Acad. Sci. U.S.A. 56, 1778–1785.
Mills, D. R., Kramer, F. R., & Spiegelman, S. (1973) *Science 180*, 916–927.
Mills, D. R., Dobkin, C., & Kramer, F. R. (1978) *Cell 15*, 541–550.
Nonoyama, M., & Ikeda, Y. (1964) *J. Mol. Biol. 9*, 763–771.
Randerath, K., & Randerath, E. (1967) *Methods Enzymol. 12A*, 323–348.
Sanger, F., Brownlee, G. G., & Barrell, B. G. (1965) *J. Mol. Biol. 13*, 373–398.
Spiegelman, S., Pace, N. R., Mills, D. R., Levisohn, R., Eikhom, T. S., Taylor, M. M., Peterson, R. L., & Bishop, D. H. L. (1968) *Cold Spring Harbor Symp. Quant. Biol. 33*, 101–124.
Weissmann, C., Borst, P., Burdon, R. H., Billeter, M. A., & Ochoa, S. (1964) *Proc. Natl. Acad. Sci. U.S.A. 51*, 682–690.
Weissmann, C., Feix, G., & Slor, H. (1968) *Cold Spring Harbor Symp. Quant. Biol. 33*, 83–100.

Effect of Estrogen on Preprolactin Messenger Ribonucleic Acid Sequences†

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ABSTRACT: A complementary DNA (cDNA) probe has been synthesized for rat preprolactin messenger RNA (mRNA). Following preparative gel electrophoresis, the cDNA was characterized and estimated to be ~75% pure. The reverse transcript is estimated to be greater than 1000 nucleotides long and, therefore, is a full-length copy of the preprolactin mRNA. It is a faithful transcript, as evidenced by back-hybridization with the mRNA. By use of the cDNA as a hybridization probe, the levels of preprolactin mRNA in the pituitary of male and female rats were monitored and found to increase following estrogen treatment. Increases in preprolactin mRNA activity, assayed by in vitro translation in the wheat germ system, were paralleled by increases in hybridization to the cDNA probe after estrogen treatment.

Prolactin synthesis in the rat pituitary is responsive to estrogens (Gersten & Baker, 1970; Lu et al., 1971; Maurer & Gorski, 1977). Preprolactin messenger RNA (pPRL mRNA) from rat pituitaries and its translation product have been partially characterized (Maurer et al., 1976, 1977; McKean & Maurer, 1978). Induced prolactin synthesis is closely paralleled by corresponding increases in pPRL mRNA activity (Stone et al., 1977). Whether this represents an increase in the number of pPRL mRNA molecules or the liberation of a preexisting, formerly untranslatable pool of pPRL mRNA has yet to be demonstrated. The use of molecular hybridization allows the quantitation of specific mRNA within a total RNA population. This approach has shown that increases in translatable mRNA following hormone stimulation are accompanied by increased hybridization to a cDNA probe (McKnight et al., 1975; Shapiro & Baker, 1977; Harris et al., 1975; Evans et al., 1978; Martial et al., 1977; Matusik & Rosen, 1978). We have synthesized a cDNA probe to rat pPRL mRNA and used it to monitor pPRL mRNA levels in the pituitary of estrogen-induced male and female rats. The number of pPRL mRNA sequences within the pituitary increases after estrogen treatment.

Experimental Procedure

Preparation of cDNA. Essentially the method of Kacian & Myers (1976) was used to synthesize cDNA in the initial experiments. Each reaction volume was 50 μL and contained the following final concentrations: 50 mM Tris-HCl, pH 8.3, 8 mM $MgCl_2$, 50 mM KCl, 1 mM dithioerythritol, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP, 0.2 mM [^{3}H]dCTP (2.5 Ci/mmol) or 0.2 mM [^{32}P]dCTP (15 Ci/mmol), 4 mM sodium pyrophosphate, 25 μg/mL oligo(dT)$_{12-18}$, 30–60 μg/mL mRNA, and 60 units/mL reverse transcriptase. For preparative syntheses, the following modifications were made: 0.015 mM [^{32}P]dCTP (50 Ci/mol) and 320 units/mL reverse transcriptase.

Reactions were initiated by the addition of enzyme and allowed to incubate for 75 min at 37 °C. The microliters of 1 N NaOH were then added, and the mixture was incubated at 68 °C for 30 min. After neutralization with acetic acid, the solution was extracted with 2 volumes of phenol–chloroform–isoamyl alcohol (100:98:2). The aqueous phase was removed and the organic phase reextracted with 0.5 volume of sterile water. The aqueous phases were pooled and chromatographed on Sephadex G-100 to remove unreacted [^{32}P]dCTP from the cDNA. To the eluted cDNA pool was added 2.5 volumes of 80% EtOH–20% 0.2 M NaCl, and the solution was placed at −20 °C overnight. The cDNA was pelleted by centrifugation, redissolved in 100 μL of sterile water, and stored at −20 °C.

Preparative Gel Electrophoresis. Typically, 100 μL of cDNA in sterile water was combined with 50 μL of a solution containing 0.2% sodium dodecyl sulfate ($NaDodSO_4$), 100 mM EDTA, 50% glycerol, 0.2 mg/mL bromophenol blue (BPB), and 0.6X Peacock's buffer (Peacock & Dingman, 1968). The mixture was heated in a boiling water bath for 2 min and applied to a 4%:10% composite acrylamide slab gel (Peacock & Dingman, 1968). Electrophoresis was begun at 50 V and continued until the BPB dye had migrated approximately 1 cm into the 4% gel. The remainder of the run was carried out at 120 V, until the bromophenol blue had migrated 12 cm. The gel was then covered with saran wrap

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and autoradiographed with Kodak NS2T X-ray film. Appropriate bands were located on the gel and excised.

The gel fragments were homogenized in a Dounce homogenizer in 2 mL of 0.5 M ammonium acetate, 0.01 M magnesium acetate, and 0.1% sodium dodecyl sulfate. After centrifugation at 500*g* for 4 min, the supernatant was removed, and the pellet was similarly extracted 2 additional times. The supernatants were pooled and centrifuged at 30000 rpm in a Ti50 rotor for 30 min to remove any residual acrylamide polymer. To this supernatant was added 2.5 volumes of 80% EtOH–20% 0.2 M NaCl, and the solution was placed at −20 °C for 24 h. After centrifugation in a Beckman Type 30 rotor for 60 min at 20000 rpm, the pellet was resuspended in sterile water. This cDNA solution was used directly for hybridizations.

Hybridization of cDNA to mRNA. Hybridizations were performed in 50-μL volumes in 0.4 mL of polypropylene microfuge tubes. The reaction mixture contained 10 mM Tris-HCl, pH 7.1, 400 mM NaCl, 2 mM EDTA, 0.1% sodium dodecyl sulfate, 2 μg of soluble RNA from liver, and 4000–5000 cpm of [^{32}P]cDNA (~25 pg of cDNA). Desired R_0t values (the product of the concentration of mRNA in moles of nucleotide per liter and the time in seconds) were obtained by varying both the mRNA concentration and the time of incubation.

The samples were overlayed with paraffin oil, heated to 100 °C for 2 min, and incubated at 68 °C for the desired period. Reactions were terminated by freezing. The percent hybridization was measured with S_1 nuclease, as previously described (McKnight & Schimke, 1974).

Purification of pPRL mRNA. Pituitaries were obtained from retired breeder female rats (Holtzman). The preparation of polysomes from brain tissue has been described (Palmiter, 1974). The total RNA was deproteinized by centrifugation of the polysomes through cesium chloride (Glisin et al., 1974). Poly(A)-containing RNA was separated from the ribosomal RNA (rRNA) by chromatography on oligo(dT)–cellulose (Aviv & Leder, 1972). Finally, the poly(A)-containing RNA was centrifuged on 5–20% sucrose gradients. Sucrose solutions were prepared in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 1% $NaDodSO_4$. The peak tubes of pPRL mRNA were pooled and ethanol precipitated. The pellet was extensively washed with 80% ethanol–20% 0.2 M NaCl (to remove the $NaDodSO_4$) and used for cDNA synthesis.

Cell-Free mRNA Translation. In vitro translation was performed with the wheat germ system. The details of this procedure have been previously described (Stone et al., 1976).

Prolactin Synthesis in Pituitaries. Anterior pituitaries were incubated in HeLa medium containing [^{3}H]leucine according to Maurer & Gorski (1977). Prolactin content was determined by gel electrophoresis (Maurer & Gorski, 1977).

Results

The synthesis of full-length cDNA by avian myeloma virus reverse transcriptase has been observed to be influenced by the deoxynucleotide triphosphate concentration of the reaction (Efstratiadis et al., 1975; Weiss et al., 1976) and the use of nuclease inhibitors (Kacian & Myers, 1976). The present study has employed the basic procedure described by Kacian & Myers (1976). Figure 1 shows the cDNA from a typical reaction after sedimentation through a 5–20% alkaline sucrose gradient. It can be seen that without sodium pyrophosphate in the reaction, a definite shoulder accompanies the main peak of radioactivity. With the addition of sodium pyrophosphate, a more homogeneous peak results, and only the higher molecular species is present.

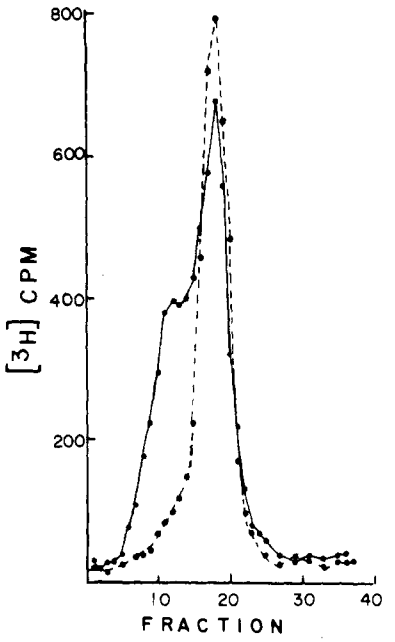

FIGURE 1: Alkaline sucrose gradients of cDNA synthesized from purified pPRL mRNA. [^{3}H]cDNA was synthesized in the presence (---) or absence (—) of 4 mM sodium pyrophosphate. Aliquots of the reaction mixtures were layered on 5–20% alkaline sucrose gradients (Studier, 1965) and centrifuged at 40000 rpm for 16 h. The direction of sedimentation was from left to right.

In early experiments, deoxynucleotide concentrations of 200 μM were used with 60 units/mL reverse transcriptase. However, when the dCTP concentration was lowered to 15 μM and the enzyme concentration raised to ~350 units/mL, the yield of apparently full-length cDNA was conserved (Friedman & Rosbash, 1977). A comparison of cDNA prepared under these different conditions is shown in Figure 2. Both procedures are efficient in producing a predominantly single-length cDNA from globin mRNA and pPRL mRNA. Since the lower dCTP concentration resulted in a cDNA of higher specific activity, it was used routinely for preparative synthesis.

The size of the pPRL cDNA was estimated on a 4% acrylamide cylindrical gel, by using single-stranded cDNA markers. Purified full-length rabbit globin cDNA (575 nucleotides) and a Hae III restriction digest of this cDNA (325, 125, and 120 nucleotides) were used as standards (Maniatis et al., 1976). The gel was sliced into 1.5-mm sections and counted. The relative mobility of the ^{32}P-labeled cDNA fragments (0.349, 0.440, and 0.587, respectively) was plotted vs. the log of the nucleotide length. (While autoradiography on a slab gel showed two bands corresponding to the lengths of 120 and 125 nucleotides, the two fragments were not resolved on the cylindrical gel. This resulted in a single $R_f = 0.587$. Therefore, this R_f was plotted against a nucleotide length of 125.) The three markers generated a straight standard line from which the length of the pPRL cDNA was estimated to be about 1300 nucleotides ($R_f = 0.22$). This must be regarded as only an estimate, since single-stranded fragments larger than the pPRL cDNA were not employed for the determination. Furthermore, this probably represents an overestimate, since the length of the pPRL mRNA has been calculated to be about 1150 nucleotides (unpublished experiments). Taken together, the cDNA length and relative homogeneity strongly suggest that a full-length copy of the pPRL mRNA has been synthesized.

To enhance the final purity of the cDNA, the major band of radioactivity obtained after gel electrophoresis was extracted

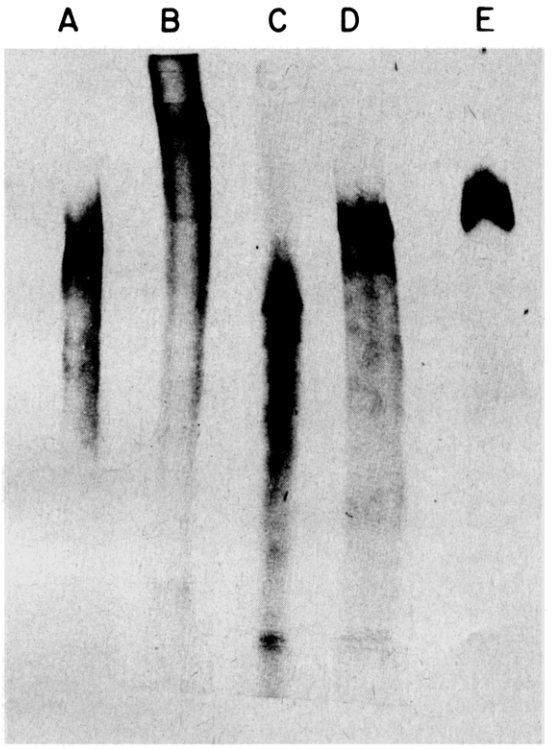

FIGURE 2: Electrophoresis of [^{32}P]cDNA synthesized from purified pPRL mRNA and globin mRNA. The cDNA reaction mixture was base hydrolyzed, phenol–chloroform extracted, passed over Sephadex G-100, and ethanol precipitated (see Experimental Procedure). Aliquots of the resuspended pellet were electrophoresced on a 4%:10% composite acrylamide gel. Lanes A and B: globin cDNA and pPRL cDNA, respectively, were synthesized with 0.2 mM dCTP and 60 units/mL reverse transcriptase. Lanes C and D: globin cDNA and pPRL cDNA, respectively, were synthesized with 0.015 mM dCTP and 320 units/mL reverse transcriptase. Lane E: pPRL cDNA was extracted from a preparative gel and reelectrophoresced.

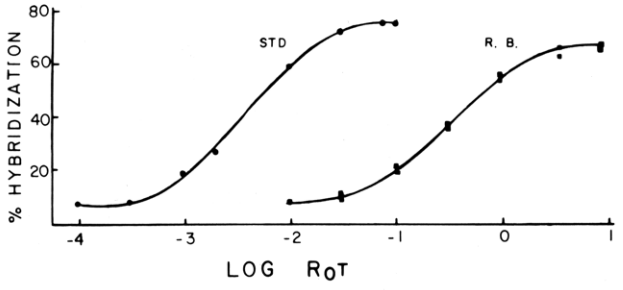

FIGURE 3: Hybridization of pPRL cDNA to its homologous mRNA template (STD) and to a total RNA preparation from the same biological source, retired breeder females (R. B.). RNA excess reactions were incubated at 68 °C until the indicated R_0t values were reached. Aliquots were assayed for acid-precipitable radioactivity and for S_1 nuclease resistance to determine the extent of hybridization. R_0t is the product of the RNA nucleotide concentration (mol/L) and the time (s).

from the gel. Figure 2 shows this cDNA when rerun on an identical acrylamide gel. With this procedure, we now have selectively refined the size of the cDNA, giving us a full-length hybridization probe.

That the cDNA represents a faithful copy of the pPRL mRNA was tested by back-hybridization (Figure 3). The $R_0t_{1/2}$ for this reaction is 0.00376 M s. Also shown in Figure 3 is the hybridization of the cDNA to total pituitary RNA, the source of the purified pPRL mRNA. Comparison of the $R_0t_{1/2}$ for this reaction (0.355 M s) to the $R_0t_{1/2}$ for the purified mRNA (0.00376 M s) shows that the pPRL mRNA has been purified about 95-fold. Assuming that the total cellular mRNA compromises approximately 2% of the total RNA

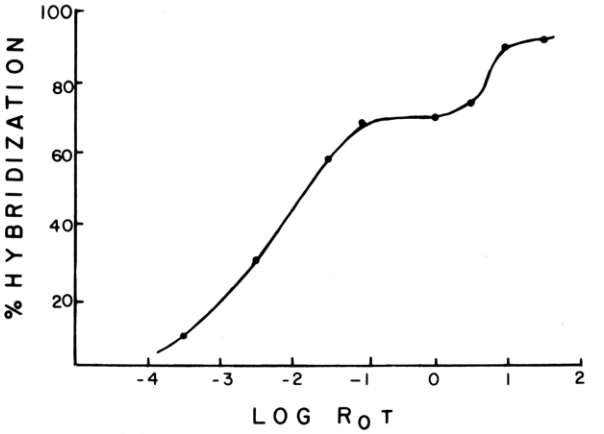

FIGURE 4: Hybridization of pPRL cDNA to its homologous mRNA template. The hybridization was extended to log R_0t = 1.5. The hybridization was performed in RNA excess as in Figure 3.

Table I: Purity of pPRL mRNA According to in Vitro Translation[a]

	mRNA act. (cpm/μg of RNA)	% of total
purified mRNA, total Cl_3AcOH precipitate	85 000 ± 700	100
PRL immunoprecipitate	63 000 ± 300	74
GH immunoprecipitate	11 500 ± 700	13

[a] Pituitary mRNA was purified as described under Experimental Procedure and translated in the wheat germ system. A 25-μL aliquot of the in vitro translation reaction was assayed for immunoprecipitate prolactin or growth hormone or total Cl_3AcOH precipitable radioactivity. Data are expressed as the mean ± SEM.

(Woo et al., 1975; McDonald et al., 1977) and that prolactin mRNA constitutes about 35% of the pituitary mRNA (Maurer & Gorski, 1977), one can calculate an expected purification of 140-fold for homogeneous pPRL mRNA. By use of these assumptions, the pPRL cDNA would be nearly 70% purified.

It was of interest to determine whether additional hybridization could be achieved at higher R_0t values. Figure 4 shows that an additional component was observed. The biphasic curve suggests the presence of two hybridizing species which have relative proportions of ~75–80% and 20–25%, respectively. The purity of the mRNA from which the cDNA was prepared has been estimated by cell-free translation to be 75% preprolactin mRNA activity with approximately 10–15% pregrowth hormone mRNA (GH mRNA) activity (Table I). The pPRL and GH mRNAs are similar in length (unpublished experiments), and assuming equal reverse transcription of both mRNAs, their respective cDNAs are not likely to be completely separated by the electrophoretic isolation used in this study. Thus, it is possible that some GH cDNA was present in our probe. In order to test this possibility, the cDNA was annealed to an enriched GH mRNA prepared from pituitaries of male rats injected with triiodothyronine and dexamethasone. Hybridization was not observed until R_0t values were greater than 10, and 75–80% hybridization occurred at a $R_0t_{1/2}$ of 50.1 M s. This could be accounted for by a 3% content of pPRL mRNA in the male pituitary RNA and is reasonably close to estimates of prolactin synthesis in male pituitaries (Maurer & Gorski, 1977). This suggests that GH cDNA is present only as a minor contaminant, if it is present at all. Thus, the cDNA preparation used in this study has a low R_0t component which is pPRL cDNA and accounts for 75–80% of the total R_0t curve. The wide separation of this component on the R_0t curve from the

Table II: Stimulation of Prolactin Synthesis by Estrogen[a]

	hybridization		translation		pituitary prolactin synthesis: protein synthesis		
treatment	$R_0t_{1/2}$ (mol s L^{-1})	stimulation	mRNA act. (cpm/μg of RNA)	stimulation	cpm × 10^{-2}/ mg of protein	% total	stimulation
			Males				
C	0.417		15 000 ± 650		35.0	7.1	
E_3	0.266	1.57	21 800 ± 720	1.45	50.2	10.0	1.41
E_6	0.199	2.10	34 600 ± 625	2.30	69.8	14.2	2.10
			Females				
C	0.473		6 980 ± 220		19.2	4.3	
E	0.083	5.68	37 400 ± 698	5.36	96.4	22.0	5.12

[a] Mature male rats were treated for 0 (C), 3 (E_3), or 6 (E_6) days with 10 μg of estradiol 17-β. Immature females were treated for either 0 (C) or 5 (E) days with 10 μg of estradiol 17-β. Two sets of animals were contained in each treatment group. Total RNA preparations were made from each set, and aliquots were taken for cDNA–RNA hybridizations and for wheat germ translation assays (10 μg per reaction). The $R_0t_{1/2}$ is the point at which 50% of the hybridization was attained. Prolactin messenger activity was determined as immunoprecipitable preprolactin synthesized in duplicate cell-free reactions per microgram of RNA assayed. Data is expressed as the mean ± SEM. In parallel groups of pituitaries, prolactin synthesis was calculated as the incorporation of radioactive leucine into protein migrating as prolactin on NaDodSO$_4$ gels. Data are expressed as radioactivity per milligram of protein or as percent total protein synthesis.

contaminating cDNAs permits its use as a hybridization probe in the experiments described below.

The effect of estrogen on pituitary pPRL mRNA in male rats was investigated. Total pituitary RNA was extracted at 3- and 6-day periods following daily injections of 10 μg of estradiol (E_2). The pPRL mRNA levels were quantitated by hybridization to the cDNA probe, as well as cell-free translation in the wheat germ system. The hybridization curves are presented in Figure 5A, and the appropriate $R_0t_{1/2}$ values are summarized in Table II. Estrogen stimulated the levels of hybridizable pPRL mRNA 1.57- and 2.1-fold, after 3 and 6 days, respectively. Corresponding increases of 1.45- and 2.3-fold were found by cell-free translation. The increase in both translatable pPRL mRNA and pituitary prolactin synthesis coincides with an increase in the number of pPRL mRNA sequences (Table II).

When immature female rats were administered daily injections of E_2 for 5 days, a more dramatic response was obtained (Figure 5B). Pituitary pPRL mRNA was elevated more than fivefold when assayed by cell-free translation (Table II). Once again, the increase in translation activity was accompanied by a nearly identical increase in mRNA sequences. Prolactin biosynthesis in whole pituitaries from similarly treated rats was also in accord with the cDNA hybridization and cell-free translation data (Table II).

In these same experiments, growth hormone mRNA activity was 22 350 ± 500 cpm/μg of RNA for male rats and 8675 ± 295 cpm/μg of RNA for immature females and did not vary significantly with estrogen treatment. This is additional evidence for the specificity of the prolactin response to estrogen. It also supports the idea that the shift in $R_0t_{1/2}$ values observed for the low R_0t component with estrogen treatment is due to prolactin mRNA since the other major messenger species is not affected.

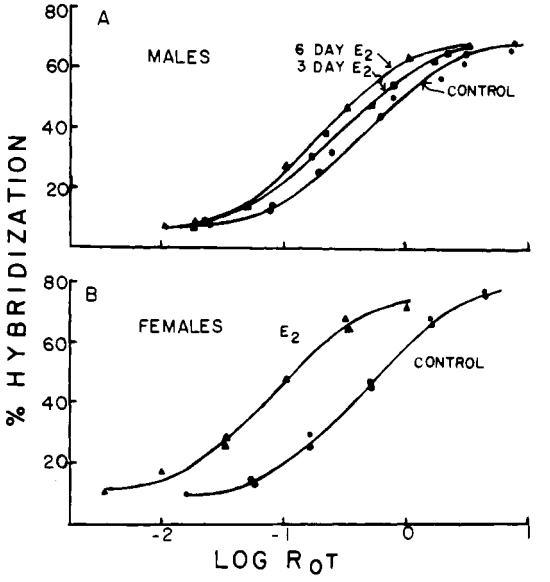

FIGURE 5: Hybridization of pPRL cDNA to total RNA from control and estrogen-treated rats. In panel A, 30-day-old males were treated for either 0 (○), 3 (□), or 6 days (△) with 10 μg of estradiol 17-β; in panel B, immature females (19 days) were injected either with control vehicle (C) or with 10 μg of estradiol 17-β for 5 days (E_2). Hybridizations were performed in RNA excess as in Figure 3. Each data point represents an RNA preparation from 6 pooled pituitaries.

Discussion

We have prepared a highly purified, full-length cDNA probe for preprolactin mRNA. The hybridization of this cDNA with purified pPRL mRNA occurs over two log R_0t intervals (Figure 3) and is estimated to be 75–80% pure. This estimation agrees well with the purity of the pPRL mRNA as determined by cell-free translation. The similar shapes of the hybridization curves for pPRL cDNA with purified and unpurified pPRL mRNA (Figure 3) indicate homogeneity of the system. If the back-hybridization is extended to large R_0t values ($R_0t > 0$), an additional hybridizing component is observed (Figure 4). The two phases of the hybridization curve are widely separated, and the higher R_0t component contributes only 15–20% of the total hybridization at complete saturation. The pPRL cDNA appears adequate for the studies presented in this work.

When estrogen was administered daily to male rats, the levels of translatable pPRL mRNA were elevated 1.45-fold and 2.3-fold after 3 and 6 days of treatment. Corresponding increases in the hybridization to the cDNA probe were also observed (Table II). Similarly, when immature female rats were given 5 daily injections of E_2, 5.36-fold increases in pPRL mRNA were measured by cell-free translation (Table II). Again, these increases were paralleled by increased hybridization to the cDNA. This greater responsiveness of the immature females vs. male rats is in agreement with previously reported patterns of prolactin secretion with estrogen administration (Neill, 1972) and may be due, in part, to hypothalamic development processes determined shortly after birth. However, such a sexual difference in response to es-

trogen was not observed in biosynthesis studies performed with males and ovariectomized mature females in our laboratory (Maurer & Gorski, 1977). Whether animal age and/or prior exposure to sex hormones is sufficient to account for this discrepancy has not been established.

Thus, the number of pPRL mRNA sequences appears to be elevated in response to estrogen rather than a modification of sequestered sequences to an actively translated form. Thyrotropin-releasing hormone has also been observed by Evans et al. (1978) to cause higher rates of prolactin synthesis via an increased number of pPRL mRNA sequences. Such results do not prove that there are increased rates of transcription of the prolactin gene, however, since only the accumulation of total message is being measured. Hormonal regulation could occur at several points to bring about final higher levels of this mRNA. The half-life of cytoplasmic message might be affected by steroid treatment. There is some evidence from translation studies that estrogen administration increases the stability of ovalbumin mRNA, but the effect is not sufficient to account for the total increase in mRNA sequences observed (Palmiter & Carey, 1974). Transport of message from nucleus to cytoplasm has not been well investigated but could also be a factor in determining final mRNA levels. The finding that many messengers are synthesized as larger primary transcripts within the nucleus (Van Voorthuizen et al., 1978; Strair et al., 1978; Tilghman et al., 1978) has opened up the possibility of nuclear processing steps playing a critical role in mRNA induction. One of the most obvious control points is that of increased transcription. While higher levels of transcription with estrogen administration have been implied in other systems by increased RNA polymerase activity (Mohla et al., 1972) and chromatin binding sites (Tsai et al., 1976), an increase in transcription of a particular gene has not been demonstrated.

Thus, further investigation will have to be focused at the nuclear level to quantitate the number of primary prolactin gene transcripts and any changes in processing of nuclear to cytoplasmic message with hormone induction. Complete studies will require measurement not only of the absolute number of transcripts but also of the relative half-lives of any processing intermediates.

Acknowledgments

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References

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408.

Efstratiadis, A., Maniatis, T., Kafatos, F. C., Jeffrey, A., & Vouruakis, J. N. (1975) *Cell 4*, 367.

Evans, G. A., David, D. N., & Rosenfeld, M. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1294.

Friedman, E. Y., & Rosbash, M. (1977) *Nucleic Acid Res. 4*, 3455.

Gersten, B. E., & Baker, B. L. (1970) *Am. J. Anat. 128*, 1.

Glisin, V., Crkvenjakov, R., & Byus, C. (1974) *Biochemistry 13*, 2633.

Harris, S. E., Rosen, J. M., Means, A. R., & O'Malley, B. W. (1975) *Biochemistry 14*, 2072.

Kacian, D. L., & Myers, J. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2191.

Lu, K., Koch, Y., & Meites, J. (1971) *Endocrinology 89*, 229.

Maniatis, T., Kee, S. G., Efstratiadis, A., & Kafatos, F. C. (1976) *Cell 8*, 163.

Martial, J. A., Baxter, J. D., Goodman, H. M., & Seeburg, P. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1816.

Matusik, R. J., & Rosen, J. M. (1978) *J. Biol. Chem. 253*, 2343.

Maurer, R. A., & Gorski, J. (1977) *Endocrinology 101*, 76.

Maurer, R. A., Stone, R., & Gorski, J. (1976) *J. Biol. Chem. 251*, 2801.

Maurer, R. A., Gorski, J., & McKean, D. J. (1977) *Biochem. J. 161*, 189.

McDonald, R. J., Przybyla, A. A., & Rutter, W. J. (1977) *J. Biol. Chem. 252*, 5522.

McKean, D. J., & Maurer, R. A. (1978) *Biochemistry 17*, 5215.

McKnight, G. S., & Schimke, R. T. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4327.

McKnight, G. S., Pennequin, P., & Schimke, R. T. (1975) *J. Biol. Chem. 250*, 8105.

Mohla, S., DeSombre, E. R., & Jensen, E. V. (1972) *Biochem. Biophys. Res. Commun. 46*, 661.

Neill, J. D. (1972) *Endocrinology 90*, 1154.

Palmiter, R. D. (1974) *Biochemistry 13*, 3606.

Palmiter, R. D., & Carey, N. H. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2357.

Peacock, A. C., & Dingman, C. W. (1968) *Biochemistry 7*, 668.

Shapiro, D. J., & Baker, H. J. (1977) *J. Biol. Chem. 252*, 5244.

Stone, R. T., Maurer, R. A., & Gorski, J. (1977) *Biochemistry 16*, 4915.

Strair, R. K., Yap, S. H., Madal-Ginard, B., & Shafritz, D. A. (1978) *J. Biol. Chem. 253*, 1328.

Studier, W. F. (1965) *J. Mol. Biol. 11*, 373.

Tilghman, S. M., Curtis, P. J., Tiemeier, D. C., Leder, P., & Weisman, C. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1309.

Tsai, M. J., Towle, H. C., Harris, S. E., & O'Malley, B. W. (1976) *J. Biol. Chem. 251*, 1960.

Van Voorthuizen, W. F., Dinsart, C., Flavell, R. A., VeVijlder, J. J., & Vassart, G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 74.

Weiss, G. B., Wilson, G. N., Steggles, A. W., & Anderson, W. F. (1976) *J. Biol. Chem. 251*, 3425.

Woo, S. L. C., Rosen, J. M., Liarkos, C. D., Robberson, D. L., Choy, Y. C., Busch, H., Means, A. R., & O'Malley, B. W. (1975) *J. Biol. Chem. 250*, 7027.