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Translation of Xenopus Liver Messenger RNA in Xenopus Oocytes: Vitellogenin Synthesis and Conversion to Yolk Platelet Proteins

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Summary

Xenopus liver vitellogenin and albumin mRNAs injected into Xenopus oocytes are correctly translated, as shown by specific immunoprecipitation and co-electrophoresis with purified Xenopus vitellogenin (molecular weight 210,000 daltons) and albumin (molecular weight 72,000 daltons). Vitellogenin made in oocytes under the direction of injected liver mRNA is unstable compared to other proteins made on injected messengers (such as albumin and globin) and endogenous oocyte proteins (including actin), the half-life of newly made vitellogenin being about 8 hr. Pulse-chase experiments with ^{35}S-methionine show vitellogenin to be a precursor to yolk platelet lipovitellin (molecular weight 120,000 daltons), while ^{3}H-serine labeling demonstrates conversion to phosvitin (molecular weight 34,000 daltons). In contrast, injected ^{3}H-serine ^{35}S-methionine-labeled Xenopus vitellogenin protein is not converted to yolk platelet proteins and is degraded rather slowly (half-life, 23-29 hr).

Phosphorylation of serine residues in phosvitin can be detected in oocytes injected with $^{32}PO_4$ or γ-^{32}P-ATP; thus exogenously derived yolk platelet protein is further modified, or turned over, once it is within the oocyte. Moreover, vitellogenin made in oocytes programmed with liver mRNA is phosphorylated. Thus phosphorylation, assembly into yolk platelets, and cleavage are events that do not require vitellogenin supplied by the normal pathways involved in yolk formation (synthesis and post-translational modification in the liver, transport in the serum, and follicle cell-dependent pinocytosis).

Vitellogenin mRNA sediments at about 29S in a sucrose-SDS gradient, while albumin messenger peaks at 16S; both species contain poly(A). These liver mRNAs are functionally stable in oocytes for at least 5 days. Vitellogenin-forming activity, relative to albumin, actin, or total endogenous activity, increases with time, and the final rate of 2-2.5 times the initial rate is only reached 3 days after injection. The potentiation effect probably stems from an increase in the efficiency of translation of vitellogenin mRNA.

The availability of homologous mRNAs now permits injected messenger to be used as a valid probe of oocyte function: the biological activity of mRNA from a non-ovarian Xenopus tissue proves that some at least of the translational systems within the Xenopus oocyte are not cell type-specific. Moreover, the whole cell system is eminently suitable for assaying putative translational (and possibly transcriptional) control elements from frog liver.

Introduction

The hormone-controlled synthesis of a multicomponent precursor polypeptide, vitellogenin, takes place in Xenopus liver (Wallace and Dumont, 1968; Wallace and Jared, 1969; Redshaw and Follett, 1971; Wallace et al., 1972), and the system offers many advantages for the study of the control of gene expression (Berridge et al., 1976; Wangh and Knowland, 1975; Green and Tata, 1976). Vitellogenin is secreted by the liver and transported in the blood as a complex glycolipophosphoprotein to the ovary, where it is absorbed and converted into yolk platelet protein (Wallace, Jared, and Nelson, 1970; Wallace et al., 1972). Endogenous synthesis of yolk proteins by the oocyte is small—less than 1% of the amount derived from absorbed vitellogenin (Wallace et al., 1972). Thus the important post-translational events take place in the oocyte, in which they can be studied using microinjection techniques combined with biochemical analysis.

In this paper, we describe the preparation of crude liver vitellogenin messenger, its translation in oocytes, and conversion of the initial polypeptide formed into egg yolk proteins. Such studies reveal the nature and specificity of the translational and post-translational systems within the oocyte; the use of homologous messenger yields final proof that some of the translational systems within the oocyte are not cell type-specific (Lane, Marbaix, and Gurdon, 1971). The messenger-directed synthesis of yolk platelet protein shows that phosphorylation, platelet assembly, and cleavage are not dependent upon vitellogenin taken up by pinocytosis. Nonetheless, we confirm (Dehn and Wallace, 1973) that injected labeled vitellogenin does not appear to be processed into yolk platelet proteins, and find that the injected protein is degraded rather slowly (half-life, 23-29 hr) in healthy oocytes. The messenger-directed product has a half-life of about 8 hr. Thus the Xenopus oocyte system can now be used to assay and study liver vitellogenin messenger and the factors which affect its translation in a normal living cell. We show that albumin messenger and even the large 29S vitellogenin messenger are functionally stable in oocyte cytoplasm for a period of at least 5 days, and that several days may elapse

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and then immunoprecipitating at various times after the beginning of the effective chase. Figure 3A shows that the cytoplasmic half-life is about 8 hr, a value of 5–8 hr having been obtained in an earlier experiment.

To test the possibility that the above results are due to the injection of a liver mRNA that codes for the enzyme responsible for the specific cleavage of vitellogenin, the experiments were repeated using partially purified vitellogenin mRNA. Thus poly(A)+ rich liver RNA from treated females was heat-denatured and fractionated on sucrose–SDS gradients. Figure 4 shows the location of vitellogenin and albumin mRNA activities recovered from the gradient. Partially purified vitellogenin messenger RNA (Figure 4, fractions 10 and 11) sedimenting at 28S–30S was injected into oocytes. Vitellogenin was synthesized but, as before, a chase experiment revealed a half-life of less than 12 hr. The distribution of both albumin and vitellogenin mRNA activities shows that little aggregation took place on the sucrose–SDS gradient, and since there are few

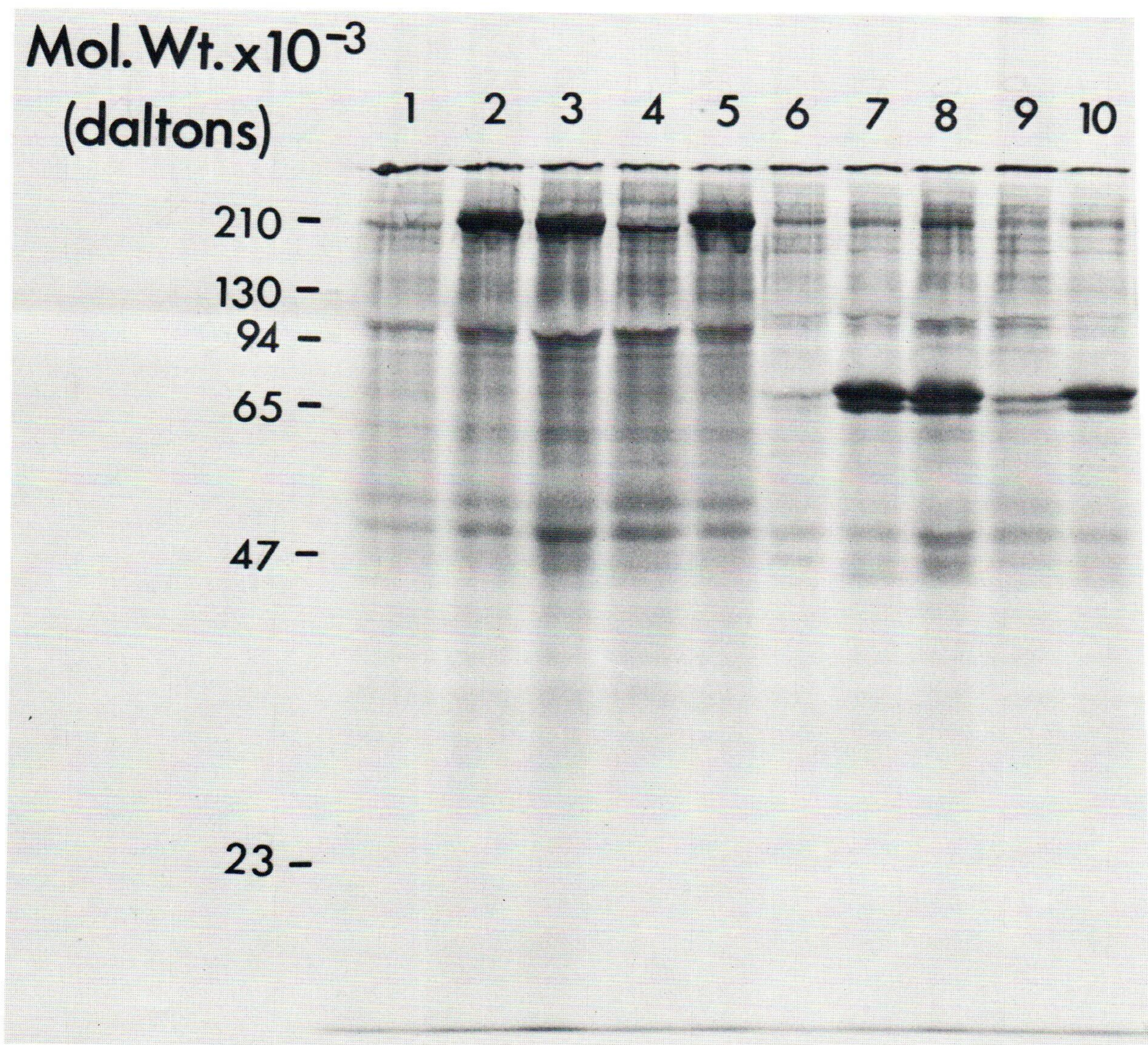

Figure 1. Synthesis of Vitellogenin and Albumin in Xenopus Oocytes Injected with Liver RNA

Batches of 35–40 oocytes were injected with 30–50 nl of RNA dissolved in injection medium and labeled with 100 μCi ^{35}S–methionine at 22°C for 22 hr. Each batch was divided into two, and soluble oocyte proteins were extracted and immunoprecipitated with either antivitellogenin (slots 1–5) or antialbumin (slots 6–10) antibodies (see Experimental Procedures). Immunoprecipitates were electrophoresed on a 10% slab gel which was then autoradiographed.

Slots 1 and 6—uninjected oocytes; slots 2, 3, 7, 8—oocytes injected with total Xenopus liver RNA (200 A_{260}/ml); slots 4 and 9—oocytes injected with (dT) flow through RNA (150 A_{260}/ml); slots 5 and 10—oocytes injected with poly(A)-containing RNA (35 A_{260}/ml).

known degradative enzymes coded for by such large messengers, these results suggest, but do not prove, that processing of vitellogenin stems from intrinsic properties of the oocyte.

Oocytes Programmed with Liver RNA Synthesize Vitellogenin Which Is Converted to Yolk Platelet Proteins

Vitellogenin absorbed from the blood stream is specifically processed (Bergink and Wallace, 1974) into lipovitellin (two polypeptides of 120,000 and 30,000 daltons) and phosvitin (34,000 daltons), yet vitellogenin injected into oocytes is degraded (Dehn and Wallace, 1973). Thus the vitellogenin made under the direction of injected messenger permits one to test further the hypothesis that the pinocytotic mode of entry (Wallace et al., 1970; Dumont, 1972) is required for correct processing. Figure 5 shows that ^{35}S-methionine radioactivity is chased out of vitellogenin (made on injected messenger) and can be accounted for by the appearance of labeled yolk platelet lipovitellin (120,000 dalton component). Thus slots 1 and 3 of Figure 5 show that over the 19 hr labeling period, there is little incorporation into lipovitellin, in mRNA-injected or in uninjected oocytes. However, as can be seen from slots 4–6 of Figure 5, a 4 day chase revealed labeled lipovitellin in platelets from mRNA-injected oocytes, but not in those from controls (Figure 5, slot 2). No radioactivity enters phosvitin, because this serine-rich phosphoprotein lacks methionine (Redshaw and Follett, 1971). Table 1 summarizes another pulse-chase experiment: ^{3}H-serine labeling now reveals increased radioactivity in yolk platelet phosvitin and a corresponding decrease in soluble cytoplasmic vitellogenin. The more sensitive technique of fluorography (Bonner and Laskey, 1974) confirms the rise in phosvitin labeling and also reveals radioactivity chased into lipovitellin (120,000 dalton component).

Table 1. The Chase of Serine Radioactivity from Vitellogenin into Phosvitin in Oocytes Programmed with Liver RNA

Duration of Chase with 2 mM Serine (hr)	Labeled Vitellogenin per 10 Oocytes (cpm)	Labeled Albumin per 10 Oocytes (cpm)	Labeled Phosvitin per 10 Oocytes (cpm)
	16,300	2460	500
32	1,900	1580	27,000

Each of a batch of 400 oocytes was injected with total liver RNA (200 A_{260}/ml) and labeled with ^{3}H-serine (250 μCi) for 16 hr before being transferred to medium containing 2 mM serine. Control oocytes were not injected with RNA. Radioactivity in vitellogenin and albumin was measured by slicing gels of immunoprecipitates, while slicing yolk platelet protein gels gave values for phosvitin. Radioactivity in the vitellogenin, albumin, and phosvitin regions of gels from control oocytes has been subtracted from the values obtained with RNA-injected oocytes. Such background values for a given gel region remain very nearly constant throughout the experiment, and all the effects represent changes of several times the background values.

Vitellogenin Injected into Oocytes Is Not Converted into Yolk Platelet Protein

The injection of vitellogenin into oocytes tests some of the restrictions on correct processing. Pinocytotically absorbed or endogenously synthesized vitellogenin clearly has access to the processing machinery of the oocyte, but we confirm the results of Dehn and Wallace (1973) showing that injected vitellogenin is not, apparently, converted into yolk platelet proteins. Thus ^{3}H-serine ^{35}S-methionine-labeled vitellogenin was prepared from female frogs, and about 1 μg of protein was injected into each of about 200 oocytes. Figure 3B shows that radioactivity associated with the yolk platelets actually declines with time, and the amount found probably results from adsorption, since the maximum is reached after a 1 min incubation. The viscous vitellogenin solution leaves a substantial injection wound, and within 48 hr more than two thirds of the total radioactivity may leak out, after which the level tends to stabilize. However, expressing the radioactivity in platelets as a proportion of total radioactivity or of TCA-precipitable radioactivity (the latter two are equal) still leads to the same conclusion: there is no evidence for conversion of vitellogenin

Figure 2. Stability of Vitellogenin and Albumin Synthesized in Xenopus Oocytes under the Direction of Liver RNA

Oocytes were injected with 30–50 nl of liver RNA (200 A_{260}/ml), dissolved in injection medium, and then labeled for 21 hr with 200 μCi of ^{35}S-methionine. Control oocytes were labeled but not injected; subsequent studies show that injection with medium lacking RNA yields similar results. Oocytes were then transferred to nonradioactive medium containing 2 mM methionine, and batches of 20 oocytes were removed at daily intervals. Soluble oocyte proteins were extracted and immunoprecipitated, first with antivitellogenin (slots A1–A8), then with antialbumin (slots B1–B8) antibody. Immunoprecipitates equivalent to 3.1×10^6 total TCA-precipitable counts (except for slots A8 and B8, which correspond to only 2.0×10^6 cpm) were electrophoresed on 10% gels. The marker tadpole tail extract, containing actin (molecular weight 47,000 daltons), was run in the slot labeled B. Gels were dried and then autoradiographed.

Slot (A and B Series)	1	2	3	4	5	6	7	8
mRNA	–	–	–	+	+	+	+	+
Label	21 hr	21 hr	21 hr	21 hr	21 hr	21 hr	21 hr	21 hr
Chase	–	24 hr	48 hr	–	24 hr	48 hr	72 hr	96 hr

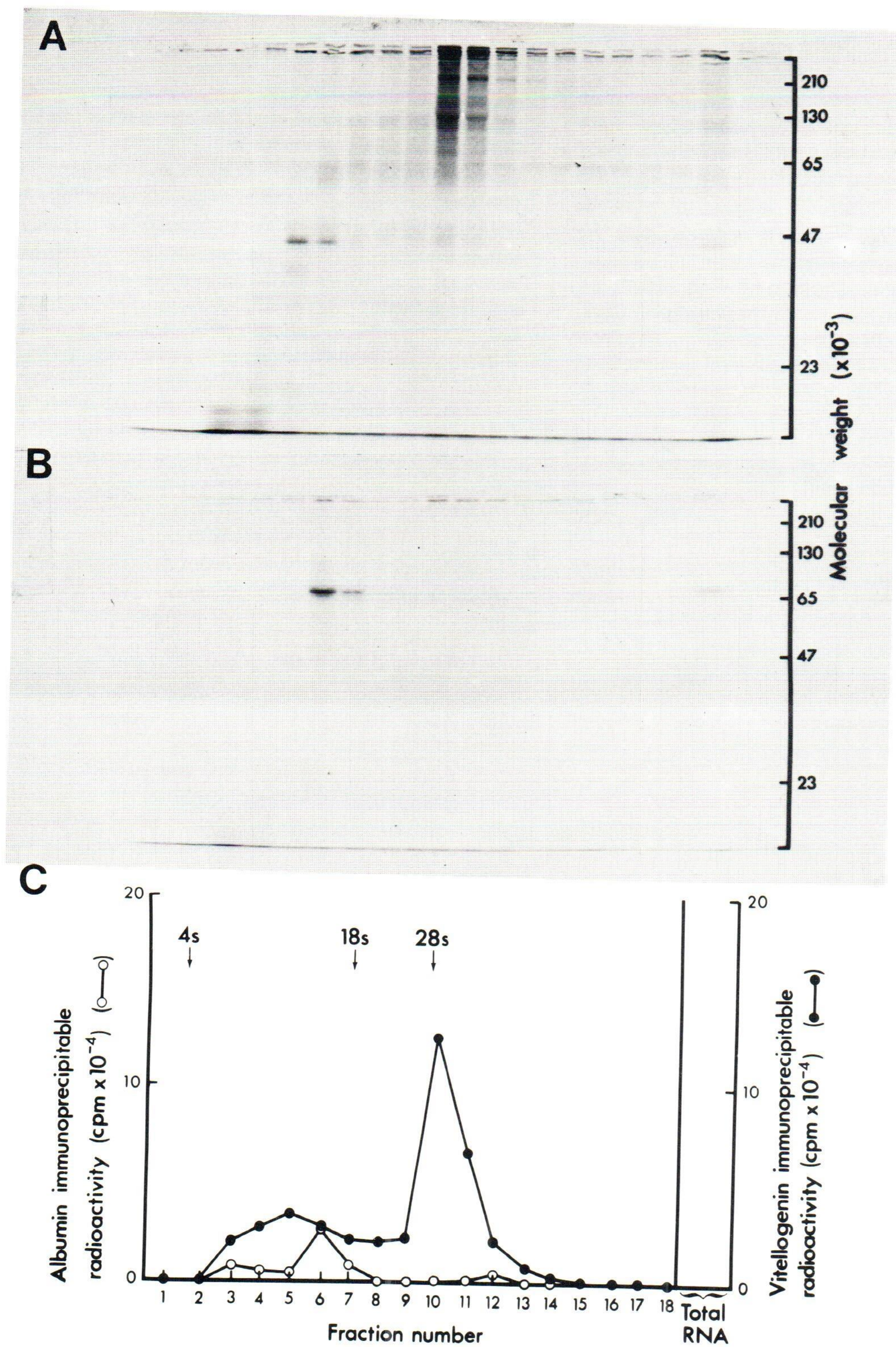

Figure 4. Purification of Active 29S Xenopus Liver Vitellogenin mRNA

Poly(A)-containing RNA was prepared from the livers of estradiol-treated female frogs and then fractionated on an SDS-sucrose gradient. RNA from each gradient fraction was translated in a rabbit reticulocyte lysate; vitellogenin and albumin immunoprecipitates were fractionated on gels.

(A) autoradiograph of gel of vitellogenin immunoprecipitates;

(B) autoradiograph of gel of albumin immunoprecipitates;

(C) TCA-precipitable radioactivity in one tenth part of the vitellogenin (●——●) or albumin (○——○) immunoprecipitates.

Wheat germ tRNA (4S) and Xenopus ribosomal RNAs (18S and 28S) were used as markers in the SDS-sucrose gradient. Molecular weight markers for the gel are as described in Experimental Procedures.

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