An estrogen-dependent polysomal protein binds to the 5′ untranslated region of the chicken vitellogenin mRNA

Haimin Liang and Jean-Pierre Jost*
Friedrich-Miescher Institute, PO Box 2543, CH-4002 Basel, Switzerland

Received February 19, 1991; Revised and Accepted March 25, 1991

ABSTRACT

An estrogen-dependent protein present in chicken liver polysomes binds to the 5′ untranslated region of the chicken vitellogenin II mRNA. Competition binding assays with different RNAs indicate that the binding of the polysomal protein to this region is sequence specific. Of the tissues tested, this RNA binding activity is liver specific. *In vivo* kinetics of appearance of the binding activity following a single injection of estrogen to immature chicks are similar to the rate of accumulation of vitellogenin mRNA. The molecular weight of the polysomal protein has been estimated to be 66,000 on the basis of UV crosslinking and subsequent SDS polyacrylamide gel electrophoresis. *In vitro* RNA decay assays carried out with a mini-vitellogenin mRNA suggest that the estrogen-dependent polysomal protein may be involved in the estrogen-mediated stabilization of the chicken vitellogenin II mRNA.

INTRODUCTION

Vitellogenin is the precursor of egg yolk proteins and is synthesised in the liver of all egg-laying oviparous vertebrates (1, 2). The expression and accumulation of the vitellogenin mRNA is under tissue specific and hormonal control (3,4). In chickens the vitellogenin is encoded by three different genes. The gene for vitellogenin II can be fully activated in roosters and in immature chicks by a single injection of estrogen (5). Previous studies have demonstrated that estrogen-dependent induction of vitellogenin mRNA in both avian (6) and amphibian (7,8) livers is regulated at two levels: transcription of the relevant gene and stabilization of the mRNA. In an avian system, an *in vivo* analysis based on the kinetics of accumulation of vitellogenin II mRNAs following estrogen treatment as well as their rate of degradation following acute hormone withdrawal has shown that the mRNAs were stabilized 8−10 fold during the induction. The half-life in the presence of hormone was estimated to be 22−26 hours, decreasing to 2 to 3 hours following estrogen withdrawal. More dramatically, in cultured *Xenopus* liver cells the vitellogenin mRNA has a half-life of approximately 500 hours in the presence of estrogen and 16 hours in the absence of estrogen; when this mRNA undergoes cytoplasmic degradation, it can be restabilized by the addition of estrogen, indicating that vitellogenin mRNA stabilization is a reversible effect of estrogen.

To date, over twenty eucaryotic mRNAs are known to have a regulated mRNA stabilization (reviews 9, 10, 11, 12). Although the mechanism that regulates the stability of each mRNA has unique features, it seems that in each case, specific RNA sequences, mainly located in 5′ and 3′ untranslated region (UTR), and their recognition by specific factors are required (10). In the case of *Xenopus* vitellogenin mRNA, Nielson and Shapiro have shown that the 5′ and 3′ terminal regions of the mRNA are involved in estrogen mediated mRNA stabilization (13). But the mechanism of such a hormone mediated stabilization remains unknown. Is it possible that specific factors regulated by estrogen bind to the 5′ or 3′ UTRs of vitellogenin mRNA and protect it from degradation? To answer this question, we used UV crosslinking and subsequent SDS PAGE to seek estrogen-dependent RNA binding proteins. Using the 5′ UTR of chicken vitellogenin mRNA as a probe, we found a protein of 66 kDa which binds with high specificity to this region. The binding activity in polysomes is estrogen-dependent and liver specific. The kinetics of the estrogen-dependent induction and *in vitro* decay assay suggest that this protein may be involved in the stabilization of vitellogenin mRNA.

MATERIALS AND METHODS

Materials

T_7 and Sp_6 RNA polymerase and restriction enzymes were supplied by Boehringer Mannheim. (alpha-^{32}P)UTP (800 Ci/mmol) was from Amersham Corp. Oligonucleotides were synthesized on an Applied Biosystems 380A synthesizer and purified as described by Jost et al.(14).

Hormone Treatment of Animals

Mature white Leghorn roosters, hens and immature chicks (200−250 gr body weight) were treated for the time indicated with a single intramuscular injection of 17 beta-estradiol (estrogen) (40 mg/ml in propylenglycol, 40 mg/kg of body weight).

* To whom correspondence should be addressed

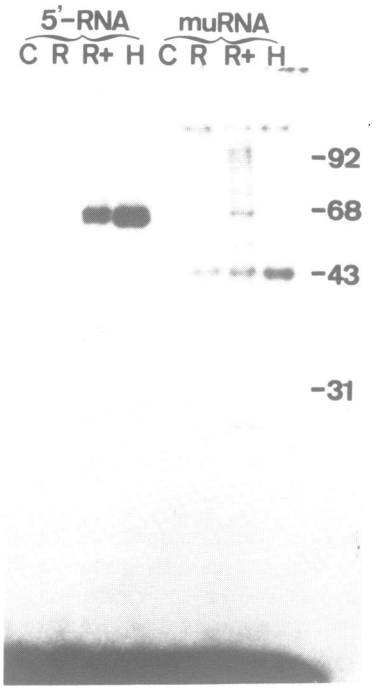

Fig. 1. UV crosslinking of the polysomal protein to a mini-vitellogenin mRNA. Protein (2.5 μg) from rooster (R), estrogen treated rooster (R+) or hen (H) polysome extracts or protein buffer (C) was UV crosslinked to ^{32}P labeled 5'-RNA (5×10^4), 5'GGGCGAAUUCAUUCACCUUCGCUAUGAAUAUUC 3' (5'UTR underlined) or to the corresponding anti-sense RNA (muRNA). The reaction products were separated by electrophoresis on a 10% SDS polyacrylamide gel.

Fractionation of Liver Cytoplasm

(a) The cytoplasmic fraction from chicken livers was prepared as described by Feavers et al. (15). This was further fractionated into cytosol, free polysome, smooth (SER) and rough (RER) endoplasmic reticulum as described by Murray et al. (16). Briefly, the post-nuclear and mitochondrial fraction (centrifugation at 17000×g for 10 minutes) was adjusted to 1.31 M sucrose and overlaid on a 10 ml 2 M sucrose cushion in a SW 28 Beckman centrifuge tube; 3 ml 0.8 M sucrose buffer was then layered on top of this solution and centrifugation was performed for 18 hours at 25,000 rpm, 4°C. SER and RER fractions were diluted in 0.2 M sucrose, 5 mM $MgCl_2$, 50 mM Tris-Cl pH 8.0, 1 mM Phenylmethylsulfonylfluoride (PMSF) and centrifuged for 40 minutes at 30,000 rpm. Polysome, SER and RER fractions were extracted with a buffer containing 50 mM Tris-Cl pH 8.0, 1 M KCl, 10 mM EDTA pH 8.0, 1 mM PMSF for 30 minutes on ice. Following centrifugation for 3 hours at 40,000 rpm in a SW 65 Beckman rotor at 0°C, the supernatant was 65% saturated with ammonium sulfate. The precipitated protein was collected by centrifugation and resuspended in a small volume of buffer containing 20 mM Hepes pH 8.0, 50 mM KCl, 0.1 mM EDTA pH 8.0, 12.5 mM $MgCl_2$, 10% glycerol, 0.5 mM PMSF, 2 mM 2-mercaptoethanol (protein buffer) and dialyzed 3 hours against 2×500 ml of the same buffer.

(b) To isolate total polysomes (both free and bound) from liver, kidney, oviduct, heart or muscle, the post nuclear and mitochondrial fraction was loaded on a 1.31 M sucrose buffer cushion in a SW 28 centrifuge tube. The centrifugation was performed for 3 hours at 25,000 rpm, 4°C. The polysome pellet was extracted as described in (a).

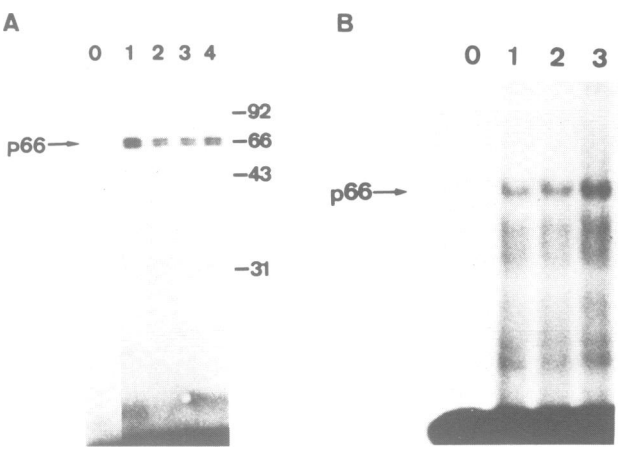

Fig. 2. p66 is not estrogen receptor. (A) U.V. crosslinking assay of p66 to the 5'UTR in the presence of 5 μg of BSA (lane 2), 5 μg of monoclonal antibody against calf estrogen receptor (lane 3) or 500 ng of synthetic estrogen response element (lane 4). Lane 1 is the control of the interaction of p66 with 5' UTR. (B) Native gel-shift assay of p66 to the 5' UTR under same condition as described in (A).

In Vitro RNA Synthesis

(A) The 28 bp long oligonucleotide 5'GAATTCATTCACCTTCGCTATGAAGCTT 3' containing the entire 5' UTR region in the first exon (underlined) of the chicken vitellogenin mRNA (17) was cloned into the vector pGEM-3Z (Pharmacia) such that it was flanked 5' by a T_7 promoter and 3' by a Sp_6 promoter. The resulting plasmid was digested with HindIII or EcoRI and run-off RNAs were generated *in vitro* with T_7 RNA polymerase (for messenger-like RNA) or Sp_6 RNA polymerase (for anti-sense RNA) in the presence of ATP, GTP, CTP and (alpha-^{32}P) UTP as described previously (18). The transcripts were purified on a 15% sequencing gel, eluted and recovered as described by Jost et al.(14).

(B) The 57 bp long oligonucleotide 5'AATTATTCACCTTCGCTATTCACCTTCGCTATTCACCTTCGCT$(A)_{15}$3' and 5'AATTAAAGTGGAAGCGAAAAGTGGAAGCGAAAAGTGGAAGCGA$(A)_{15}$3' have been cloned into the vector pGEM-4Z, respectively such that it was flanked 5' by Sp_6 promotor. The run-off RNAs were generated as described in (A).

Protein-RNA Crosslinking

Protein-RNA crosslinking with UV irradiation was performed essentially as described by Moore et al. (19). *In vitro* synthesized RNA (5×10^4 cpm) was heated at 90°C for 3 minutes, chilled quickly on ice before incubation with 2.5 μg of polysome extract and 0.5 μg of poly(A) as non-specific competitor. After 10 minutes of incubation at room temperature, the reaction mixture was irradiated for 20 minutes on ice using a U.V. lamp (Philips TUV 15W G15 T8) at a distance of 15 cm. The samples were treated with a mixture of RNase A (1 μg/μl) and RNase T_1 (2.5 U/μl) for 20 minutes at 37°C before analysis on a 10% discontinuous SDS polyacrylamide gel. The gel was stained with Coomassie Brilliant Blue G-250 (Serva) and electro-destained at 70 V for 6 minutes. The autoradiography was carried out after the gel was dried.

In Vitro RNA Decay Assay

In vitro synthesized RNA (6×10^4 cpm) was incubated with 12 μg protein from rooster liver cytoplasmic extract (major source

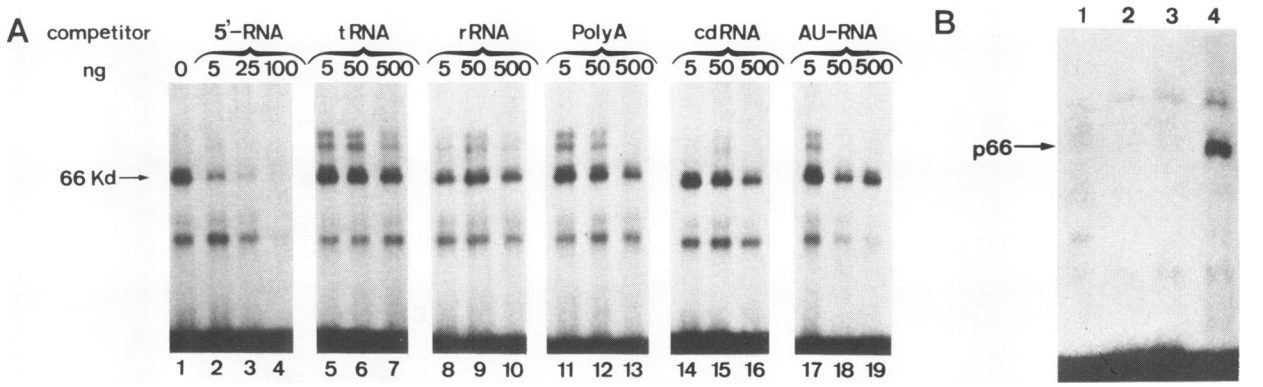

Fig. 3. p66 binds specifically to the 5'UTR. (A) Hen liver polysomal protein (1 μg) was incubated with ^{32}P labeled 5'-RNA (0.5ng, Lane 1) in the presence of non labeled 5'-RNA (lanes 2–4), *E. coli* tRNA (lanes 5–7), chicken rRNA (lanes 8–10), polyA (lanes 11–13), TMV first open reading frame mRNA (cdRNA, lanes 14–16) or AU rich RNA (AU-RNA, lanes 17–19). The reaction products were UV and RNase treated and separated on a 10% SDS polyacrylamide gel. Unlabeled competitor RNA is given in nanogram per reaction mixture as indicated above. The arrow indicates the p66. (B) U.V. crosslinking assay of p66 to the 5' UTR 5'UUCACCUUCGCU3' (lane 4), mutants 5'GAAGCCUUCGCU3' (lane 1), 5'UUCAAGAGCGCU3' (lane 2) or 5'UUCACCUUAUAG3' (lane 3).

of RNases) and 30 μg of either bovine serum albumin or hen polysome extract in 150 μl binding reaction buffer. Aliquots from the reaction mixture were removed at different times and quenched by the addition of 25 μl acid phenol. After phenol and chloroform extraction, each sample was mixed with 5 μl loading buffer (90% Formamide, 1×TBE, 0.02% Bromophenol blue, 0.02% Xylene cyanol) and the RNA was resolved by electrophoresis on a 10% sequencing gel. Gels were then dried and subjected to autoradiography.

Heparin-Sepharose chromatograph

Polysomal protein (25 mg) were applied onto a column of Heparin-Sepharose (1 ml bed volume, Pharmacia) equilibrated with 0.1 M KCl protein buffer (as above). Fractionation was achived by elution with a stepped KCl gradient on FPLC. The fractions were 65% saturated with ammonium sulfate and the precipitate was collected and resuspented in protein buffer. Following extensive dialysis against the protein buffer, the protein fractions were assayed for RNA binding activity using the UV cross-linking assay.

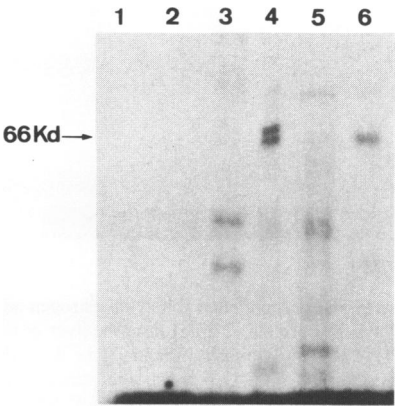

Fig. 4. Tissue distribution of p66. Hen polysomal protein (2.5 μg) from heart (lane 1), muscle (lane 2), Kidney (lane 3), liver (lane 4) or oviduct (lane 5) was incubated and UV crosslinked to ^{32}P labeled 5'-RNA (5×10^4 cpm) and separated on a 10% SDS polyacrylamide gel. A purified p66 (Heparin-Sepharose fraction) was also included in the assay (lane 6) to indicate the position of p66.

RESULTS

Detection of an estrogen-dependent protein binding to the 5'UTR of chicken vitellogenin mRNA

The cytoplasmic fraction from hen liver was further fractionated into cytosol, smooth and rough endoplasmic reticulum (SER and RER) and free polysomes by centrifugation on a discontinuous sucrose gradient. Since no RNase inhibitors were added during any part of the preparation, a large part of the polysomes was released from the RER and hence most of the vitellogenin polysomes were found in the free polysome fraction (Jost; unpublished results). The SER, RER and free polysome fractions were further extracted by 1 M KCl and RNA binding activities in the four fractions were tested by UV crosslinking to *in vitro* synthesized RNA containing the 5' UTR of the chicken vitellogenin mRNA (henceforth denoted 5'-RNA). The RNA binding activity was found in all the fractions except SER (data not shown). The highest binding activity was present in the polysomal fraction; thus the polysomal protein was chosen for further characterization.

To investigate whether or not the RNA binding activity present in polysomes is estrogen-dependent, we treated roosters for 24 hours with estrogen. Total polysomes were isolated, extracted and UV crosslinked to the 5'-RNA or to the corresponding antisense RNA (muRNA). Figure 1 shows that a protein of 66 kDa is found in polysome extracts from hens and estrogen-treated roosters but not from non-treated ones, suggesting that the p66 polysomal protein is estrogen-dependent. The p66 binds strongly to the 5'-RNA and only slightly to the antisense RNA, suggesting that the binding may be sequence specific. The double bands observed in the crosslinking of the polysomal protein could be due to incomplete RNase digestion or could even represent an important modification of this protein.

The possible relationship between this estrogen-dependent p66 (it has the same apparent molecular weight as estrogen receptor) with estrogen receptor was tested by means of a specific monoclonal antibody directed against the calf estrogen receptor (20, 21). This antibody can efficiently cross-react with chicken estrogen receptor as tested previously (22). Figure 2 shows that this antibody neither prevented the formation of the protein-RNA

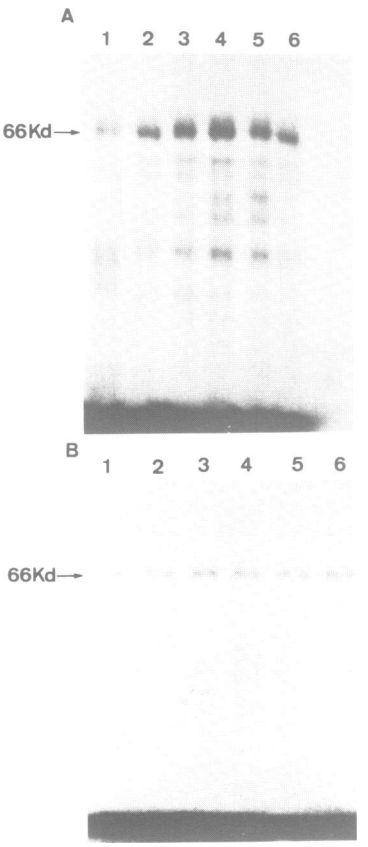

Fig. 5. Time course of p66 accumulation following estrogen administration to immature chicks. Polysomal protein (2.5 μg) from the liver of immature chicks at zero hour (lane 1), 24 hours (lane 2), 48 hours (lane 3), 3 days (lane 4), 4 days (lane 5) or 5 days (lane 6) after 17 beta-estradiol administration (A) or non treated control chicks (B) was UV crosslinked to the 5'-RNA.

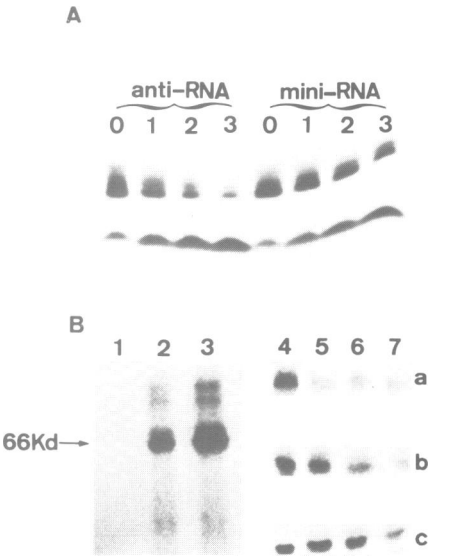

Fig. 6. *In vitro* kinetics of RNA degradation in the presence or absence of the estrogen-induced polysomal p66. (A) The mini-poly(A) vitellogenin mRNA 5'GAAUACGAAUUAUUCACCUUCGCUAUUCACCUUCGCUAUUCACCUUCGCU$(A)_{15}$3' (mini-RNA, 6×10^4 cpm) or the corresponding anti-sense poly(A) mRNA 5'GAAUACGAAUUAAAGUGGAAGCGAAAAGUGGAAGCGAAAAGUGGAAGCGA$(A)_{15}$3 (anti-RNA) were incubated with 12 μg rooster cytoplasmic extract (as the main source of RNases) and 30 μg protein from hen polysomal extract at room temperature. After 0, 10, 20 or 30 minutes (lanes 0, 1, 2, 3) of incubation aliquots were removed and treated as described in 'Materials and Methods'. (B) The 5' UTR binding activity of BSA (lane 1), hen polysomal protein (lane 2) or hen polysomal protein fractionated by Heparin-Sepharose (0.2 M KCl fraction) (lane 3) was tested by UV crosslinking assay. BSA (a), crude polysomal protein (b) or the 0.2 M Heparin-Sepharose fraction (c) were used in the decay assay performed for 0, 30, 60 and 90 minutes (lanes 4, 5, 6 and 7) as described in figure 5A.

complex when incubated with the polysomal extract before UV crosslinking (Fig.2A) nor changed the complex size in a native gel-shift assay (Fig.2B), suggesting that the p66 is not estrogen receptor.

p66 polysomal protein binds specifically to the 5'UTR of the chicken vitellogenin mRNA

The specificity of the binding of p66 polysomal protein to the 5' UTR was further tested by competition assays with various unlabeled RNAs. The polysomal extract from hen livers was incubated with 0.5 ng ^{32}P labeled 5'-RNA in the presence of increasing amounts of unlabeled competitor RNAs, followed by a UV crosslinking assay and electrophoresis separation. Transfer RNA and ribosomal RNA do not compete, even when incubated in a weight excess of 1000 times greater than the labeled probe. PolyA, TMV first ORF mRNA and AU rich RNA give only slight competition; while the 5' UTR competes completely at 50 fold weight excess (Fig.3A). These competition experiments clearly show that p66 binds to the 5' UTR in a sequence specific manner. Furthermore p66 binds very poorly to the following mutants: 5'GAAGCCUUCGCU3', 5'UUCAAGAGCGCU3' and 5'UUCACCUUAUAG3' (Fig.3B), indicating that the whole 5' UTR (5'UUCACCUUCGCT3') are required for the binding.

p66 polysomal protein is liver specific

As we already know, the vitellogenin gene is only expressed in the liver. Therefore if the function of p66 is related to vitellogenin mRNA, we would not expect to see this protein in tissues where vitellogenin gene is not expressed. To investigate the tissue specificity of p66, total polysomes from hen kidney, heart, muscle, oviduct and liver were isolated, extracted and UV crosslinked to 5'-RNA. Figure 4 shows that strong specific binding activity is observed only in liver polysome extract (lane 4), very weak activity is observed in oviduct polysome extract (lane 5), and none at all (in the p66 position) in heart (lane 1), muscle (lane 2) or kidney (lane 3) polysome extracts, indicating that p66 polysomal protein is liver specific.

In vivo kinetics of appearance of p66 RNA binding activity following a single injection of estrogen to immature chicks

Since p66 is estrogen-dependent and binds specifically to the 5' UTR of vitellogenin mRNA, it is possible that this protein may play a role in the stabilization of the mRNA. In this case, it would be interesting to see whether or not the appearance of estrogen induced p66 activity follows the *in vivo* kinetics of the rate of accumulation of vitellogenin mRNA. We and other laboratories have already shown that upon a single injection of estrogen to immature chicks, the rate of accumulation of vitellogenin mRNA rises to a maximum within 3 days and decays thereafter (6,23,24,25). To study the *in vivo* kinetics of appearance of estrogen induced p66, immature chicks were treated with estrogen. At the time indicated in figure 5A, total liver polysomes were isolated, extracted and tested for the p66 binding activity by UV crosslinking to 5'-RNA, followed by SDS-PAGE. As shown in figure 5A the p66 binding activity can be induced about 8 fold within 48 hours (compare lanes 1 and 3), reaches a

maximum in 3 days (lane 4) and subsequently declines, while in non-treated controls, it remains at a constant low level (Fig. 5B). The kinetics of p66 induction are therefore very similar to those of the vitellogenin mRNA accumulation.

Possible role of the estrogen-dependent p66

The positive correlation between the accumulation of p66 and vitellogenin mRNA suggests a role of this protein in the posttranscriptional regulation of the mRNA. It could either stimulate the initiation of translation or stabilize the mRNA against degradation. To investigate the second hypothesis we performed an *in vitro* decay assay using endogenous liver ribonucleases. A mini-vitellogenin mRNA containing three p66 binding sites was used as labeled RNA substrate and the corresponding antisense RNA was used as a control. The labeled RNAs were tested *in vitro* in the presence of the polysomal protein and ribonucleases (from rooster liver cytoplasmic extracts). For practical reasons a mini-RNA was used rather than a full size vitellogenin mRNA. *In vivo* we may have several targets for mRNA stabilization (5′ and 3′ ends), whereas with the mini-RNA we can study more easily the effect of one particular protein: the polysomal p66. Figure 6A shows that the mini-RNA can be protected against ribonuclease degradation in the presence of the polysomal protein, while the antisense RNA which does not bind p66 is not protected. Furthermore when hen liver polysomal extracts were fractionated by Heparin-Sepharose chromatography, the p66 activity was increased approximately 7 fold (Fig. 6B, compare lanes 2 and 3). At same time the purified fraction had a better protective effect against degradation (Fig. 6B, compare b and c). These results strongly suggest that the binding of estrogen-dependent p66 protects the vitellogenin mRNA from endogenous ribonuclease degradation.

DISCUSSION

Specific sequences that control the turnover of mRNA have been identified for several genes. A conserved 51 base long AU rich sequence located in the 3′ UTR of GM-CSF mRNA is involved in the selective degradation of the mRNA (26). The 3′ noncoding region of histone mRNA (27) and of transferrin receptor mRNA (28) have been shown to promote mRNA decay. In the case of transferrin receptor mRNA, iron-response elements (IRE), which form 5 stem-loop structures in the 3′ untranslated region, have been shown to be critical elements in mRNA stabilization (29, 30). In this system, a 90 kDa cytoplasmic protein binds to the IRE of the transferrin receptor mRNA and protects the mRNA from degradation by blocking endonucleolytic cleavage at an unmapped site within the 3′ untranslated domain (31,32). Sequences at the 5′ end of the mRNA are also involved in the decay process. In the *E. coli ompA* mRNA, a 5′ leader segment that includes the ribosomal binding site and the first few codons determines the half-life of the mRNA (33). In eucaryotes the 5′ UTR has been shown to be involved in the stability of c-myc mRNA (34, 35, 36) and of human H3 histone mRNA (37). 5′ exonuclease has already been found in yeast (38). Thus it is clear that 5′ UTR can influence mRNA stability.

The stability of chicken vitellogenin mRNA has been demonstrated to increase 8 to 10 fold upon estrogen treatment (6). Our *in vitro* decay assays have shown that the half-life of a mini-RNA with three p66 binding sites was approximately three times as long in the presence of p66 as in its absence, while an antisense RNA which does not contain p66 binding sites cannot be protected. The difference between the *in vivo* and *in vitro* mRNA half-life could be due to other factors which may play a role *in vivo*. Nielsen and Shapiro have shown by deletion experiments that 5′ and 3′ terminal regions of the *Xenopus* vitellogenin mRNA are required for the stabilization (13). Blume and Shapiro have examined the effect of protein synthesis and of ribosome-loading on vitellogenin mRNA stabilization, and have concluded that maintaining a high density of ribosomes on the vitellogenin mRNA is necessary for estrogen mediated stabilization (39). Our results show that a 66 kDa estrogen-dependent protein from liver polysomal extract binds with high affinity to the 5′ UTR of the chicken vitellogenin mRNA. It is conceivable that the p66 polysomal protein interacts with the 40s ribosomal subunit *in vivo* so that the binding of p66 to the 5′ UTR of the mRNA could increase the loading of ribosomes on the vitellogenin mRNA and thus protect it. In the case of *E. coli ompA* mRNA, the stabilization effect appears to involve ribosome interaction, as suggested by the fact that a hybrid mRNA with a stop codon at the end of the 5′ leader segment is not stabilized (33). Another possibility is that p66 facilitates the translation of vitellogenin mRNA. These hypotheses will be investigated with purified p66.

It has been demonstrated (8, 13) that estradiol is absolutely required for the stabilization of vitellogenin mRNA. However, under our experimental conditions no estradiol binding activity has been found in the polysomal extract, and also *in vitro* RNA binding activity of the polysomal protein to RNA *in vitro* was not changed by the addition of 10^{-7}M estradiol, suggesting that some cytosolic factors might be involved in the estrogen-mediated induction. More interestingly, a cytosolic protein of 64−66 kDa which also binds specifically to the 5′ UTR of chicken vitellogenin mRNA has been found in both rooster and hen livers (Liang; unpublished results). Whether these proteins are the same or a modified form of polysomal protein is under investigation. There are two possible mechanisms for the estrogen-mediated induction of polysomal p66: first, posttranslational modification triggered by estrogen could increase the RNA binding affinity of the protein, secondly, estrogen could increase the synthesis of p66. These hypotheses are currently under investigation.

ACKNOWLEDGEMENTS

We thank Drs. T. Hohn, A. Kraemer, H. Rothnie, and J.M. Zingg for critical reading of the manuscript, W. Zuercher for the synthesis of oligonucleotides, I. Obergfoell for help with the photography, B. Burgin for proofreading of the manuscript.

REFERENCES

1. Ryffel,G.U. (1978) *Mol.Cell.Endocrinol.*, **12**, 237−246.
2. Wahli,W., Dawid,I.B., Ryffel,G.U. and Weber,R. (1981) *Science*, **212**, 298−304.
3. Deeley,R.G., Gordon,J.I., Burns,A.T.H., Mullinix,K.P., Bina-Stein,M. and Goldberger,R.F. (1977) *J.Biol.Chem.*, **252**, 8310−8319.
4. Jost,J.P., Ohno,T., Panyim,S. and Schuerch,R. (1978) *Eur.J.Biochem.*, **84**. 355−361.
5. Wang,S.Y. and Williamy,D.L. (1980) *Biochemistry*, **19**, 1557−1563.
6. Wiskocil,R., Bensky,P., Dower,W., Goldberger,R., Gordon,J. and Deeley,R.G. (1980) *Proc.Natl.Acad.Sci.USA*, **77**, 4474−4478.
7. Brock,M.L. and Shapiro,D.J. (1983) *J.Biol.Chem.*, **258**, 5449−5455.
8. Brock,M.L. and Shapiro,D.J. (1983) *Cell*, **34**, 207−214.
9. Brawerman,G. (1989) *Cell*, **57**, 9−10.
10. Cleveland,D.W. (1989) *Curr. Opin. Cell Biol.*, **1**, 1148−1153.
11. Raghow,R. (1987) *Trends Biochem. Sci.*, **12**, 358−360.
12. Ross,J. (1989) *Sci.Am.*, **260(4)**, 48−55.

13. Nielsen,D.A. and Shapiro,D.J. (1990) *Mol.Cell.Biol.*, **10**, 371–376.
14. Jost,J.P., Saluz,H.P., Jiricny,J. and Moncharmont,M. (1987) *J.Cell. Biochem.*, **35**, 69–82.
15. Feavers,I.M., McEwan,I.J., Liang,H and Jost,J.P. (1989) *J.Biol.Chem.*, **264**, 9114–9117.
16. Murray,R.K., Suess,R. and Pitot,H.C. (1967) In Busch, H. (ed),Methods in Cancer Research Academic Press New York Vol.II, pp. 239.
17. van het Schip,F.D., Samallo,J., Broos,J., Ophuis,J., Mojet,M., Gruber,M. and AB,G. (1987) *J.Mol.Biol.*, **196**, 245–260.
18. Davis,L.G., Dibner,M.D. and Battey,J.F. (1986) Basic methods in molecular biology. Elsevier, Amsterdam, pp.152–156.
19. Moore,C.L., Chen,J. and Whoriskey,J. (1988) *EMBO.J.*, **7**, 3159–3169.
20. Moncharmont,B., Anderson,W.L., Rosenberg,B. and Parikh,I. (1984) *Biochemistry*, **23**, 3907–3912.
21. Moncharmont,B., Su,J.L. and Parikh,I. (1982) *Biochemistry*, **21**, 6916–6921.
22. Jost,J.P., Moncharmont,B., Jiricny,J., Saluz,H. and Hertner,T. (1986) *Proc.Natl.Acad.Sci.USA*, **83**, 43–47.
23. Cochrane,A.W. and Deeley,R.G. (1988) *J.Mol.Biol.*, **203**, 555–567.
24. Deeley,R.G., Udell,D.S., Burns,A.T.H., Gordon,J.I. and Goldberger,R.F. (1977) *J.Biol.Chem.*, **252**, 7913–7915.
25. Jost,J.P., Saluz,H.P., McEwan,I., Feavers,I.M., Hughes,M.J., Reiber,S., Liang,H.M. and Vaccaro,M. (1990) *Phil.Trans.R.Spc.Lond.*, B **326**, 231–240.
26. Shaw,G. and Kamen,R. (1986) *Cell*, **46**, 659–667.
27. Levine,B.J., Chodchoy,N., Marzluff,W.F. and Skoultchi,A.I. (1987) *Proc.Natl.Acad.Sci.USA*, **84**, 6189–6193.
28. Owen,D. and Kuehn,L.C. (1987) *EMBO J.*, **6**, 1287–1293.
29. Casey,J.L., Hentze,M.W., Koeller, D.M., Caughman,S.W. Rouault,T.A., Klausner,R.D. and Hardford,J.B. (1988) *Science*, **240**, 924–928.
30. Mueller,E.N. and Kuehn,L.C. (1988) *Cell*, **53**, 815–825.
31. Koeller,D.M., Casey,J.L., Hentze,M.W., Gerhardt,E.H., Chan,L.N., Klausner,R.D. and Hardford,J.B. (1989) *Proc.Natl.Acad.Sci.USA*, **86**, 3574–3578.
32. Mullner,E.N., Neupert,B. and Kuehn,L.C. (1989) *Cell*, **58**, 373–382.
33. Belasco,J.G., Nilsson,G., von Gabin,A. and Cohen,S.N. (1986) *Cell*, **46**, 245–251.
34. Eick,D., Piechaczyk,M., Henglein,B., Blanchard,J.M., Traub,B., Kofler,E., Wiest,S., Lenoir,G.M. and Bornkamm,G.W. (1985) *EMBO J.*, **4**, 3717–3725.
35. Piechaczyk,M., Bonnien,A., Eick,D., Remmers,E., Yang,J.Q., Marcu,K., Jeanteur,P. and Blanchard,J.M. (1986) *Curr.Top.Microbiol.Immunol.*, **132**, 331–338.
36. Rabbitts,P.H., Forster,A., Stinson,M.A. and Rabbitts,T.H. (1989) *EMBO J.*, **4**, 3727–3733.
37. Morris,T., Marashi,F., Weber,L., Hickey,E., Greenspan,D., Bonner,J., Stein,J. and Stein,G. (1986) *Proc.Natl.Acad.Sci.USA*, **83**, 981–985.
38. Stevens,A. (1980) *J.Biol.Chem.*, **255**, 3080–3085.
39. Blume,J.E. and Shapiro,D.J. (1989) *Nucleic Acids Res.*, **17**, 9003–9015.