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Subcellular Redistribution of Seryl-Transfer RNA during Estrogen-Induced Phosvitin Synthesis and Specificity of the Estrogen Effect†

Pekka H. Mäenpää‡ and Merton R. Bernfield*

ABSTRACT: The estrogen-induced hepatic synthesis of the serine-rich protein, phosvitin, in chickens is accompanied by an increase in the serine acceptance of total hepatic tRNA, which is limited to two serine isoacceptors. To investigate the role of the tRNA alterations in the synthesis of phosvitin, the relative amounts of various seryl-tRNA species in isolated nuclei and in free and membrane-bound ribosomes were determined. Estrogen treatment causes a shift in the subcellular distribution of hepatic seryl-tRNA. Of the four serine isoacceptors, the amount of $tRNA^{Ser}_{AGU,AGC}$ was specifically increased in nuclei and in membrane-bound ribosomes. Changes in total hepatic tRNA occurring during physiologic estrogenization were compared with those occurring by varying steroid hormones, times after estrogen administration, estrogen doses, animal ages, and tisssue types. The changes observed demonstrate that the seryl-tRNA alterations are closely correlated with the synthesis of phosvitin. The coincident change in seryl-tRNA levels and in phosvitin synthesis, together with the specific change in cellular localization, suggests that the amount and subcellular distribution of each tRNA species are separately controlled in a manner dependent upon its frequency of use in translation.

Alterations in the levels of specific transfer RNA species have been noted in association with changes in the rate of specific protein synthesis (for a recent review, see Garel, 1974). A well-documented example of this phenomenon is the increase in levels of liver seryl-tRNA associated with increased rates of hepatic synthesis of the serine-rich yolk protein, phosvitin, in laying hens and in chicks and roosters after estrogen administration (Carlsen et al., 1964; Mäenpää and Bernfield, 1969; Beck et al., 1970). Increased tRNA acceptance of additional amino acids has also been found (Busby and Hele, 1970; Klyde and Bernfield, 1973) and it has not been demonstrated whether the increase in seryl-tRNA level is related in a physiologically significant way to the augmented synthesis of phosvitin. Indeed, no change in the amount of liver seryl-tRNA was observed during the estrogen induction of a phosvitin-like protein in *Xenopus* liver (Wittliff et al., 1972), and despite the increase in total liver seryl-tRNA induced in chicks by estrogen, no change was observed in the *rate* of total liver $tRNA^{Ser}$ synthesis relative to the rate of total tRNA synthesis (Klyde and Bernfield, 1973).

In cell-free systems the relative abundance of a specific aminoacyl-tRNA controls the rate of messenger translation (Anderson, 1969), and a similar relationship has been observed in experiments using tissue explants (Sharma et al., 1973). The present experiments were performed to assess the mechanism by which estrogen evokes seryl-tRNA changes and the possible role of these changes in phosvitin synthesis. The distribution of seryl-tRNA was assessed in tRNA derived from isolated hepatic nuclei and from free and membrane-bound ribosomes. The pattern of total hepatic seryl-tRNA alteration was established in laying hens to determine the nature of the seryl-tRNA alterations during physiological estrogenization and phosvitin synthesis. This specific pattern of seryl-tRNA levels was then assessed in several other circumstances to determine whether the pattern of seryl-tRNA levels changes coincidentally with increased phosvitin synthesis.

Materials and Methods

Animals. White leghorn roosters and 1-week-old male chicks were obtained from local hatcheries and fed *ad libitum.* Hormones were dissolved (by heating if necessary) in sesame oil and injected into leg muscles. Heparinized blood samples were analyzed for protein phosphate as previously described (Mäenpää and Bernfield, 1969).

Chemicals. Estradiol benzoate [estra-1,3,5(10)-triene-3,17β-diol 3-benzoate] and ethynyl estradiol [estra-

† From the Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland (P.H.M.), and the Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305 (M.R.B.). *Received March 21, 1975.* Part of this study was performed during the visit of P.M. to the Stanford University School of Medicine. Research was supported in part by grants from the National Institutes of Health (GM15086) and from the Sigrid Juselius Foundation, Finland.

‡ Present address: Department of Biochemistry, University of Kuopio, SF-70101 Kuopio 10, Finland.

1,3,5(10)-trien-17α-ethynyl-3,17β-diol] were from Nutritional Biochemicals Corp.; 17α-estradiol [estra-1,3,5(10)-triene-3,17α-diol], estriol [estra-1,3,5(10)-triene-3,16α,17-β-triol], and testosterone propionate [4-androsten-17β-ol-3-one 17-propionate] were from Schwarz/Mann; estrone [estra-1,3,5(10)-trien-3-ol-17-one] was from E. Merck, Darmstadt, and hydrocortisone acetate [pregn-4-ene-11β,17α,21-triol-3,20-dione 21-acetate] was from Merck, Sharp & Dohme. [^{3}H]Serine (specific activity 2230 Ci/mol) was from New England Nuclear. Benzoylated DEAE-cellulose was from Schwarz/Mann (lot W2049).

Preparation of tRNA. Transfer RNA was prepared essentially as previously described (Mäenpää and Bernfield, 1969). Animals were sacrificed by decapitating (chicks) or by injecting air into the wing vein (roosters). Livers were quickly removed, weighed, and chilled in cold saline; 1.5 volumes of cold buffered salt solution (1.0 *M* NaCl–5 m*M* NaEDTA–0.1 *M* Tris-HCl (pH 7.5)) (Brunngraber, 1962) was added and the tissue was minced with scissors. Water-saturated, cold phenol (1.5 volumes) was added to the mixture and after disruption for 15–20 min at 4° in a Waring Blendor (with intermittent cooling in ice) the mixture was centrifuged at 10,000*g* for 10 min at 4°. The aqueous layer was removed and extracted two more times with an equal volume of phenol for 45 min at 4°. The RNA was precipitated overnight at −20° by adding 0.1 volume of 2 *M* potassium acetate (pH 5.0) and 2.5 volumes of 95% ethanol. The precipitate was collected by centrifugation, dried, and deacylated in 0.1 *M* Tris-HCl (pH 8.8–9.0) for 60 min at 37°. RNA was then precipitated overnight as described above and redissolved in 0.3 *M* sodium acetate (pH 7.0). The tRNA was purified by successive 2-propanol precipitations (Zubay, 1962). 5S RNA was removed by passing the RNA through a column of Sephadex G-100 (2.5 × 100 cm) in a buffer containing 0.5 *M* NaCl, 10 m*M* $MgCl_2$, and 5 m*M* sodium acetate (pH 4.5) at 24°. The absorbancy at 254 nm was monitored and the major A_{254}-absorbing peak corresponding to tRNA was precipitated with ethanol, redissolved in water, and stored at −20° or lyophilized.

RNA from liver nuclei was prepared according to the method of Dingman et al. (1969). On electron microscopy isolated nuclei were devoid of attached cytoplasmic ribosomes. Low molecular weight RNA was further purified after deacylation at pH 8.8 by successive 2-propanol precipitations as described above. The RNA preparations were subjected to electrophoresis on 10% polyacrylamide gels according to Peacock and Dingman (1967) and scanned at 254 nm, using an ISCO gel scanner. The 5S to 4S RNA ratio was significantly higher in the nuclear RNA preparations as compared to a similar preparation from whole liver (Figure 1).

Transfer RNA from membrane-bound and free ribosomes was prepared as follows. Membrane-bound ribosomes were isolated from microsomal membranes precipitated by the Ca^{2+} method (Kamath and Rubin, 1972). One percent sodium deoxycholate plus 1% Triton X-100 (final concentration) were added and the detached ribosomes were further purified by centrifugation for 24 hr at 105,000*g* through a cushion of 2 *M* sucrose containing 0.05 *M* Tris-HCl (pH 7.5), 0.025 *M* KCl, and 0.005 *M* $MgCl_2$ (Blobel and Potter, 1967). Free ribosomes were prepared by homogenizing the liver in 2 volumes of 0.25 *M* sucrose containing 0.05 *M* Tris-HCl (pH 7.5), 0.025 *M* KCl, 0.005 *M* $MgCl_2$, and 0.1 mg/ml of sodium heparin. After centrifugation (twice) for 10 min at 10,000*g*, the free ribosomes

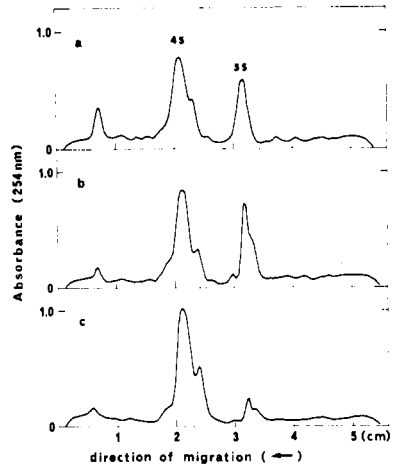

FIGURE 1: Polyacrylamide gel electrophoresis of low molecular weight RNA derived from (a) nuclei of control rooster liver, (b) nuclei of an estradiol-treated (3 days, 10 mg/kg) rooster liver, and (c) control rooster liver (whole tissue).

from the supernatant were collected by centrifugation for 24 hr through a 2 *M* sucrose cushion as described above. Transfer RNA (peptidyl-tRNA, aminoacyl-tRNA, tRNA) was prepared from the ribosome pellets by the method of Slabaugh and Morris (1970). Briefly, ribosomes were dissolved in 4 *M* urea plus 3 *M* LiCl and the resulting rRNA precipitate was removed by centrifugation. LiCl was removed by a Bio-Gel P-10 filtration and the ribosomal proteins were removed by a DEAE-cellulose chromatography. Transfer RNA was deacylated in conditions which enhance hydrolysis of N-substituted aminoacyl residues (0.1 *M* Tris-HCl (pH 9.0)–10 m*M* $MgCl_2$, 90 min at 37°) [Novogrodsky, 1971], passed through a Sephadex G-25 column, and lyophilized.

Preparation of Aminoacyl-tRNA Synthetases. Preparation of synthetases was carried out as previously described (Mäenpää and Bernfield, 1969). Synthetase preparations from untreated chicks and roosters were used in all acylations because previous results indicated that the extent of serine acceptance and the chromatographic profile are not influenced by the source of synthetase (Mäenpää and Bernfield, 1969; see also Le Meur et al., 1974).

Acylation of tRNA and Chromatographic Analysis of Seryl-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 (Mäenpää and Bernfield, 1969). The other components of the acylation reaction mixture were: 0.1 *M* Tris-HCl (pH 7.4), 10 m*M* $MgCl_2$, 10 m*M* KCl, 0.4 m*M* dithiothreitol, 5 m*M* ATP (pH 7.4), 1 m*M* CTP, 0.1 m*M* NaEDTA, 0.1 m*M* 19 nonradioactive amino acids, 0.02 m*M* radioactive serine, and synthetase. The duration of the acylation incubation at 37° and the amount of the tRNA and synthetase were varied in each case to obtain maximal acylation. Radioactive seryl-tRNA was precipitated after incubation with cold 5% trichloroacetic acid, collected on glass fiber filters (Gelman, Type A), dried, and counted in toluene-based scintillant.

Preparation of seryl-tRNA for benzoylated DEAE-cellulose chromatography was performed in 0.5-ml reaction mixtures containing optimal amounts of tRNA and synthetase protein. After incubation, the reaction mixture was treated with an equal volume of cold, water-saturated phenol, and, after a low-speed centrifugation, the aqueous phase was

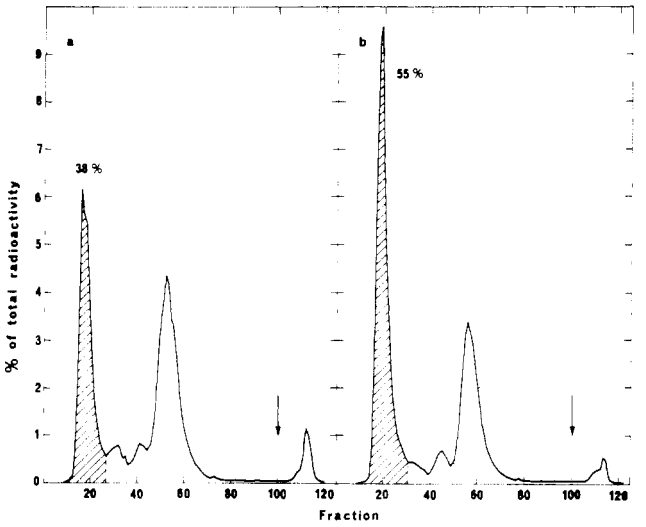

FIGURE 2: Elution profiles on benzoylated DEAE-cellulose of nuclear [^{3}H]seryl-tRNA derived from liver of (a) control rooster, and (b) rooster 3 days after injection of estradiol-17β (10 mg/kg). Each tRNA was acylated with a synthetase derived from untreated rooster liver. Columns were eluted at room temperature with a linear gradient of 0.6–1.2 *M* NaCl containing 10 m*M* $MgCl_2$ and 5 m*M* sodium acetate (pH 4.45). Elution was continued (arrow) with a linear gradient of 1.2–1.5 *M* NaCl containing 10 m*M* $MgCl_2$ and 5 m*M* sodium acetate (pH 4.45). The 1.5 *M* NaCl buffer also contained 14% ethanol. The shaded area and the indicated percentage of total seryl-tRNA represents peak I. See Figure 5 and Mäenpää and Bernfield (1969, 1970) for chromatographic pattern of total hepatic seryl-tRNA from control and estrogen-treated roosters.

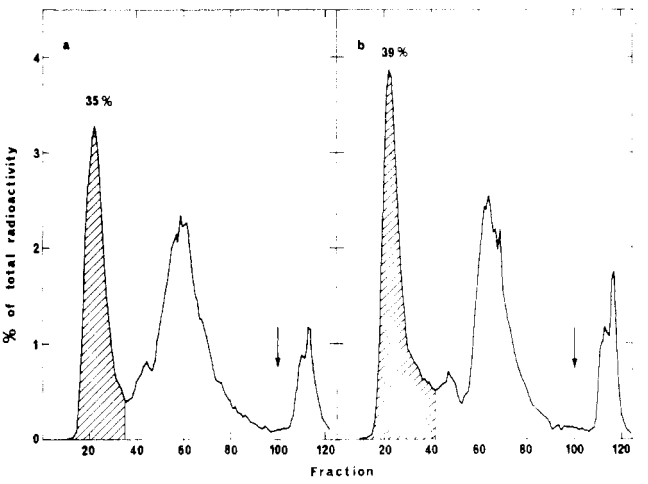

FIGURE 3: Elution profiles on benzoylated DEAE-cellulose of free ribosomal [^{3}H]seryl-tRNA derived from liver of (a) control rooster, and (b) rooster 3 days after injection of estradiol-17β (10 mg/kg). Acylation, chromatography conditions, and representation of data as in the legend to Figure 2.

passed through a Sephadex G-25 column. The fractions containing radioactive tRNA were collected, pooled, lyophilized, and stored at 4° until used in chromatography. The conditions for chromatography of seryl-tRNA have been described previously (Mäenpää and Bernfield, 1969).

Determination of Absolute Amounts of Different Seryl-tRNA Fractions. Calculations of the absolute amounts of seryl-tRNA fractions in different tRNA preparations were based upon serine accepting capacities of Sephadex G-100 purified unfractionated tRNA assuming that 1 A_{260} unit is equivalent to 1660 pmol of tRNA, and upon radioactivities under each chromatographic peak with [^{3}H]seryl-tRNAs chromatographed on benzoylated DEAE-cellulose. In each case [^{3}H]seryl-tRNA was resolved in four chromatographic fractions qualitatively similar to those published previously (Mäenpää and Bernfield, 1969). Therefore, all chromatograms are not shown in detail. Peak I binds to *Escherichia coli* ribosomes in the presence of AGU and AGC, peak II with UCG, and peak III with UCU, UCC, and UCA (Mäenpää and Bernfield, 1970; see also Hatfield and Portugal, 1970; Staehelin, 1971). Peak IV does not bind with any of the serine-specific triplets, but is probably analogous to the species that binds with UGA (Hatfield and Portugal, 1970).

Results

Subcellular Distribution of Seryl-tRNA

Nuclear Seryl-tRNA Pattern during Phosvitin Induction. Substantial differences were observed in the seryl-tRNA derived from isolated nuclei of control and estrogen-treated rooster livers. Nuclei from control animals yielded 10 A_{260} units of 4S RNA, with a serine acceptance of 3.8%, while those from estrogenized birds yielded 32 A_{260} units with an acceptance of 4.4%. Therefore, 3 days following estrogen treatment, there was a 16% increase in the relative concentration of nuclear seryl-tRNA and a 3.7-fold increase in the total amount per liver. The distribution of seryl-tRNA peaks in nuclear tRNA from control birds is similar to that of total hepatic tRNA from these animals (Figure 2). On the other hand, in estrogen-treated animals, the pattern of nuclear seryl-tRNA differs significantly from that of total hepatic tRNA. Instead of a reduction in the relative amount of peak I, nuclear seryl-tRNA shows a marked increase in this peak. Based upon these data, estrogen-evoked changes in the absolute amounts of the nuclear seryl-tRNA fractions were calculated: nuclear seryl-tRNA peak I increased from 0.24 to 1.29 nmol (5.4-fold), while the three other peaks together increased from 0.39 to 1.05 nmol (2.7-fold).

Seryl-tRNA from Free and Membrane-Bound Ribosomes. The yields of tRNA from free and membrane-bound ribosomes were low. Final yield after purification of free ribosomal tRNA from control birds was 10.6 A_{260} per liver (mean net weight of 39 g; $N = 2$) and that from estrogen-treated birds was 11.2 A_{260} per liver (mean net weight of 77 g; $N = 3$). The corresponding yields for membrane-bound ribosomal tRNA were 4.7 and 7.9 A_{260} per liver. For comparison, the yield of highly purified total tRNA per 40 g of liver averaged ca. 200 A_{260} for control birds and ca. 250 A_{260} for estrogen-treated animals. Due to the limited amount of material, comparisons of the serine acceptance of unfractionated ribosomal tRNA could not be made with confidence. Consequently, calculations of the absolute amounts of ribosomal seryl-tRNA in whole liver were not possible.

Comparison of the *relative* amounts of ribosomal seryl-tRNAs by their chromatographic distribution revealed significant differences between control and estrogen-treated birds (Figures 3 and 4). In control animals, the patterns of seryl-tRNA from free and membrane-bound ribosomes were nearly identical with that of total hepatic seryl-tRNA. Seryl-tRNA from free ribosomes of estrogen-treated birds gave a similar pattern, with peak I slightly increased. However, the relative increase in peak I of membrane-bound ribosomal tRNA from estrogen-treated birds was striking. This pattern is markedly distinct from that of the total hepatic seryl-tRNA from these animals in that an increase

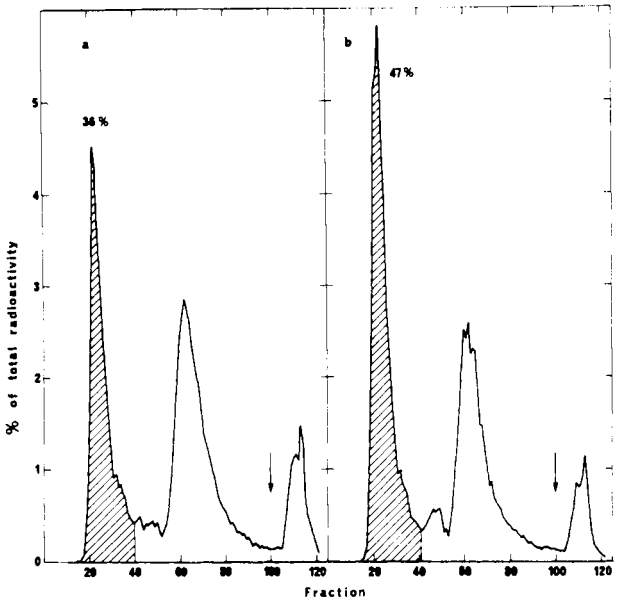

FIGURE 4: Elution profiles on benzoylated DEAE-cellulose of membrane-bound ribosomal [^{3}H]seryl-tRNA derived from liver of (a) control rooster, and (b) rooster 3 days after injection of estradiol-17β (10 mg/kg). Acylation, chromatography conditions, and representation of data as in the legend to Figure 2.

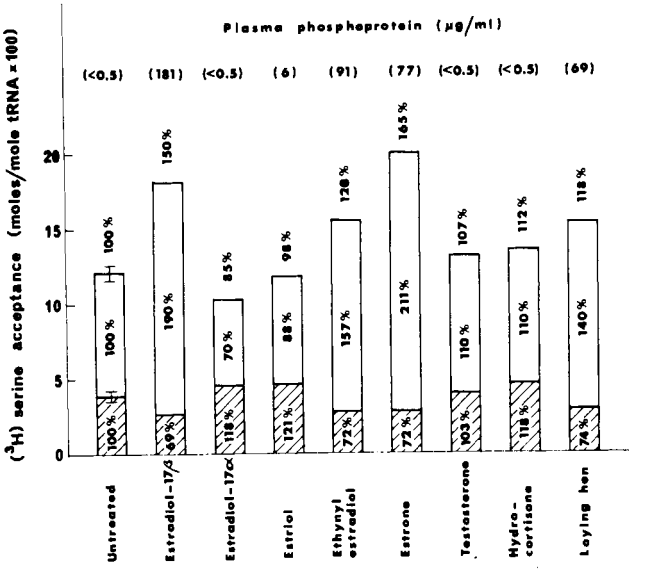

FIGURE 5: Liver [^{3}H]seryl-tRNA and plasma phosphoprotein levels in laying hens and in roosters 3 days after injection with estrogens or unrelated steroids. In each case of hormone treatment, 10 mg/kg of the hormone was injected intramuscularly in sesame oil. Each tRNA preparation was acylated with a synthetase preparation derived from untreated rooster liver. Percentages of the values for untreated roosters (mean ±SEM for four observations) are given for total seryl-tRNA (whole bar), for the sum of peaks II, III, and IV (unshaded area), and for peak I (shaded area).

rather than a decrease was observed for peak I. This difference was not due to the urea–LiCl procedure used to prepare the ribosomal tRNA since identical treatment of total tRNA did not change its chromatographic distribution.

Relationship between Phosvitin Synthesis and Seryl-tRNA Alterations

Seryl-tRNA Pattern from Laying Hen Liver. The chromatographic pattern (not shown) and the absolute amounts of liver seryl-tRNAs derived from a laying hen were almost identical with those obtained from chick and rooster liver after estradiol-17β treatment (Figure 5). The serine acceptance of unfractionated tRNA was increased 18% as compared to control roosters and 30% as compared to control chicks (cf. Figure 8), while the amount of seryl-tRNA peak I decreased 25–30% in all three cases. These data suggest that pharmacologic doses of estrogen elicit a response qualitatively and quantitatively similar to that seen under physiological conditions in laying hens.

Hormonal Specificity of Phosvitin Induction and Seryl-tRNA Alterations. The time course of the appearance of phosphoprotein in plasma after a single intramuscular injection of estradiol in chicks and roosters is shown in Figure 6. Following an equivalent dose of hormone, the extent and duration of elevated plasma phosphoprotein levels were much more limited in chicks as compared to roosters. Even a fivefold increase in estradiol dosage in chicks did not increase plasma phosphoprotein to levels found in roosters.

Plasma phosvitin and hepatic seryl-tRNA levels after injection of various estrogens, testosterone, and hydrocortisone are shown in Figure 5. Estradiol-17β, was the most effective inducer of phosvitin synthesis; estrone (which is converted to estradiol in avian liver [Gilbert, 1971]) and ethynyl estradiol were slightly less effective. Estriol (which is not converted to estradiol [Gilbert, 1971]) produced only a minimal response. Testosterone, hydrocortisone, and estradiol-17α produced no increase in plasma phosphoprotein.

Comparison of the levels of hepatic seryl-tRNA and plasma phosvitin indicates that both variables change coinci-

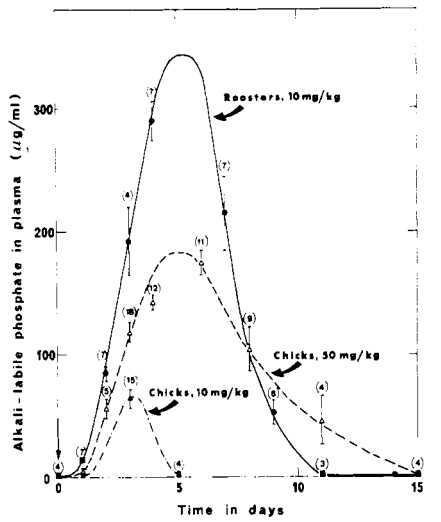

FIGURE 6: Plasma phosphoprotein levels in week-old male chicks and adult roosters after estradiol treatment. Alkali-labile phosphorus in lipid-free plasma protein was measured at designated times after an intramuscular injection of estradiol-17β benzoate in sesame oil. Number of observations are given in parentheses. Where indicated, values are means ±SEM. Data for roosters taken, in part, from Mäenpää and Bernfield (1969).

dentally, and that the changes are of similar magnitude. Of the estrogens, estradiol-17β and estrone caused the greatest change in seryl-tRNA pattern, and ethynyl estradiol was slightly less effective. Estradiol-17α and estriol resulted in a slightly changed seryl-tRNA pattern which, in contrast to that observed for estradiol-17α, showed lower total serine acceptance and higher levels of peak I than the untreated control. Testosterone and hydrocortisone somewhat increased the serine accepting capacity of unfractionated liver tRNA, but this was not a selective increase of any of the seryl-tRNA peaks.

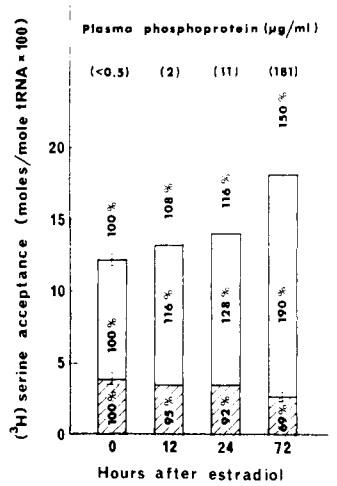

FIGURE 7: Temporal sequence of changes in liver [^{3}H]seryl-tRNA and plasma phosphoprotein in roosters after injection of 10 mg/kg of estradiol-17β benzoate. Each tRNA preparation was acylated with a synthetase preparation derived from untreated rooster liver. Where indicated, values are means ±SEM for four observations. Representation of data as in the legend to Figure 5, with the O-hr values set at 100%.

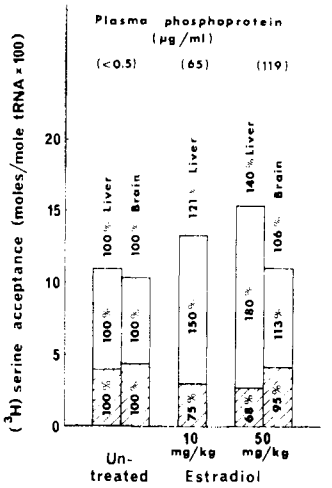

FIGURE 8: Liver and brain [^{3}H]seryl-tRNA and plasma phosphoprotein in week-old chicks 3 days after injection with various doses of estradiol-17β. Each tRNA preparation was acylated with a synthetase preparation derived from untreated chick liver or brain. Each tRNA preparation was prepared from a pooled tissue sample (N=16). Representation of data as in the legend to Figure 5, with values for untreated liver and brain set at 100%.

Temporal Sequence of Changes. Approximately 20 hr following the injection of estradiol, phosphoprotein begins to accumulate rapidly in the plasma (Greengard et al., 1965; Mäenpää and Bernfield, 1969, Beuving and Gruber, 1971b). The pattern of rooster liver seryl-tRNA is already slightly changed 12 hr after estrogen injection (Figure 7). At this time, prior to the period of rapid phosvitin synthesis, the seryl-tRNA acceptance was increased 8%; the absolute amount of peak I was decreased and that of the other peaks was increased. These alterations were subsequently enhanced so that the serine acceptance of unfractionated tRNA at 72 hr was increased by approximately 50% ($p < 0.02$). A 90% increase in the amounts of peaks II, III, and IV entirely accounted for the increase, since peak I was significantly decreased (69% of the control value).

Dose Dependence and Tissue Specificity of the Estrogen Effect. The induction of phosvitin synthesis by estradiol in chicks is dependent on the dose of hormone (Figure 6). This dose dependence was also seen in the hepatic seryl-tRNA changes (Figure 8). Seryl-tRNA isolated from brains of the same animals did not show changes comparable to those seen in hepatic seryl-tRNA.

Discussion

As shown in this and prior studies (Mäenpää and Bernfield, 1969, 1970), following the induction of phosvitin synthesis by estrogen, the amount of total hepatic seryl-tRNA increases due to a coordinate increase in the amounts of peak II ($tRNA^{Ser}_{UCG}$) and peak III ($tRNA^{Ser}_{UCX}$); peak I ($tRNA^{Ser}_{AGU,AGC}$) decreases in amount. Seryl-tRNA derived from isolated nuclei and from free and membrane bound ribosomes was investigated because of the possibility that intracellular compartmentalization of seryl-tRNA might provide an insight into the nature of the relationship between phosvitin synthesis and the seryl-tRNA alterations. In control animals, the chromatographic distribution of seryl-tRNA from these subcellular fractions was similar to the pattern of total hepatic seryl-tRNA. Following estrogen treatment, however, the pattern of seryl-tRNA in the subcellular fractions was distinct from that observed with total liver seryl-tRNA. While total hepatic tRNA from estrogen-

Table I: Subcellular Distribution of Seryl-tRNA Peak I in Rooster Liver.[a]

Source of tRNA	Peak I as a Percentage of Unfractionated Seryl-tRNA		
	Control	Estradiol[b]	% Change
Total liver	32	15	−53
Isolated nuclei	38	55	45
Free ribosomes	35	39	11
Membrane-bound ribosomes	36	47	31

[a] Summarized from data in Figures 2, 3, and 4 (subcellular components), and Figure 5 (total liver). [b] Three days after injection of estradiol-17β (10 mg/kg).

ized roosters shows a relative decrease in seryl-tRNA peak I (Table I), this decrease was not seen in any of the subcellular fractions. Rather, peak I in tRNA derived from free ribosomes showed little change and isolated nuclei and membrane-bound ribosomes showed an increase in peak I.

Membrane-bound ribosomal tRNA may be the most sensitive indicator of those tRNAs used for the synthesis of secreted proteins. Even the 31% relative increase in peak I found on membrane-bound ribosomes may be highly significant. Approximately 85% of cellular RNA is ribosomal, and, at least in rat liver, 75% of the ribosome population is membrane bound (Blobel and Potter, 1967). During estrogenic stimulation, the RNA content of chick liver increases substantially (Jost et al., 1973). Since hormonal stimulation results in increased production of proteins for export (McIndoe, 1971), the amount of membrane-bound ribosomes undoubtedly increases following estrogen treatment. Assuming that the tRNA found on ribosomes closely reflects the code words used for protein synthesis at the time of isolation, the present data indicate that $tRNA^{Ser}_{AGU,AGC}$ may be preferentially utilized in the synthesis of phosvitin.

Klyde and Bernfield (1973), using a different method of tRNA extraction, have recently determined the rate of $tRNA^{Ser}$ synthesis relative to the rate of total tRNA synthesis in the livers of estrogen-treated chicks. Despite a two-

fold increase in the amount of total tRNA, no appreciable change was found in the relative rate of $tRNA^{Ser}$ synthesis during a period when the level of seryl-tRNA continued to rise. If the nuclear tRNA pool reflects changes in $tRNA^{Ser}$ synthesis, it is difficult to reconcile these results with the increase in total nuclear seryl-tRNA and the even greater increase in $tRNA^{Ser}_{AGU,AGC}$ found in roosters in the present study. Other explanations include the possibility that nuclear tRNA represents a separate pool which is such a small proportion of the total that an increase in the synthesis of nuclear tRNA would not have been observed.

The increased proportion of peak I in nuclear and ribosomal seryl-tRNA may not have been previously apparent because these tRNA pools are a minor proportion of total cellular tRNA. In addition, failure to observe this change is possibly due to the method of tRNA extraction. "Total" liver tRNA has been prepared using Brunngraber's (1962) method, which involves a low solute volume and high salt–phenol extraction at 4°. A precipitate, which is discarded, forms at the phenol–aqueous interface during the extraction. According to Samarina et al. (1971), unbroken nuclei ("phenolic nuclei") are present in this precipitate, and thus an unknown proportion of nuclear tRNA may be absent from our "total" liver tRNA preparations. Ribosome-associated tRNA may also be trapped in the phenolic precipitate, either with denatured ribosomal protein or because of the presence of the peptidyl chain.

Since the relative proportions of the various seryl-tRNA peaks are similar in all components of control livers, selective loss of certain fractions would not alter the seryl-tRNA pattern. Following estrogen treatment, however, "total" liver tRNA prepared in this manner may be enriched in extranuclear tRNA species which are *not* being used for protein synthesis at the time of extraction (i.e., non-ribosomal tRNA). Calculating the proportion of tRNA in this pool from the final yields of purified tRNA is spurious, especially because purity and not quantity was sought and because greater relative losses occur during purification of small amounts of tRNA. Nevertheless, such calculations indicate this pool is ca. 75% of hepatic tRNA in estrogen-treated birds. This proportion is consistent with the hypothesis which suggests that peak I is preferentially utilized and peaks II and III used with less frequency during rapid phosvitin synthesis. This could account for the estrogen-induced increase in peaks II and III in "total" liver tRNA.

A correlation between estrogen-induced seryl-tRNA alterations and phosvitin synthesis was shown in several ways. Changes in unfractionated liver seryl-tRNA and in levels of plasma phosphoprotein were studied in laying hens and in chicks and roosters after treatment with various estrogens and other steroids. During physiologic estrogenization in laying hens, total serine acceptance was increased, while the absolute amount of peak I decreased significantly and the amounts of the three other seryl-tRNAs greatly increased. In roosters, phosvitin synthesis and this pattern of seryl-tRNA change can be induced only by estrogens which can be converted to estradiol-17β in avian liver. Estriol, which is not converted to estradiol (Gilbert, 1971), may be an exception, since it caused no changes in seryl-tRNA but very slightly raised the plasma phosphoprotein level. The nature of this phosphoprotein was not determined. The doses of estrogen used in this and previous studies produced responses quantitatively similar to those noted during physiological estrogenization. These findings concur with our previous data (Mäenpää and Bernfield, 1969) and the results of Beck et al. (1970). The seryl-tRNA change was already detected 12 hr after the hormone injection, thus preceding the large increase in the synthetic rate of phosvitin, which occurs at 20 hr (Mäenpää and Bernfield, 1969; Beuving and Gruber, 1971a).

Control tissue (brain) showed no comparable seryl-tRNA alterations after estrogen administration. At equivalent hormone doses, the phosvitin and seryl-tRNA response of chicks was quantitatively less than that of roosters. These data suggest that the presence of estrogen receptors may be important for the alterations in seryl-tRNA, since avian liver, but only a very small portion of brain, contains such receptors and the number of estrogen receptors in chick liver is known to be smaller than in rooster liver (Lebeau et al., 1973).

Although there are alterations in the total amounts of other aminoacyl-tRNAs in avian liver after estrogen administration (e.g., an increase in arginine and leucine acceptance and a decrease in the acceptance of histidine and tyrosine) (Busby and Hele, 1970; Klyde and Bernfield, 1973), and although there are no analogous changes in hepatic seryl-tRNA in *Xenopus* (which synthesizes a phosvitin-like protein in response to estrogen [Wittliff et al., 1972]), taken together the present data indicate a close relationship in chickens between phosvitin synthesis and seryl-tRNA alterations.

Changes in the levels of specific aminoacyl-tRNAs have been found during developmental and oncologic processes. Their significance, however, is not yet clear. Following estrogen treatment, the proportion of tRNA which is aminoacylated does not change (Shelton and Bernfield, 1973), but levels of various tRNAs, as well as levels of different serine isoacceptors do change. The data presented in this paper suggest that the seryl-tRNA changes are closely related to phosvitin synthesis, and that the mechanism by which these changes occur includes redistribution of individual isoacceptors within the cell. These results imply that the levels of isoaccepting tRNA species are under separate control which, in part, may be related to the frequency with which these species are used in translation.

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Transbilayer Exchange of Phosphatidylethanolamine for Phosphatidylcholine and *N*-Acetimidoylphosphatidylethanolamine in Single-Walled Bilayer Vesicles†

M. Roseman, B. J. Litman, and T. E. Thompson*

ABSTRACT: A preparation of small single-walled liposome vesicles containing a 9:1 mole ratio of phosphatidylcholine to phosphatidylethanolamine was subjected to reaction with the membrane-impermeable reagent, isethionyl acetimidate hydrochloride. This reagent converted 90% of the external phosphatidylethanolamine groups to the amidine derivative, leaving the mole ratio of unreacted phosphatidylethanolamine to phosphatidylcholine on the outside surface of the vesicle much lower than that on the inside surface. Equilibration of phosphatidylethanolamine across the bilayer was then measured as a function of time by monitoring the appearance of phosphatidylethanolamine on the outside surface utilizing the reaction of the amino groups with 2,4,6-trinitrobenzenesulfonic acid. The results show that no new phosphatidylethanolamine appeared on the external surface of the vesicles over a period of 12 days at 22°. A conservative estimate of the precision of the measurements is ±10%. On this basis, the estimated half-time for the equilibration of phosphatidylethanolamine across the bilayer of these vesicles must be at least 80 days at 22°.

Marked transmembrane compositional asymmetries of lipid components have been described recently in erythrocytes (Verkleij et al., 1973), rod outer segment discs (Smith and Litman, 1974), and animal cell cytoplasmic membranes (Wisnieski et al., 1974). Similar compositional asymmetries have also been reported in multicomponent single-walled phospholipid vesicles of very small radius of curvature (Michaelson et al., 1973; Litman, 1973; Huang et al., 1974; Thompson et al., 1974). The stability of such large compositional differences in the opposing faces of biological membranes and in simple bilayer systems has focussed attention on the kinetics of transbilayer movement of component lipid molecules. This so-called flip-flop motion was first examined by Kornberg and McConnell (1971) using electron spin resonance techniques, in a preparation of single-walled phospholipid vesicles. The results of these experiments suggested that the half-time for transbilayer equilibration of a spin-label phospholipid derivative was as short as 6.5 hr at 30°. Similar experiments carried out on vesicles derived from the electroplax of *Electrophorus electricus* (McNamee and McConnell, 1973) gave equilibration half-times of the order of minutes at 15°. However, recent experiments utilizing small single-walled vesicles in which a transbilayer compositional asymmetry of isotopically labeled phospholipid was established with a phospholipid exchange protein suggest that flip-flop half-times for self-exchange are much longer (Johnson et al., 1975; Rothman and Dawidowicz, 1975).

† From the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22901. *Received June 30, 1975.* This investigation was supported by grants from the National Institutes of Health, U.S. Public Health Service (GM-14628 and EY-000548).