DNA POLYMERASE ACTIVITY DURING MATURATION IN XENOPUS LAEVIS OOCYTES

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SUMMARY

DNA polymerase activity was studied during the maturation of Xenopus laevis oocytes. DNA polymerase activity is already present in extracts of oocytes at stages 5 and 6 of oogenesis. The DNA polymerase present in Xenopus laevis oocytes is not localized in the germinal vesicle. Two distinct DNA polymerase activities can be detected in homogenates of unfertilized eggs. These activities differ from each other in the isoelectric point and optimal pH.

INTRODUCTION

The work of Gurdon (1) and Gurdon et al. (2) has shown that the injection of adult frog brain nuclei or purified DNA preparations into unfertilized eggs of Xenopus laevis stimulates the incorporation of [^{3}H] dTR into DNA. The average base composition of the newly synthesized DNA resembles that of the injected DNA. On the other hand, the injection of brain nuclei or purified DNA into the cytoplasm of Xenopus laevis oocytes fails to induce a de novo synthesis of DNA (3). It has been proposed that DNA polymerase and/or functionally related enzymes, such as ligase or DNase, are not present, or are inactive, in Xenopus laevis oocytes; they would appear, or be activated, only after germinal vesicle breakdown (3).

In the present paper, experiments are reported which demonstrate that DNA polymerase is, in fact, present both in oocytes and in unfertilized eggs; but undergoes a several fold increase in the course of maturation. This, however, is not due to an activation of the oocyte polymerase but rather to the appearance of a new DNA polymerase activity. In addition, evidence is presented which indicates that the activity found in the oocytes is not localized in the germinal vesicle.

EXPERIMENTAL PROCEDURES

Ovaries were removed from Xenopus laevis females anesthetized with 222

Sandoz. Single oocytes were isolated with watchmaker forceps and follicle cells were separated according to Hanocq-Quertier et al. (4). Stages of oogenesis were classified according to Davidson (5). Ovulation in Xenopus laevis females was induced as described by Gurdon (6). The removal of the jelly which surrounds the ovulated eggs was performed as described by Dawid (7).

DNA polymerase activity was assayed as previously described (8). One unit of enzyme activity is defined as the amount catalyzing in 30 min. the incorporation of 10 nmoles of [^{3}H] dTTP (New England Nuclear) or α^{32}P dTTP (International Chemical and Nuclear Corporation, Irvine, California) into an acid-insoluble product. The radioactivity which became acid-insoluble was proportional to the amount of enzyme added between 0.05 and 0.5 unit of the enzyme. Control assays for terminal deoxynucleotidyl transferase were made by omitting dATP, dCTP, and dGTP from the reaction mixture.

RESULTS

DNA polymerase activity is detectable in extracts of both oocytes and unfertilized eggs of Xenopus laevis. Table I shows the level of DNA polymerase specific activity in oocytes at two different stages of oogenesis and in homogenates of unfertilized eggs. In experiment a, oocytes and eggs were obtained from two different animals; in experiments b and c, oocytes and eggs were obtained from the same animal. DNA polymerase specific activity markedly increases following breakdown of the germinal vesicle. The presence of DNA polymerase inhibitors in the oocytes, or of activators in the egg, is discounted by experiments showing that the activity of the mixed extracts is the sum of the activities of the individual homogenates.

Table II shows that no DNA polymerase activity is present in the germinal vesicle and that essentially all the enzyme activity is recovered in the oocyte cytoplasm. The question now arises as to whether the maturation-dependent increase of DNA-polymerase activity is due to an increased activity of the enzyme already present in the oocyte or to the appearance of a new enzyme. In order to answer this question attempts have been made to purify and characterize the crude enzymes present in oocytes and unfertilized eggs.

Fig.1 shows the isoelectric fractionation of an oocyte homogenate in a pH gradient, indicating the presence of a single DNA-polymerase activity peak with an isoelectric point at pH 6.2. On the other hand, when the same fractionation proce-

Table I

DNA polymerase specific activity during oogenesis in Xenopus laevis. Isolated oocytes or unfertilized eggs (see Methods) were ground in a loose Teflon homogenizer in two volumes of 0.2 M potassium phosphate buffer (pH 7.4) containing 2×10^{-3} M 2-mercaptoethanol. The homogenate was filtered through several layers of cheesecloth and then centrifuged for 20 min. at 15,000 rpm in the SS 34 rotor of a Servall RC 2 B centrifuge at 4°C. The supernatant was collected and DNA polymerase activity assayed in the standard incubation mixture (see Methods). Protein concentration was determined by the method of Lowry (9). In experiment a, oocytes and mature eggs were obtained from two different frogs. In experiment b and c, the ovaries were removed from animals anesthetized with 222 Sandoz and the oocytes were isolated after ovulation.

TABLE I

DNA Polymerase Specific Activity During Oogenesis in Xenopus laevis

Experiment	oocytes stage 5	oocytes stage 6	unfertilized eggs
	Units/mg Protein		
a	0.014	0.025	0.15
b	0.020	0.03	0.10
c	0.018	0.02	0.11

TABLE II

Localization of DNA Polymerase Activity in Oocytes of Xenopus laevis

Extract	Relative Activity
Whole Oocyte	100%
Germinal Vesicle	< 5%
Cytoplasm	60%

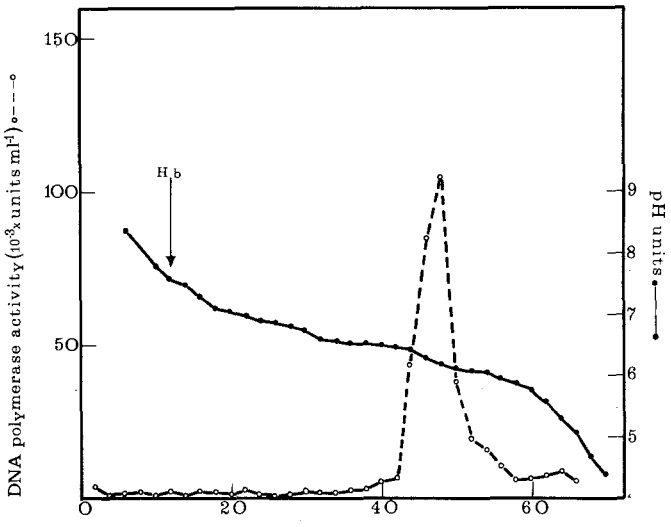

Figure 1
Isoelectric focusing of extracts from oocytes.
Oocytes were homogenized as described in Table I. The homogenate was dialyzed against 0.02 M potassium phosphate buffer (pH 7.4) containing 2×10^{-3} M 2-mercaptoethanol and 25% glycerol. After dialysis, the extract was diluted with the same buffer at a protein concentration of 10 mg/ml. A sample of 5 ml was used for the isoelectric separation. Electrofocusing was performed with the LKB 8101 electrofocusing equipment, according to Vesterberger and Svensson (10). The column (110 ml) was filled with a sucrose linear density gradient, 0 - 47% (w/v), containing 1% Ampholines (LKB-Produkter AB Sweden) for the pH range 5 - 8. A voltage of 400 volt was applied for 35 hr. At the end of the run, one ml fractions were collected and pH and DNA polymerase activity were measured. Human hemoglobin was used as a marker. All procedures were carried out at 0 - 5°.

dure is applied to homogenates of unfertilized eggs (Fig.2), two distinct activity peaks are detected: one at pH 6.4 - 6.2 and the other with isoelectric point at pH 7.0. Fig.3 shows the pH activity curve of these two enzymes. The pH curve of the crude extract of oocytes (not shown in figure) coincides with the curve of the enzyme with isoelectric point at pH 6.4 - 6.2.

Table II
Localization of DNA polymerase activity in oocytes of Xenopus laevis. Oocytes were isolated as previously described. Germinal vesicles were removed by means of two watchmaker forceps and the extracts were prepared as described in Table I. DNA polymerase activity was assayed as described in the Methods.

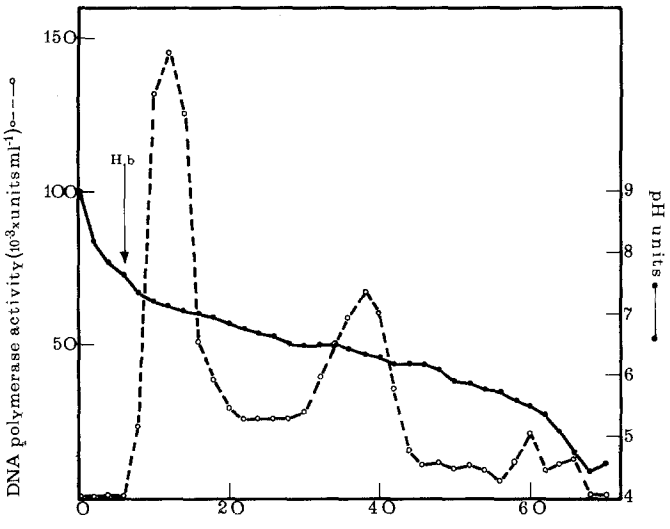

Figure 2
Isoelectric focusing of extracts from unfertilized eggs.
The eggs were homogenized and the extract dialyzed as described in Table I and in Fig.1. Electrofocusing was performed on a sample of 5 ml in the same condition as for Fig.1, except that a voltage of 400 volt was applied for 48 hr.

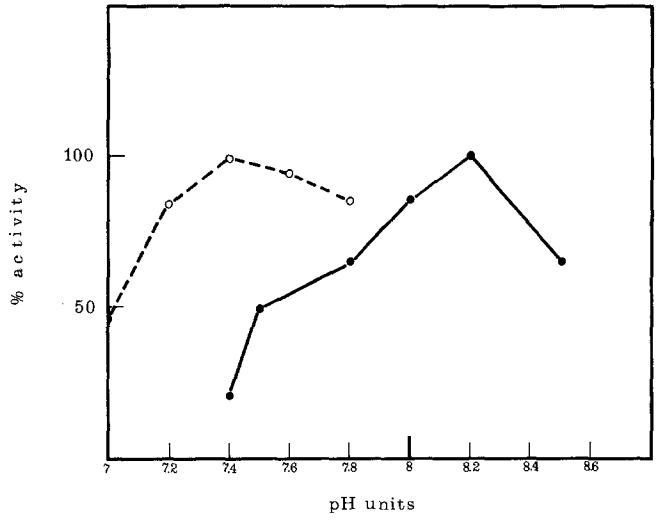

Figure 3
pH activity curve of DNA polymerases.
The enzymes used in the assays were the fractions separated at the isoelectric points of pH 7 (•——•) and pH 6.2(o---o) respectively (see Fig.2). The incubation mixture was the same as described in the Methods, except that the potassium phosphate buffer pH 7.4 was substituted with Tris-HCl buffers at the indicated pH.

DISCUSSION

The relevant point which emerges from these experiments is that, in the Xenopus egg in preparation for the onset of DNA replication, a different DNA polymerase activity appears from that present in the oocyte. Beside the precise characterization of the two enzyme activities, the most urgent question to be answered is whether the new activity in the egg is due to de novo synthesis or to activation of a pre-existing, inactive protein. Two points, however, are already established by the present experiments: a, the egg enzyme is not compartimentalized in an active form in the germinal vesicle; b, its appearance is not due to the removal of an inhibitor in the cytoplasm of the oocyte or to an activator in the cytoplasm of the unfertilized egg.

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REFERENCES

1. Gurdon, J.B., Proc. Natl. Acad. Sci. (USA) 58, 545 (1967).
2. Gurdon, J.B., Birnstiel, M.L. and Speight, V.A., Biochim. Biophys. Acta 174, 614 (1969).
3. Gurdon, J.B. and Speight, V.A., Exptl. Cell Res. 55, 253 (1969).
4. Hanocq, F., Kirsch-Volders, M., Hanocq-Quertier, J., Baltus, E. and Steinert, G., Proc. Natl. Acad. Sci. (USA) 69, 1322 (1972).
5. Davidson, E.H., Gene Activity in Early Development, Academic Press, New York, 1968.
6. Gurdon, J.B., in F. Wilt and N. Wessells, Methods in Developmental Biology, T.Y. Crowell Co., New York, 1967, p.75.
7. Dawid, J.B., J. Mol. Biol. 12, 581 (1965).
8. Grippo, P. and Richardson, C.C., J. Biol. Chem. 246, 6867 (1971).
9. Lowry, O.H., Rosebrough, N.J., Fann, A.L. and Randall, R.L., J. Biol. Chem. 193, 265 (1951).
10. Vesterberg, O. and Svensson, H., Acta Chem. Scand. 20, 820 (1966).