Acta Chemica Scandinavica B 35 (1981) 379–385

Codon-specific Serine Transfer Ribonucleic Acid Degradation in Avian Liver during Vitellogenin Induction

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The relative rates of degradation of two major $tRNA^{Ser}$ species in rooster liver were simultaneously assessed during induction by estradiol-17β benzoate of the synthesis of a serine-rich phosphoprotein, vitellogenin. The relative rate of degradation was determined by an *in vivo* pulse-chase labeling method, which included a 24-h labeling period with [5-^{3}H]orotic acid prior to and a 6-day chase period with nonradioactive orotic acid after the administration of estrogen. $tRNA^{Ser}$(AGU,C) and $tRNA^{Ser}$(UCU,C,A) were extensively purified by chromatography on benzoylated DEAE-cellulose in the presence and absence of Mg^{2+} and their radioactivities determined. In three separate labeling experiments, the difference in radioactivity of pulse-labeled and chased $tRNA^{Ser}$(AGU,C) *vs.* that of $tRNA^{Ser}$(UCU,C,A) was approximately 2-fold, suggesting a slower rate of degradation of $tRNA^{Ser}$(AGU,C) during vitellogenin induction. Calculation of the approximate half-lives of the two $tRNA^{Ser}$ species indicates that the half-life of $tRNA^{Ser}$ (AGU,C) was increased from 3.1 days to 6.2 days during vitellogenin induction, while that of $tRNA^{Ser}$ (UCU,C,A) was essentially unchanged (2.6 days). Regulation of tRNA degradation which is possibly connected with the frequency of its use in ribosomal protein synthesis may help to explain why, in many differentiated cells, the tRNA population is adapted to the amino acid composition of the synthesized proteins.

Induction of vitellogenin synthesis in avian liver is accompanied by alterations in abundance and subcellular distribution of specific serine tRNAs.[1,2] Vitellogenin is a serine-rich yolk phosphoprotein precursor whose synthesis can be induced also in male birds by estrogens.[3] Depending on the age of the animals and the dose of estrogen, vitellogenin induction is accompanied by a 16 to 50 % increase in serine acceptance of unfractionated liver tRNA.[1,2] Of the four serine isoacceptors, the amount of $tRNA^{Ser}$(AGU,C) is specifically increased in membrane-bound polyribosomes, while the other major species, $tRNA^{Ser}$(UCU,C,A), becomes preferentially a non-ribosomal tRNA species.[1,2] The relative abundances of these serine tRNA are also altered.[1] However, double-labeling experiments, which were designed to assess their relative rates of synthesis *in vivo*, have indicated that these $tRNA^{Ser}$ species are synthesized at closely similar rates during vitellogenin synthesis.[4] The present experiments were performed to determine their relative rates of degradation under similar *in vivo* conditions. The results indicate that the relative rates of degradation of the two $tRNA^{Ser}$ species are differentially regulated during vitellogenin induction.

EXPERIMENTAL

Animals and chemicals. White Leghorn roosters were obtained from a local hatchery and fed *ad libitum* with a standard diet (Hankkija, Finland). They were used at the age of about 7 weeks. Estradiol-17β benzoate was dissolved in sesame oil and injected in several portions into leg muscles. Control roosters were injected with sesame oil. Heparinized blood samples were collected from the wing vein and analyzed for alkali-labile protein phosphate as previously described.[1]

[5-^{3}H]Orotic acid (780–890 GBq/mmol) and L-[U-^{14}C]serine (6.44 GBq/mmol) were purchased from the Radiochemical Centre, Amersham. NCS-solubilizer was from Amersham/Searle. Orotic acid was from E. Merck. Estradiol-17β benzoate (estra-1,3,5-(10)-triene-3,17β-diol 3-benzoate) was purchased from Sigma Chemical Co. Benzoylated

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DEAE-cellulose (Cellex-BD) was obtained from Bio-Rad Laboratories.

Radioactive labeling of tRNA in vivo. Roosters weighing about 0.55 kg (0.30 kg in Exp. 2) were injected i.p. with 51.4 MBq (25.9 MBq in Exp. 2) of [5-^{3}H]orotic acid in 0.9% NaCl. After 24 h, the animals received i.m. 36 mg/kg of estradiol-17β benzoate in sesame oil and i.p. 11 mg of nonradioactive orotic acid in 0.9% NaCl (pH 5.5) (6 mg of orotic acid were injected in Exp.2). During the subsequent 5 days, the animals were fed a diet containing 500 mg of orotic acid/day (300 mg of orotic acid in Exp. 2). On the eighth day, a blood sample was collected from the wing vein. The animals were decapitated and liver tRNA was prepared as described below.

Preparation of tRNA. Transfer RNA was prepared from the livers as previously described.[4] Briefly, the tissue was homogenized in a buffer containing 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), 25 mM NaCl, 5 mM $MgCl_2$ and 1 mg/ml of sodium heparin. After centrifugation at 30000 g_{max} for 20 min, the supernatant was extracted with phenol and the RNA in the aqueous phase was precipitated with ethanol. tRNA was purified by gel filtration through a column of Sephadex G-100 and deacylated at pH 9.0 for 90 min (37 °C). This tRNA preparation was used in subsequent experiments.

Preparation of aminoacyl-tRNA synthetases. Preparation of synthetases from rooster liver was carried out as previously described.[1] Synthetase preparations from estrogenized animals were used in all acylation experiments since previous results have indicated that the extent of serine acceptance and the chromatographic profile are not influenced by the source of the synthetase.[1]

Acylation of tRNA. The acceptance of radioactive serine by various tRNA preparations was determined in reaction mixtures (0.05 ml) in which the amount of tRNA was limiting. The other components of the acylation reaction mixture were: 50 mM potassium phosphate (pH 7.5), 5 mM $MgCl_2$, 1 mM spermidine, 2.5 mM ATP (pH 7.5), 1 mM CTP, 0.5 mM dithiothreitol, 0.02 mM [^{14}C]serine and 0.2 to 0.5 mg/ml of synthetase protein. The duration of the acylation incubation at 37 °C and the amount of the synthetase were varied in each case to obtain maximal acylation. After incubation, [^{14}C]Ser-tRNA was precipitated with cold 10% trichloroacetic acid, collected on glass fiber filters (Whatman GF/A), washed, dried and counted for radioactivity in a toluene-based scintillant containing 4 g of 2,5-diphenyloxazole and 50 mg of *p*-bis[2-(5-phenyloxazolyl)] benzene in 1 l of toluene.

Preparation of [^{14}C]Ser-tRNA for benzoylated DEAE-cellulose chromatography was performed in 0.5 ml reaction mixtures containing optimal amounts of tRNA and synthetase. After acylation, [^{14}C]Ser-tRNA was purified by a step-wise elution from DEAE-cellulose as previously described and used immediately for chromatography.[1]

Purification of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) by benzoylated DEAE-cellulose chromatography in the presence and absence of Mg^{2+}. These two major serine tRNA species were purified free of other tRNA species by a two-step purification procedure on benzoylated DEAE-cellulose essentially as described before.[4] The procedure is based on the selective ability of tRNA^{Ser} to absorb very tightly to benzoylated DEAE-cellulose in the absence of Mg^{2+}.[5] The tRNA^{Ser} species labeled *in vivo* with [^{3}H]orotic acid were chromatographed separately, eluted with a buffer containing ethanol, the fractions precipitated with 10% trichloroacetic acid and counted for radioactivity. Thus, a comparison can be made of the total [^{3}H]radioactivity present in each tRNA^{Ser} species from a particular liver.

RESULTS

Estrogen responsiveness of the roosters. The estrogen responsiveness was evaluated on the basis of protein-bound phosphate in plasma, increase in total liver tRNA and liver weight and appearance. Six days after the hormone injection plasma protein-bound phosphate levels were 5.8 mmol/l (Exp. 1), 3.2 mmol/l (Exp. 2) and 4.7 mmol/l (Exp. 3) (essentially no phosphoprotein was present in plasma samples from control animals). When compared to control animals, the amount of total liver tRNA increased about 1.7-fold, liver weight increased about 1.8-fold and its color changed from red-brown to yellow. These changes were indicative of an induction comparable to that reported in previous studies.[1,2,4]

Purification of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A). Four peaks are observed when rooster liver [^{14}C]Ser-tRNA is chromatographed on benzoylated DEAE-cellulose in the presence of 10 mM Mg^{2+}.[1,2] Binding studies with *E. coli* ribosomes and the six trinucleotide codons assigned to serine have indicated that one of the major [^{14}C]Ser-tNRA species binds with AGU and AGC, while the other major [^{14}C]Ser-tRNA species binds with UCU, UCC and UCA.[6]

The assessment of the relative rates of degradation of the individual tRNA^{Ser} species is dependent on the purity of the tRNA^{Ser} preparations. Liver tRNA was pulse-labeled with [5-^{3}H]orotic acid and the tRNA was isolated after

24 h or 7 days and purified through the Sephadex G-100 step. It was subsequently chromatographed on benzoylated DEAE-cellulose (Fig. 1) in the presence of 10 mM Mg^{2+} and the fractions corresponding to tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) were separately pooled, desalted and concentrated in small DEAE-cellulose columns. A sample of [^{14}C]Ser-tRNA was chromatographed under identical conditions to identify the individual tRNA^{Ser} species. The two major tRNA^{Ser} species

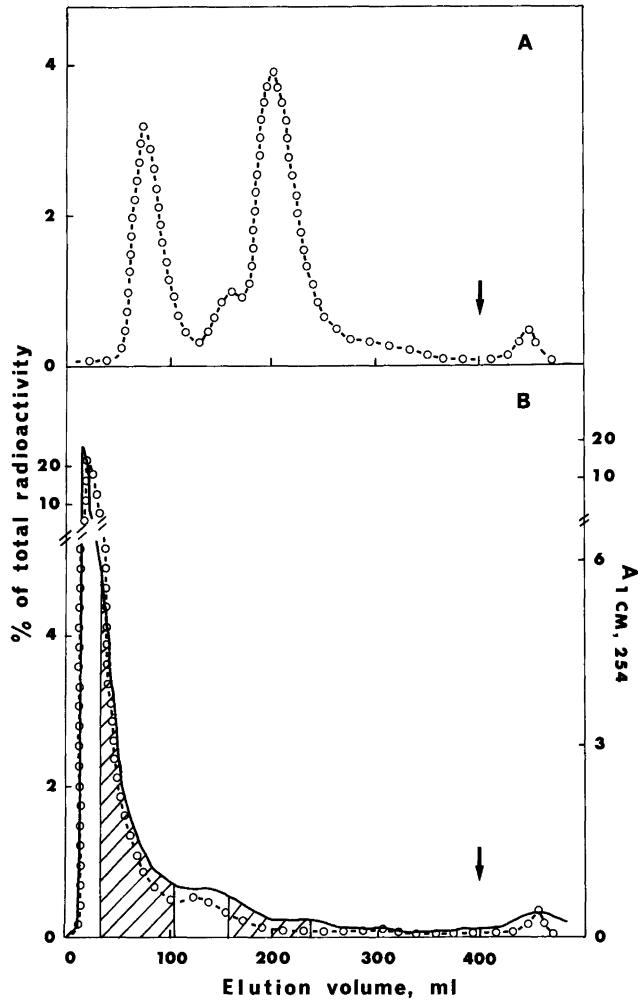

Fig. 1. Chromatography of *in vivo* labeled rooster liver tRNA on benzoylated DEAE-cellulose in the presence of Mg^{2+}. 290 A_{260} units of unacylated liver tRNA pulse-labeled with [5-^{3}H]orotic acid for 24 h and chased with nonradioactive orotic acid for 6 days were chromatographed on benzoylated DEAE-cellulose as follows. Columns were eluted at room temperature with 400 ml of a linear gradient of 0.6 – 1.1 M NaCl containing 10 mM $MgCl_2$ and 5 mM sodium acetate (pH 4.43). Elution was continued (arrow) with 80 ml of a linear gradient of 1.1 – 1.5 M NaCl containing 10 mM $MgCl_2$ and 5 mM sodium acetate (pH 4.43). The 1.5 M NaCl buffer also contained 14 % ethanol. (A) Chromatography of marker [^{14}C]Ser-tRNA. A similar column and identical conditions were used as in B. (○) [^{14}C]Serine radioactivity. (B) Chromatography of endogenously labeled [^{3}H]tRNA. Shaded areas represent fractions which were pooled for further purification of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A). (–) Absorbance at 254 nm; (○) [^{3}H]-radioactivity determined from 1-ml aliquots of each fraction.

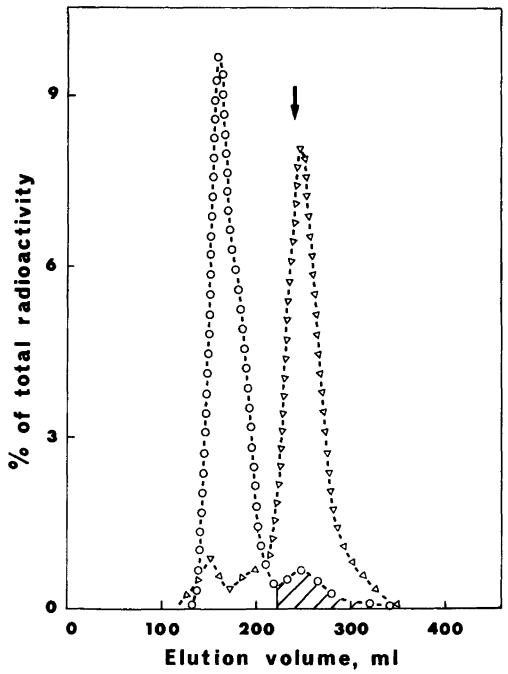

Fig. 2. Purification of $tRNA^{Ser}$(AGU,C) on benzoylated DEAE-cellulose chromatography in the absence of Mg^{2+}. Pooled, desalted and concentrated $tRNA^{Ser}$(AGU,C) from Fig. 1B was chromatographed as follows. The column was eluted at 4 °C with 400 ml of a linear gradient of 0.2 −2.0 M NaCl containing 10 mM sodium acetate (pH 4.5) and 1 mM mercaptoethanol. After 240 ml of the eluate had been collected, ethanol was added (arrow) to the 2 M gradient solution to a final concentration of 7%. (○) Each fraction was separately precipitated and counted for [^{3}H]radioactivity. The total [^{3}H]radioactivity indicated by the shaded area was used in further calculations (see Table 1). (△) Marker [^{14}C]-Ser-tRNA (AGU,C) was chromatographed on a parallel column.

were further purified on benzoylated DEAE-cellulose columns in the absence of Mg^{2+} (Figs. 2 and 3). Under these conditions, serine tRNA are strongly attached to the ion exchange material and can be eluted with an ethanol-NaCl gradient, while most other tRNAs are eluted with the NaCl-gradient alone (with some batches of benzoylated DEAE-cellulose, the elution of $tRNA^{Ser}$ begins slightly before the addition of ethanol).[4,5] This purification procedure has been shown to yield avian liver $tRNA^{Ser}$(AGU,A) and $tRNA^{Ser}$-

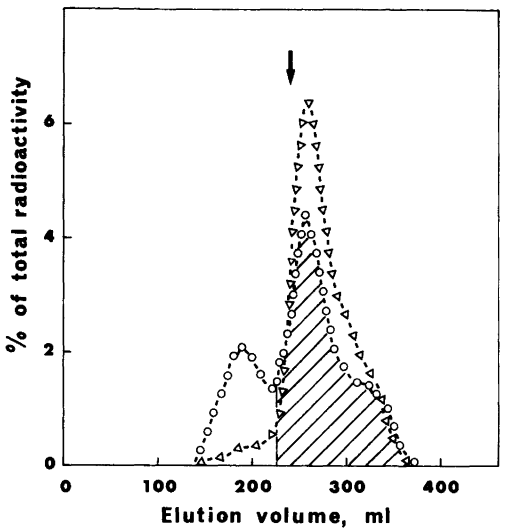

Fig. 3. Purification of $tRNA^{Ser}$(UCU,C,A) on benzoylated DEAE-cellulose in the absence of Mg^{2+}. [^{3}H]tRNA from the second pool in Fig. 1B was chromatographed and counted for radioactivity as in Fig. 2. (○) [^{3}H]radioactivity. The total [^{3}H]radioactivity indicated by the shaded area was used in further calculations (see Table 1). (△) Marker [^{14}C]Ser-tRNA (UCU,C,A) was chromatographed on a parallel column.

(UCU,C,A) which are more than 90 % pure.[4]

Comparison of the relative rates of degradation of $tRNA^{Ser}$ species during vitellogenin induction. Table 1 shows the radioactivity values and ratios of the two major $tRNA^{Ser}$ species isolated and extensively purified as described above. The absolute radioactivity values vary in these experiments, which is probably due to biological, technical and individual sources of variation connected with the *in vivo* labeling procedure. However, the radioactivity values in a particular experiment represent labeling and isolation of the two $tRNA^{Ser}$ species under identical conditions.

Comparison of the isotope ratios of $tRNA^{Ser}$(AGU,C) and $tRNA^{Ser}$(UCU,C,A) from control and induced animals indicates that a differential regulation of degradation of these $tRNA^{Ser}$ species takes place during vitellogenin induction. The difference in radioactivity of pulse-labeled and chased $tRNA^{Ser}$(AGU,C) *vs.* that of $tRNA^{Ser}$(UCU,C,A) was 1.97, while in control animals the ratio was 0.97. This suggests that the rate of degradation of $tRNA^{Ser}$(AGU,C) relative to

Table 1. Relative degradation rates of purified tRNA^{Ser} species during vitellogenin induction. The labeling procedure included a 24-h pulse-labeling with [5-^{3}H]orotic acid prior to, and a 6-day chase with nonradioactive orotic acid after the administration of estrogen. The tRNA^{Ser} species were purified by chromatography on benzoylated DEAE-cellulose as shown in Figs. 1 to 3. The radioactive labeling in Exps. 1 and 3 was performed with single animals while in Exp. 2 two animals were used in each group.

Experiment	tRNA radioactivity (dpm)					
	Pulse (24 h)		Chase (6 d)			
			Control		Induced	
(1) $tRNA^{Ser}_{AGU,C}$	100100	(0.52)[a]	27200	(0.79)	68300	(1.50)
$tRNA^{Ser}_{UCU,C,A}$	193600		34600		45600	
(2) $tRNA^{Ser}_{AGU,C}$	105900	(0.83)	34700	(1.15)	44400	(1.75)
$tRNA^{Ser}_{UCU,C,A}$	126900		30100		25400	
(3) $tRNA^{Ser}_{AGU,C}$	113000	(0.99)	19900	(0.97)	34800	(2.66)
$tRNA^{Ser}_{UCU,C,A}$	113900		20600		13100	
		(0.78)		(0.97)		(1.97)

[a] The radioactivity ratios of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) are indicated in parentheses.

that of tRNA^{Ser}(UCU,C,A) is slowed down during vitellogenin induction. Moreover, calculation of the approximate half-lives of these two tRNA^{Ser} species from the pulse-labeled (24 h) and pulse-labeled and chased (6 d) tRNA^{Ser} radioactivity values indicates that the half-life of tRNA^{Ser}(AGU,C) was increased from 3.1 to 6.2 days (+100 %) while the half-life of tRNA^{Ser}(UCU,C,A) was essentially unchanged (2.6 days) during vitellogenin induction.

DISCUSSION

The concentration of tRNA in the cell is not much higher than the concentration of ribosomes. There are typically 10–15 tRNAs per ribosome in *E. coli*, while in rabbit reticulocytes there are 4–5 tRNAs per ribosome.[7] These estimations and the alterations in the tRNA population under various physiological and pathological conditions have led to suggestions that tRNA may play a regulatory role in protein synthesis.[7] Control of translation by the availability of tRNA may act both negatively and positively. Limiting amounts of certain tRNA species may delay translation at codons where they are required. On the other hand, enrichment of certain tRNA species may facilitate the synthesis of proteins whose translation would have been limiting previously.[7] It has been frequently found that in cells committed to synthesis of one or only a few types of proteins, the tRNA population correlates with the amino acid composition of the synthesized proteins. This process has been called "functional adaptation of tRNA population" or "tRNA specialization".[7,8] Such observations have been made *e.g.* in silk gland of *Bombyx mori* (fibroin synthesis), in chick embryo tissues and rat granulation tissue (collagen synthesis), in cell lines synthesizing specific immunoglobulins, in rabbit reticulocytes (hemoglobin synthesis) and in avian liver stimulated by estrogens (vitellogenin synthesis).[7–9]

Induction of vitellogenin synthesis by estrogens is an attractive model system for the study of mechanism of action of steroid hormones, since neither vitellogenin nor vitellogenin messenger RNA is detectable in the livers of normal roosters.[3] Depending on the age of the animals and the dose of estrogen, the hepatic synthesis of vitellogenin is accompanied by a 16 to 50 % increase in the serine acceptance of hepatic tRNA.[1,2,10] Essentially no cell proliferation takes place during vitellogenin induction in the liver.[3] Total liver tRNA increases 1.3- to 2.0-fold during the induction.[1,2,10] Of the four serine isoacceptors, tRNA^{Ser} recognizing UCU, UCC and UCA is relatively enriched in the tRNA

preparation containing predominantly cytosolic tRNA, while the $tRNA^{Ser}$ species recognizing AGU and AGC is enriched in the membrane-bound ribosomes.[2]

No appreciable change in the relative rate of unfractionated $tRNA^{Ser}$ synthesis has been found in double-labeling experiments during a period when the level of $tRNA^{Ser}$ continues to increase.[10] Further, when the rates of synthesis of the two major $tRNA^{Ser}$ species were determined separately, they were found to be closely similar.[4] In contrast, the present results indicate a decline in the rate of degradation of $tRNA^{Ser}$(AGU,C) relative to that of $tRNA^{Ser}$(UCU,C,A) during the induction of vitellogenin synthesis. This finding is in agreement with our previous results on the abundance of these two $tRNA^{Ser}$ species during the primary and secondary stimulation by estrogens, which showed that the relative abundance of $tRNA^{Ser}$(UCU,C,A) is decreased during the declining part of the plasma vitellogenin curve and immediately after the vitellogenin induction.[1] Taken together, our studies have indicated that the $tRNA^{Ser}$ isoacceptors adapt to vitellogenin synthesis by selective changes in the rate of degradation in addition to a general increase in tRNA transcription.

There are only a few other studies on the regulation of synthesis and degradation of specific tRNA species in eukaryotic cells. In the silk gland of the silkworm *Bombyx mori*, the levels of a number of tRNA species are adapted to the amino acid composition of fibroin.[9] Starvation and refeeding experiments have suggested that the rates of synthesis of different tRNAs vary according to the intracellular levels of each tRNA species and that specific tRNA levels in this system are controlled at the transcriptional or pre-tRNA maturation level.[11] This conclusion has been questioned, however, on the basis of observations made in Friend leukemia cells during starvation for a specific amino acid.[12]

Friend leukemia cells respond to deprivation of specific amino acids by increasing their relative concentration of the cognate tRNA.[12,13] Studies with $tRNA^{Phe}$ have recently indicated that deprivation of phenylalanine has no effect on the relative rate of synthesis of $tRNA^{Phe}$, but that it induces a decline in the relative rate of degradation of $tRNA^{Phe}$, which can explain the increase in the relative abundance of $tRNA^{Phe}$ in these cells.[12] A suggestion has been made that the extent of aminoacylation might play a role in controlling tRNA concentrations.[12,13] If an amino acid is not being used for protein synthesis, its cognate tRNA tends to become fully aminoacylated, but if an amino acid is in great demand, the aminoacylation level of its cognate tRNA tends to decrease.[12,13] Accordingly, the degradation of a particular tRNA species would be inhibited when the extent of deacylation of that tRNA species is increased. The present findings are in general agreement with this hypothesis and extend it to isoaccepting tRNA species, since our results have indicated that the relative abundance of the particular $tRNA^{Ser}$ species, which is preferentially bound to ribosomes is increased and its degradation is simultaneously slowed down. Regulation of tRNA degradation, which is possibly connected with the frequency of its use in ribosomal protein synthesis, may help to clarify the mechanism of "functional adaptation of tRNA population".[7,8] The molecular mechanisms involved in the process of tRNA degradation and its regulation are unknown, although a change in ribonuclease and ribonuclease inhibitor activity has been observed in avian liver during vitellogenin induction.[14,15] Interestingly, a stabilization of vitellogenin mRNA and apoVLDLII mRNA, which is different from that of albumin mRNA, has been recently observed during vitellogenin induction.[16]

Acknowledgements. This work was supported in part by grants from the Sigrid Jusélius Foundation, the Research Council for Natural Sciences of the Academy of Finland and the Emil Aaltonen Foundation. The technical assistance of Mrs. Maija Hiltunen is gratefully acknowledged.

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Received February 16, 1981.