. 5, the RNA was labelled with ^{32}P, it was also possible to demonstrate that 90% of the labelled RNA was sensitive to digestion by RNAase A and RNAase T_1. The bulk of the residual ethanol-precipitable radioactivity migrated as a component of 3–5S, thus revealing the expected 3′-terminal polyadenylate residues, 100–150 nucleotides in length (Darnell *et al.*, 1973).

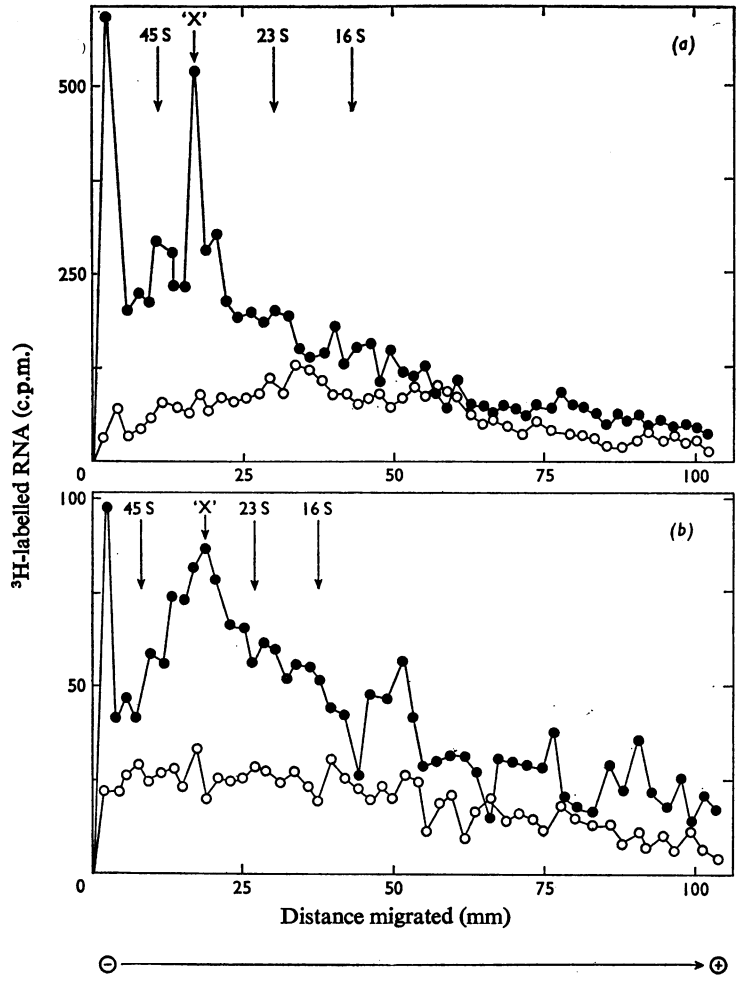

Fig. 4. *Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of total and poly(A)-rich nuclear RNA from liver of control and oestradiol-treated male Xenopus*

RNA was labelled with 400 μCi of [^{3}H]orotic acid injected 16h before death into two groups of three male frogs, one of which served as control (○) and the other had been treated with 1 mg of oestradiol (●), 11 h before death. Nuclei were prepared by the glycerol–Triton X-100 procedure and RNA was extracted from them immediately as described. The total nuclear RNA was resolved into poly(A)-poor and poly(A)-rich RNA by chromatography on oligo(dT)-cellulose. The latter fraction was made up of material eluted successively with 0.1 M-KCl, water and 90% formamide. The pooled RNA was reprecipitated with 2 vol. of ethanol and the size distribution compared with that in total nuclear RNA by electrophoresis on sodium dodecyl sulphate–2.3% polyacrylamide disc gels. After electrophoresis, the gels were cut into 2mm slices and the radioactivity was measured in each segment. Separate gels bearing 23S and 16S *E. coli* rRNA as markers were run simultaneously. The estimated position of 45S RNA is also indicated by arrows. (*a*) Total nuclear RNA; (*b*) poly(A)-rich RNA separated from total nuclear RNA. 'X', position of major component induced in hormone-treated animals.

On the basis of work on other sytems, particularly in HeLa cells (Darnell *et al.*, 1971, 1973; Nakazato *et al.*, 1974; Molloy *et al.*, 1974), it can be assumed that mRNA sequences coding for vitellogenin may be present in this RNA fraction. The results of several electrophoretic analyses of the kind illustrated in Figs. 4 and 5 revealed that the most predominant change provoked by oestrogen treatment could be found in poly(A)-rich nuclear RNA, depicted by the peak 'X', and corresponding to a size of 35–36S (Fig. 6). If this component, estimated to have a mean molecular weight of $2.05\times10^6\pm0.15\times10^6$, were to

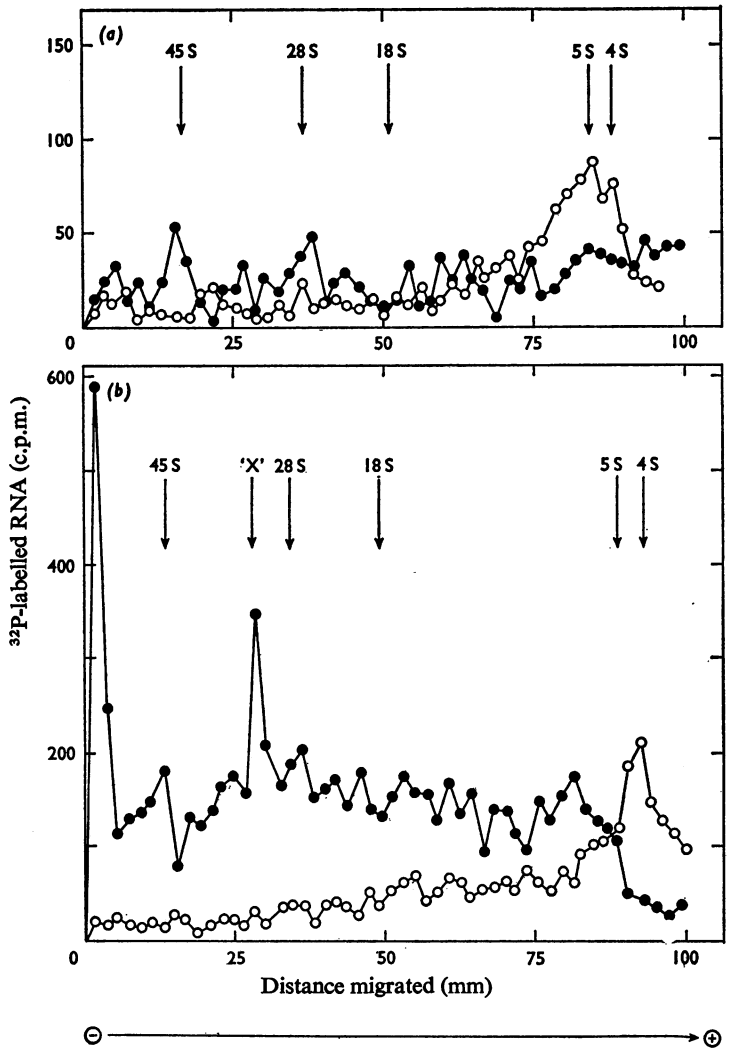

Fig. 5. *Electrophoretic analysis on 99% formamide–polyacrylamide gels of ^{32}P-labelled nuclear RNA from livers of control oestradiol-treated male Xenopus*

One group of three male frogs was injected with 1 mg of oestradiol-17β 22.8 h before killing, and another group served as the untreated control. All animals received 300 μCi of ^{32}P, 2.8 h before death. ^{32}P-labelled RNA was extracted from nuclei pooled from three livers and the poly(A)-rich RNA eluted from oligo(dT)-cellulose columns with 0.01 M-Tris–HCl buffer, pH 7.6. A portion (20%) of the total of this RNA was used directly for electrophoresis (●), whereas another sample, representing 60% of the RNA, was incubated with RNAase A and T_1, and the ethanol-insoluble fraction, mainly representing poly(A) tracts (○), was subjected to electrophoresis. Electrophoresis was carried out in 3.5% polyacrylamide disc gels in 99% formamide as described by Duesberg & Vogt (1973). Marker RNA species, indicated by their S values, were electrophoresed simultaneously. 'X' indicates major RNA species induced by the hormone. The gels were cut into 2 mm slices and the radioactivity was measured in a toluene–Triton X-100-based scintillation fluid with an efficiency of 90%. (*a*) RNA from untreated male frogs; (*b*) RNA from animals treated with oestradiol-17β.

contain sequences of the nuclear precursor of cytoplasmic mRNA, then it seems that the most stable polyadenylated precursor is only marginally larger than the functional mRNA in polyribosomes, which would be expected to be about 30 S in size, since the vitellogenin subunit of *Xenopus* is about 200000 daltons in size (see Clemens, 1974). There is no *a priori* reason why nuclear precursor RNA mole-

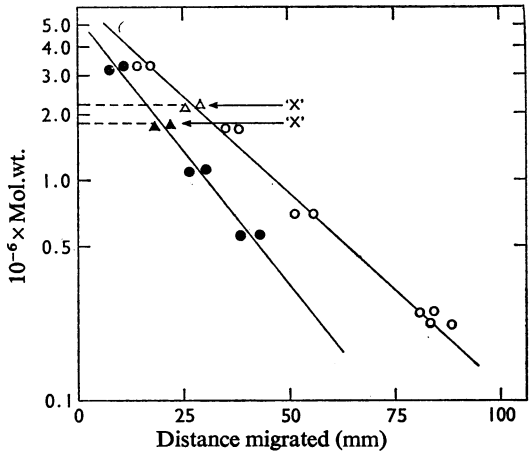

Fig. 6. *Estimation of size of major polyadenylated nRNA component in liver of male Xenopus on induction of vitellogenin synthesis by oestradiol-17β*

The data are derived from polyacrylamide-gel electrophoretic analysis in the presence of 0.5% sodium dodecyl sulphate (●, ▲) and 99% formamide (○, △) and refer to the peak of labelled RNA marked 'X' in Figs. 4 and 5. The calibration curve was derived from the following marker RNA species (○,●): *E. coli* 23S and 16S rRNA; rat liver 45S pre-ribosomal RNA; rat liver 28S, 18S and 5S rRNA; *E. coli* 4S RNA. △, ▲, Electrophoretic mobility and estimated molecular weight (2.05×10^6) of the RNA under peak marked 'X' in Figs. 4 and 5.

cules have to be considerably larger than the cytoplasmic mRNA products for all proteins, especially in view of the findings of Firtel & Lodish (1973) that in the slime mould, *Dictyostelium discoideum*, the nuclear precursor may only be slightly bigger than cytoplasmic mRNA species. That the nuclear precursor to cytoplasmic mRNA species in higher-animal cells may not be larger than the cytoplasmic message is also suggested by the work on the nuclear precursor to ovalbumin mRNA in chick oviduct (McKnight & Schimke, 1974) and in rabbit and duck erythroid cells (Lane *et al.*, 1973; C. D. Lane, personal communication). Although the results of our studies show that oestrogen administration to male frogs did indeed considerably enhance the synthesis of poly(A)-rich RNA in the nucleus, particularly that associated with euchromatin, it will be necessary, as a next step, to determine whether or not specific mRNA coding sequences were included in the large amount of additional poly(A)-rich high-molecular-weight RNA induced by the hormone. A definitive answer to the question of size of pre-mRNA molecules in the nucleus will also require the characterization and size of cytoplasmic mRNA for *Xenopus* vitellogenin. In parallel studies in our laboratory, the mRNA coding for vitellogenin has been isolated (M. V. Berridge, S. R. Farmer, C. D. Green, E. C. Henshaw & J. R. Tata, unpublished work) and, once DNA complementary to it is available, it will be possible to answer more fundamental questions, such as whether or not there exist any vitellogenin mRNA sequences present in normal untreated male *Xenopus* liver nuclear RNA, and whether these are synthesized *de novo* only on oestrogen treatment or whether the hormone determines the extent to which polyadenylated nuclear precursor to mRNA is further processed and transported to the cytoplasm.

We thank Dr. M. V. Berridge for the gift of oligo(dT)-cellulose and Dr. C. D. Lane for teaching us the technique of electrophoresis on polyacrylamide gels in 99% formamide.

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