# *POLYSOMES OF BOVINE ANTERIOR PITUITARY GLAND AND THEIR ROLE IN HORMONE AND PROTEIN BIOSYNTHESIS**

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This laboratory has shown that a cell-free ribosomal system from bovine anterior pituitary glands is capable of incorporating labeled amino acids not only into protein, but also into adrenocorticotropin, growth hormone, and prolactin fractions.[1, 2] An investigation of the physicochemical and biosynthetic characteristics of this system strongly suggested the presence of functional polysomes.[3] In the present study, the pituitary polysomes were resolved into fractions of discrete particle size, and the various factors that affect this function and organization were examined. The polysomes were shown to promote the biosynthesis of prolactin and growth hormone, as well as mixed proteins. Attempts have been made to correlate the aggregate size of the polysomes with the chain length of the hormones synthesized.

*Materials and Methods.*—(1) Radioactive compounds: 3,4-$H^3$-L-proline (5 c/mmole), 4,5-L-$H^3$-leucine (5 c/mmole), and gl-L-$H^3$-phenylalanine (4.25 c/mmole) were all purchased from New England Nuclear Corp. $H^3$-poly U (0.025 c/mmole) and nonlabeled poly U were products of Miles Chemical Co., and Sigma Chemical Co., respectively. (2) Biochemicals: puromycin was obtained from Nutritional Biochemical Corp., and cycloheximide was a gift of Upjohn Co. Crystalline pancreatic ribonuclease (RNase) was a product of the Worthington Biochemical Corp. The sources of the other biochemicals used were those described previously.[1] (3) Hormone standards: bovine prolactin (NIH-P-B1) and growth hormone (NIH-GH-B10) preparations were provided by the Endocrine Study Section, National Institutes of Health. (4) Polysomes and other components of the pituitary cell-free system: these were prepared from fresh bovine anterior pituitary glands.[1, 3]

*Density gradient analysis of polysomes:* All operations were conducted at 0–4°. The polysomal preparations were resolved into components of discrete particle size by sucrose density-gradient fractionation. Linear gradients (15–35% w/v in Medium M[1] minus mercaptoethanol, 25 ml vol) were layered on cushions of 5 ml of 50% sucrose in Medium M, using 1 × 3 in. cellulose nitrate tubes. The polysomal preparation in 1–1.5 ml of Medium S[1] was gently layered on top of the gradient. The tube was centrifuged for 270 min at 25,000 rpm in a Spinco model L ultracentrifuge equipped with an SW25.1 swinging-bucket rotor. Fractions of 0.7 ml were then collected using the Isco model 180 density gradient fractionator, Instrument Specialties Co. Ultraviolet (UV) absorbancies were either monitored continuously at 254 mμ with an Isco model UA-2 UV analyzer equipped with a recorder, or measured at 260 mμ on each fraction (after dilution to 1 ml with water) in a Beckman DU spectrophotometer. Presumptive identification of the polysomal species separated on the gradient was made by direct electron microscopy examination, or by sedimentation analysis in a Spinco model E ultracentrifuge.

*$H^3$-poly U binding to ribosomes:* Measurements were made as previously described.[3]

*Incorporation of $H^3$-amino acids into protein and hormones:* Previous methods[3, 4] were used, except that prolactin was purified by column chromatography on *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) rather than by the isoelectric precipitation step at pH 5.7.

*RNA and protein:* These were determined as described in earlier papers.[1, 3]

*Electron microscopy:* Polysome suspensions (suitably diluted) were deposited directly

onto collodion-carbon-coated grids. The latter were washed successively with 20, 10, and 5% sucrose solutions, followed by Medium M. The preparations were then shadowed with a platinum-carbon mixture at a 20° angle, and observed at 50 kv in a Hitachi HU-11A electron microscope.

*Results and Discussion.—Polysome resolution and protein synthesis by isolated aggregates:* Examined in the electron microscope, pituitary polysome preparations appeared to consist almost exclusively of roughly spherical particles, about 200 Å in diameter. Figure 1*A* shows a spectrum of ribosomal clusters, in addition to monosomes (single ribosomes). Individual peaks from sucrose gradient fractionation appeared more homogeneous when viewed in the electron microscope. In Figure 1*B*, which corresponds to the pentamer fraction, clusters of five particles were more abundant than in other gradient fractions. In Figure 1*C*, which corresponds to the heptamer-octamer region, a preponderance of large aggregates was always present in the fields.

The resolution achieved with the polysome preparation by the sucrose density-

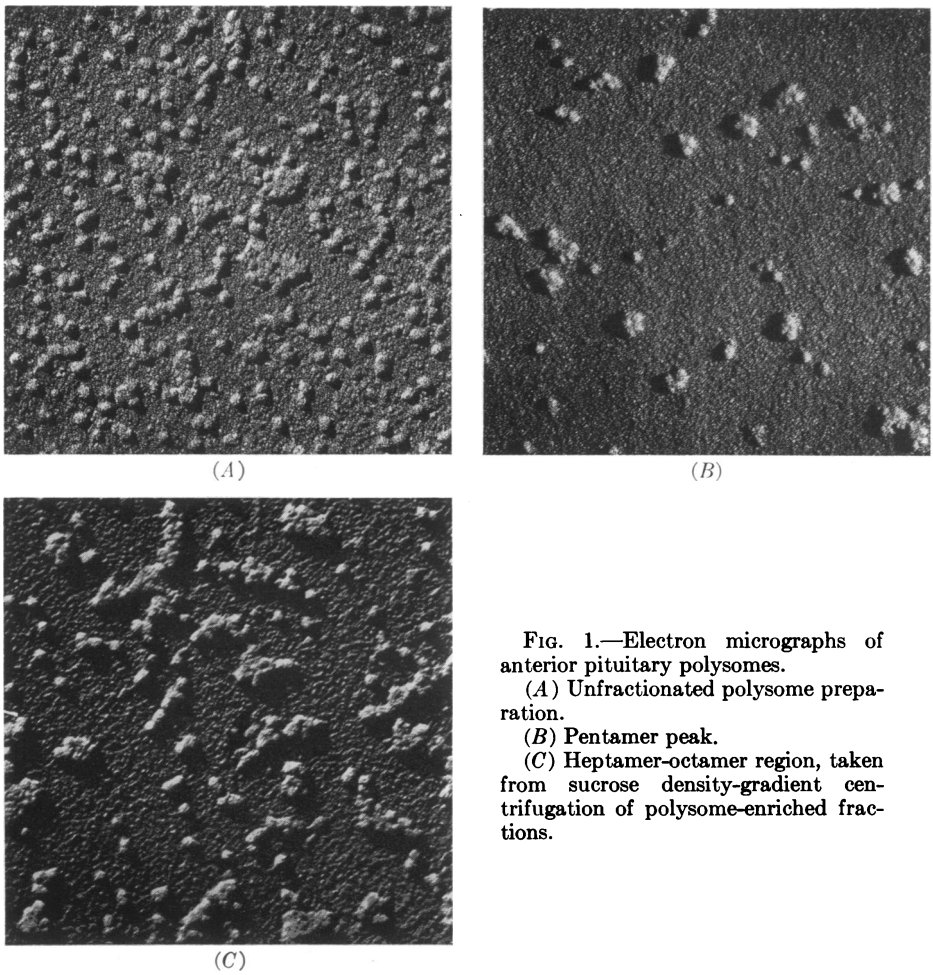


FIG. 1.—Electron micrographs of anterior pituitary polysomes.

(*A*) Unfractionated polysome preparation.

(*B*) Pentamer peak.

(*C*) Heptamer-octamer region, taken from sucrose density-gradient centrifugation of polysome-enriched fractions.

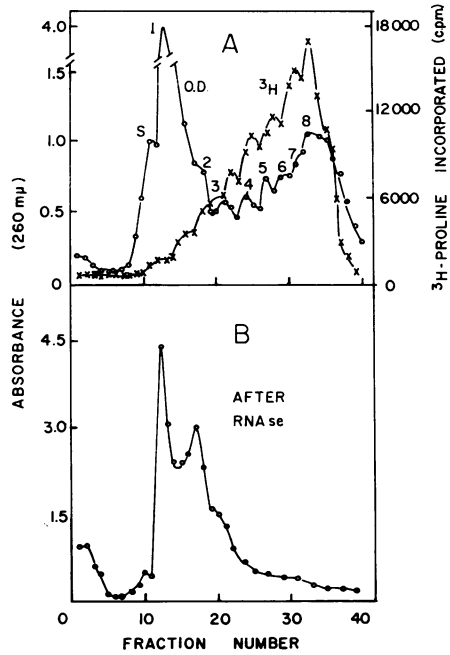


FIG. 2.—(*A*) Resolution of anterior pituitary polysomes on a sucrose density gradient, and biosynthetic activity of individual fractions.

(*B*) Sucrose gradient pattern after preliminary treatment of polysome preparation (0.7 mg rRNA) with 1 μg of crystalline pancreatic RNase for 5 min at 0°.

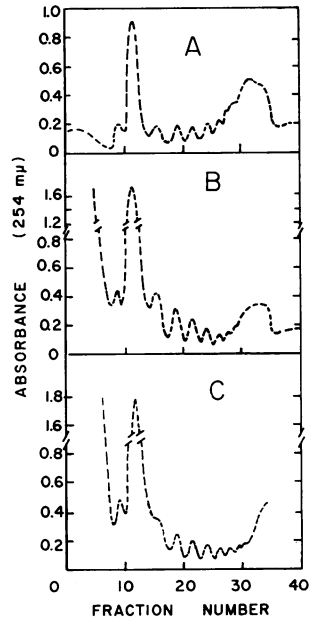


FIG. 3.—Stability of polysomes upon incubation for 1 hr at 37°.

(*A*) Isolated polysomes in Medium M without incubation (control).

(*B*) Polysomes in cell-free system minus pH 5 enzymes and pH 5 supernatant factor.

(*C*) Polysomes in complete cell-free system.

gradient method is shown in Figure 2*A*. As in sedimentation (schlieren) patterns,[3] monosomes were the predominant component. These monomers represented (on the basis of rRNA content) about 35–40 per cent of the material resolved on the gradient, and 25–30 per cent of the input. Seven heavier entities (ranging up to octasomes) were detected on the gradient profile as UV-absorbing peaks (numbered 2–8). The small peak (*S*) preceding the monosome region may correspond to the native 55*S* peak observed in schlieren patterns.[3] It is possible that the polydisperse region at the bottom of the gradient represented aggregation due to neutralization of ribosomal charges by the high ionic environment.[5, 6]

In order to test the biosynthetic ability of the isolated polysomes, fractions taken along the entire sucrose gradient were individually incubated for one hour in the complete amino acid-incorporating system (containing H$^3$-proline), and subsequently the radioactivity associated with the isolated protein preparations was measured. Figure 2*A* shows a progressive increase in the labeling of protein with increasing aggregate size up to peak 8, followed by a decline at the

heaviest region. Only a low level of radioactivity appeared in the monosome peak, in agreement with earlier results.[3]

When specific incorporative activity (cpm in protein/mg rRNA) was compared with polysome size, a similar pattern was obtained, except that the labeling reached a plateau at peak 7 (data not included).

*Effect of RNase on polysome stability:* To rule out the possibility that the heavier ribosomal species fractionated on the gradient were contaminated with random aggregates of monosomes, the effect of a minute quantity of RNase on the polysome profile was tested. Figure 2*B* shows almost complete disappearance of heavier aggregates and an increase in monomers, dimers, and some trimers. In addition, an increase in UV-absorbing material was found at the top of the gradient, equivalent to about 2–3 per cent of the total input. This component probably reflected nucleotides released by the RNase action. The small amounts of dimers and trimers remaining after RNase action is in accord with the results of Manner *et al.*,[7] and may reflect the presence of RNase-insensitive types of bonds.[8]

*Influence of incubation in a cell-free system on polysome structure and function:* Figure 3*A* gives the sucrose gradient profile of a control polysome preparation without incubation. It may be seen that the individual aggregates maintained their integrity. Incubation in the presence of all cofactors, but without pH 5.0 enzymes and pH 5.0 supernatant factor (Fig. 3*B*), led to a considerable decrease in heavier aggregates, and a noticeable increment in lighter particles. In the presence of the complete cell-free system (Fig. 3*C*), this breakdown was accentuated throughout the gradient, with a further increase in lighter components, particularly monosomes. Thus, it appeared that protein synthesis was correlated with polysome breakdown.

The labeling pattern as a function of time was studied by incubating polysomes in the complete cell-free system containing $H^3$-proline. Figure 4 reveals a progressive breakdown of polysomes, and an enhancement in protein synthesis. By 90 minutes the labeling in the polysome region approached a steady state. It is noteworthy that polysomes of rat liver,[9] HeLa cells,[10] and rabbit reticulocytes[11] have been observed to break down within 30 minutes under conditions of protein synthesis. In the pituitary system, a considerable proportion of polysome peaks was evident even after 90 minutes. This greater stability as compared to the above-mentioned polysomes may have reflected RNase removal through the use of bentonite in the preparative procedure. Alternatively, the polysomes in the pituitary gland may be more stable, or more readily reformed from monosomes, during protein synthesis.

*$H^3$-poly U binding and $H^3$-phenylalanine incorporation by polysomes:* A previous paper[3] reported that bovine pituitary 75*S* particles could bind radioactive poly U, and promote the incorporation of $H^3$-phenylalanine into polyphenylalanine, under the influence of (nonlabeled) synthetic polynucleotide. When these experiments were extended to polysomes (Fig. 5), the addition of poly U prior to gradient fractionation gave no enhancement of $H^3$-phenylalanine incorporation in the polysome region. However, a pronounced stimulation of labeling was perceptible in the monosome region (Fig. 5*B*), in agreement with

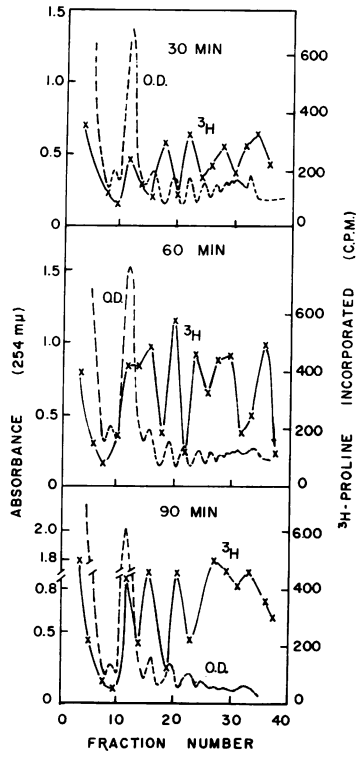


FIG. 4.—Polysome breakdown and protein synthesis in the cell-free system with increasing times of incubation. Subsequently the labeled preparations were subjected to sucrose gradient fractionation.

earlier results. Furthermore, only monomeric ribosomes were capable of binding the synthetic messenger to any great extent (Fig. 5*C*).

These results suggested that the polysome fractions were already associated with an endogenous polynucleotide, presumably mRNA. In accordance with this observation, unlabeled poly U (added before sucrose density-gradient fractionation) stimulated $H^3$-phenylalanine incorporation in assays with monosomes, but not with polysomes. The apparent binding of poly U in the polydisperse region was possibly due to an aggregation of a number of monosome particles, each holding a strand of poly U, or else reflected a cluster of ribosomes attached to a single strand of poly U.[5]

Puromycin was earlier shown[1, 2] to block effectively the incorporation of labeled amino acid into mixed pituitary proteins, as well as into specific hormones, in the bovine cell-free system. This drug releases unfinished peptide chains from ribosomes.[12] Such "stripped" structures, no longer capable of forming peptides, are then detached from polyribosomes.[7] Figure 6*A* shows that puromycin completely degraded polysomes to ribosomes during incubation in the standard pituitary cell-free system.

Cycloheximide, which also inhibits protein synthesis in mammalian and microbial systems,[13–15] appears to have at least two concentration-dependent effects on polyribosomes:[16] very low concentrations protect polysome structure, while higher levels of the drug promote polysome breakdown. Similarly in the

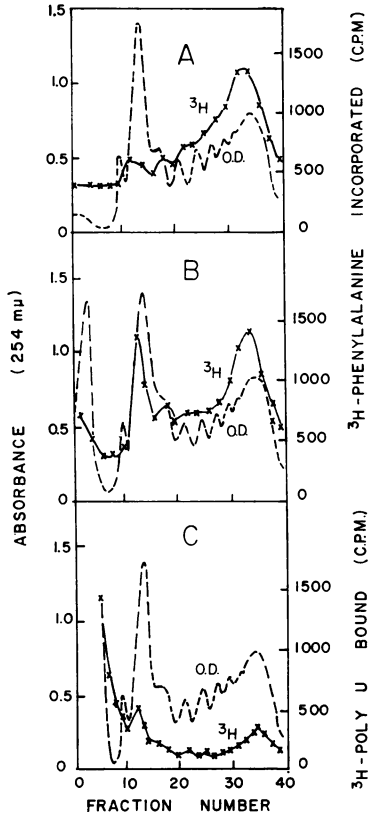


FIG. 5.—Interaction of polysomes with poly U, and the incorporation of $H^3$-phenylalanine in the presence, and in the absence of poly U.

(*A*) Polysomes incubated in the cell-free system with 5 μc of $H^3$-phenylalanine, but in absence of poly U (control).

(*B*) Same as (*A*), but with 100 μg poly U added before fractionation on the sucrose gradient.

(*C*) Polysomes treated with 50 cc of $H^3$-poly U and analyzed on the gradient.

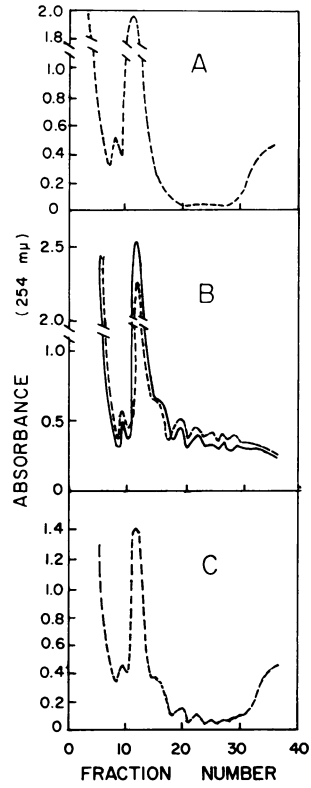


FIG. 6.—Effect of inhibitors of protein synthesis on polysome stability during incubation.

(*A*) Sucrose gradient profile after 1 hr incubation at 37° in the cell-free system containing 50 μg/ml of puromycin.

(*B*) Sucrose gradient pattern of polysomes after 1 hr incubation in the presence (*broken line*) and absence (*solid line*) of 50 μg/ml of cycloheximide.

(*C*) Same as (*A*), but with 1 mg/ml of cycloheximide.

pituitary system, a low concentration of cycloheximide protects the polysome peaks significantly (Fig. 6*B*), while a high level enhances the disintegration of polysomes (Fig. 6*C*). These observations support the conclusion that polysomes are indeed functional in the pituitary cell-free system.

*Polysome size and hormone synthesis:* It was of considerable interest to measure the incorporation of a labeled amino acid into specific hormones under the influence of polysome aggregates of different sizes. The data in Table 1 show that the radioactivity recovered in growth hormone, prolactin, and protein frac-

TABLE 1. *Hormone and protein synthesis with polysome fractions of varying size.*

| Tube no. | Polysome aggregate no. | Isotope Incorporated in One Hour with Cell-Free Assay System (cpm)* Growth hormone | Prolactin | Protein† |
|---|---|---|---|---|
| 9–11 | 55*S* subunit | 260 | 500 | 6,900 |
| 12–14 | 1 | 700 | 2,200 | 15,000 |
| 15–17 | 1–2 | 1,100 | 3,400 | 25,600 |
| 18–20 | 2 | 1,600 | 6,800 | 47,400 |
| 21–23 | 3 | 3,500 | 11,200 | 60,100 |
| 24–26 | 4 | 4,600 | 20,300 | 80,200 |
| 27–29 | 5 | 7,000 | 27,800 | 98,800 |
| 30–32 | 6–7 | 8,200 | 33,500 | 107,180 |
| 33–35 | 7–9 | 5,900 | 21,000 | 82,200 |
| 36–38 | Polydisperse region (above 9) | 4,900 | 14,500 | 35,800 |
| 38–41 | Bottom of gradient | 700 | 2,500 | 25,200 |

The corresponding fractions from three separate sucrose gradients were pooled in order to provide adequate quantities of polysomes.

* Fifteen $\mu$c $H^3$-leucine used per tube.

† Residue VIII of Rao *et al.*[4]

tion rose progressively with increasing size of the polysome clusters, and reached a maximum at the hexamer to heptamer level. Thereafter the quantities of isotope declined. In another experiment in which radioactive leucine, proline, and phenylalanine were used simultaneously, labeling in total protein was highest in heptamers to nonamers, again followed by a decline in synthesis above this region (data not shown).

*Correlation of RNP aggregate size with molecular weight of hormones:* The current hypothesis is that the maximum aggregate size of a functional polysome should be compatible with that of the synthesized protein.[17] Studies concerned with such correlations have been undertaken with myosin,[18] collagen,[7] $\beta$-galactosidase,[19] hemoglobin,[17] and immunoglobulin heavy and light chains.[20] Hemoglobin chains (mol wt 17,000) are synthesized on polysomes containing five to six ribosomes,[21] and immunoglobulin light chains (mol wt 20,000–23,000) on polysomes containing seven to eight ribosomes.[20]

From these studies it was anticipated that pituitary aggregates in the range of heptasomes to octasomes would be most effective in the biosynthesis of bovine prolactin and growth hormone, with molecular weights of 21,000 and 26,000, respectively.[22] In effect, it was found that the synthesis of the two hormones was most active with polysomes containing six to seven ribosomal units. The labeling obtained with polysomes of smaller aggregate size (possibly arising from breakdown of larger clusters) may have reflected the completion of nascent peptides. The rather similar results obtained for general protein synthesis suggest that the average molecular weight of the mixed proteins synthesized in the cell-free system was likewise in the approximate range of 20,000–30,000. The reduced biosynthetic activity in the regions of higher polysome aggregate number suggests that monocistronic, rather than polycistronic messengers, were involved in the synthesis of the two hormones studied.

*Summary.*—Polysome-enriched preparations from bovine anterior pituitary gland were resolved by sucrose density-gradient fractionation into a number of

peaks of discrete particle size, and certain of these aggregates were examined in the electron microscope. Several types of evidence supported the view that the polysomes were functional in protein synthesis: (1) the sensitivity of these structures to RNase treatment; (2) the progressive breakdown of polysomes observed during protein synthesis in a cell-free system; (3) the fact that polysomes were more active than monosomes in general protein synthesis; (4) the lesser ability of polysomes, as compared to monosomes, to bind poly U and to promote poly U-directed labeled phenylalanine incorporation into polypeptide; (5) and the finding that puromycin caused a complete breakdown of polysomes to monosomes. In studying the incorporation of radioactive leucine into prolactin and growth hormone, it was shown that the synthesis in both cases was most active with polysomes containing six to seven ribosomal units. This finding correlates well with the predicted polysome size requirement for the synthesis of proteins with molecular weights of approximately 20,000.

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