Poly(adenosine diphosphate-ribose) polymerase in quail oviduct. Changes during estrogen and progesterone induction

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ABSTRACT

The activities of the following enzymes have been determined in nuclei of quail oviducts in response to exogenous stimulation of the birds with diethylstilbestrol, used as an estrogen analogue and progesterone : DNA dependent DNA polymerase, DNA dependent RNA polymerase I and II and poly(adenosine diphosphate-ribose) [=poly(ADP-Rib)] polymerase.

During primary stimulation with the estrogen analogue the activities of the four DNA dependent polymerases increase to about the same degree. Upon withdrawal of the hormones the levels of the enzymes drop to values known from nuclei from unstimulated quail oviducts. The secondary stimulation with the estrogen analogue causes a significant increase only of the RNA polymerase II.

The in vivo induction of avidin by progesterone in oviduct mucosa cells from quails, during the period of primary estrogen stimulation, is accompanied by an increase of RNA polymerase II activity and a marked decrease of poly(ADP-Rib) polymerase activity. The activities of RNA polymerase I and of poly(ADP-Rib) polymerase are not affected significantly by an exogenous administration of progesterone.

INTRODUCTION

The existence of poly(adenosine diphosphate-ribose) [=poly(ADP-Rib)] in eukaryotic cells has been described by Chambon et al. (1). The polymer with a chain length of up to 30 ADP-Rib units (2) occurs in nuclei and is covalently attached to nuclear proteins (3). Poly(ADP-Rib) is synthesized from NAD^+ by the poly(ADP-Rib) polymerase, which seems to be firmly associated with the DNA (4). The enzyme converts NAD^+ into poly(ADP-Rib) by elimination of nicotinamide and formation of a 1'-2' glycosidic linkage between the ADP-Rib units (see review, 5).

The biological function of poly(ADP-Rib) in vivo has so far not been investigated extensively.The polymer is synthesized predominantly in two short-lived bursts during the S-phase of mouse L-cells (6). Reports dealing with the biological activity of poly(ADP-Rib) bearing on DNA synthesis revealed 1) that in intact cells a correlation exists between DNA and poly(ADP-Rib) synthesis (6), 2) that the

activity of poly(ADP-Rib) polymerase varies inversely to the rate of DNA synthesis (7), 3) that there is no correlation between poly(ADP-Rib) formation and DNA synthesis (8), and 4) that ADP-ribosylation may play a role in modulation of DNA synthesis in normal and regenerating tissue while any regulatory function of this polymer is ineffective in tumor cells (9). One reason for these contradictory results may come from the different biological systems used. Nevertheless it seems very likely that poly(ADP-Rib) formation is involved in some way in the regulation of DNA synthesis through modification of nuclear proteins.

Whether this new type of biochemical modification of nuclear proteins (ADP-ribosylation, oligo ADP-ribosylation, poly ADP-ribosylation) has in addition to the known reactions, such as methylation, phosphorylation and acetylation (5), some significance in gene expression is not yet known. The experiments of Haines et al. (10) revealed the highest poly(ADP-Rib) polymerase activity in nuclei involved in RNA synthesis.

In the present paper the in vivo biological system of avian oviduct has been used to study the function of poly(ADP-Rib). In immature birds, estrogen causes a dramatic increase of cell proliferation and an onset of cytodifferentiation in the epithelial cells of the mucosa of the oviduct (11). Another steroid hormone, progesterone, then induces a special type of gene expression (synthesis of avidin) without affecting DNA synthesis (11). These two biological event chains, one triggered by estrogen the other by progesterone, seem to be appropriate tools to check the role of poly(ADP-Rib) synthesis first, in proliferating and differentiating cells and second, in cells whose RNA synthesis is uniquely stimulated. Instead of the natural estrogen hormone, diethylstilbestrol has been administered to the quails. This synthetic substance has been shown to be a specific competitor of estradiol in the intracellular hormone binding system (Müller et al., 12). The determination of poly(ADP-Rib) polymerase activity has been performed in purified nuclei simultaneously with determinations of the following enzyme activities: DNA dependent DNA polymerase, DNA dependent RNA polymerase I and DNA dependent RNA polymerase II.

MATERIALS

Source of the materials: Unlabeled ribo- and deoxyribonucleosidetriphosphates, NAD^+, creatine phosphate, creatine phosphokinase and micrococcal nuclease (from Staphylococcus aureus, 8,000 units/mg), from Boehringer, Mannheim (Germany); diethylstilb-

estrol and progesterone from Serva, Heidelberg (Germany); dithiothreitol from Calbiochem, Los Angeles (USA); nicotinamide-[^{14}C]-adenine dinucleotide (specific activity 260 mCi/mmole), [^{3}H] dTTP (specific activity 30 Ci/mmole) and [^{3}H] ATP (specific activity 21 Ci/mmole) from The Radiochemical Centre, Amersham (England); DNase I (beef pancreas, 2,500 units/mg), RNase A (beef pancreas, 3,000 units/mg) and phosphodiesterase I (Crotalus adamanteus) from Worthington Biochemical Corp., Freehold (USA); GF/C filters from Hormuth and Vetter, Heidelberg (Germany).

METHODS

Animals. Immature female japanese quails (Coturnix japonica), with an average weight of about 100 g at an age of 35 days were obtained from J. Bökamp, Schloß Holte (Germany).

Treatment of quails. 5 mg diethylstilbestrol in 0.25 ml sesame oil were injected into quails subcutaneously on five consecutive days (= primary stimulation), followed by a period of withdrawal for 16 days; then the quails were injected again for four days with diethylstilbestrol for secondary stimulation. Hormones were given 15 hours prior to decapitation. 25 oviducts from untreated quails and 5 oviducts each, from animals treated with hormones, were used for the determinations.

In one set of experiments avidin synthesis was induced in diethylstilbestrol-stimulated oviducts in response to progesterone. Quails treated for five days with diethylstilbestrol (primary stimulation) were injected subsequently for three days subcutaneously with progesterone (5 mg progesterone dissolved in 0.25 ml sesame oil) and diethylstilbestrol or with diethylstilbestrol alone.

Nuclei. Oviduct nuclei were isolated according to McGuire et al. (13). The packed, purified nuclei were suspended in 10 mM Tris-HCl, pH 8.0 and 250 mM sucrose. Preparations containing 3.6 mg DNA/ml, 2.7 mg RNA/ml and 24 mg protein/ml were assayed for the different polymerase activities. The activity of DNA dependent RNA polymerase was determined immediately while for the determination of the activities of poly(ADP-Rib) polymerase and of the DNA dependent DNA polymerase preparations nuclei could be used, which had been stored previously in frozen state. The latter two enzymes do not lose activity during the freezing period.

Poly(ADP-Rib) polymerase assay. The polymerase was assayed in a reaction mixture containing in a total volume of 70 µl : 100 mM Tris-HCl, pH 8.5, 6 mM $MgCl_2$, 60 mM KCl, 4 mM dithiothreitol and 1.5 µM $[^{14}C]$ NAD^+ (0.4 mCi/mmole) and 20 µl of the enzyme sample to be assayed. The mixture was incubated for 30 min at 25°C. The reaction was stopped by the addition of 1 ml of 5% trichloroacetic acid. After 30 min at 0°C the mixture was then transferred onto GF/C filters; the filters were washed three times with 10 ml aliquots of 5% trichloroacetic acid and dried. The radioactivity was determined in a liquid scintillation spectrometer using a dioxane scintillator (14). The specific activity of the enzyme from nuclei of animals treated five days with diethylstilbestrol (primary stimulation) was 6.4 nmoles $[^{14}C]$ NAD^+ incorporated per mg protein x 30 min.

DNA dependent DNA polymerase assay. The DNA polymerase assay mixture (80 µl) contained the following components : 10 mM Tris-HCl, pH 7.5, 4 mM $MgCl_2$, 8 mM KCl, 1 mM 2-mercaptoethanol, 100 µM each of the unlabeled deoxyribonucleoside triphosphates, 10 µM $[^{3}H]$ dTTP (15 mCi/mmole) and the enzyme sample to be assayed (20 µl). The reaction was incubated for 20 min at 37°C. The acid precipitable radioactivity was collected and counted as previously described (15). The specific activity of the enzyme from nuclei of animals treated five days with diethylstilbestrol (primary stimulation) was 94 pmoles $[^{3}H]$ dTTP incorporated per mg protein x 20 min.

DNA dependent RNA polymerase I and II assay. The RNA polymerase I assay contained according to Austin et al (16) in a total volume of 100 µl the following components : 50 mM Tris-HCl, pH 7.8, 2 mM $MnCl_2$, 40 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 320 µM each of the unlabeled ribonucleoside triphosphates, 10 µM $[^{3}H]$ ATP (10 mCi/mmole), 2 mM creatine phosphate, 20 µg/ml creatine phosphokinase and the enzyme sample to be assayed (20 µl). The RNA polymerase II was assayed using 80 mM $(NH_4)_2SO_4$ instead of 40 mM $(NH_4)_2SO_4$.
The reaction was incubated for 10 min at 37°C. The acid precipitable radioactivity was collected and counted as described for poly(ADP-Rib) polymerase. The specific activity of enzymes from nuclei of animals treated five days with diethylstilbestrol (primary stimulation) was : RNA polymerase I 85 pmoles $[^{3}H]$ ATP incorporated per mg protein x 10 min; RNA polymerase II 116 pmoles/mg x 10 min.

The incorporation of the labeled precursors into acid insoluble material in the different enzyme assays, is expressed throughout this communication in dpm/mg protein. Each value in Fig. 1 and Fig. 2 (mean $\pm$ S.D.) comes from 5 parallel assays.

Chemical determinations. For protein determinations the method of Lowry et al. (17) was used. RNA was determined as described (18), DNA by the procedure of Kissane et al. (19).

RESULTS

Characterization of the product synthesized by poly(ADP-Rib) polymerase. The sensitivity of the product, synthesized in vitro by poly(ADP-Rib) polymerase, towards various enzymes has been determined (Table 1). From the DNA and RNA nucleases tested, only the phosphodiesterase I from Crotalus adamanteus reduces the amount of acid precipitable material synthesized by poly(ADP-Rib) polymerase.

Change of poly(ADP-Rib) polymerase activity during estrogen induction. The oviduct wet weight depends strongly on treatment of the quails with diethylstilbestrol (Fig.1,A). During primary stimulation for five days the weight of the oviduct increases from 0.1 g to 1.7 g. In a subsequent period of withdrawal of hormone, the oviduct weight decreases to 0.3 g in the course of 16 days. The secondary stimulation produces a more pronounced growth of the oviduct.

The activity of the DNA dependent DNA polymerase as well as of the poly(ADP-Rib) polymerase follows essentially the same pattern as the growth of oviduct during primary stimulation (Fig. 1, B). The activities of both enzymes are strongly reduced during withdrawal of the hormone. During secondary stimulation the activities of the two enzymes do not change significantly.

Fig. 1, C illustrates the activity of DNA dependent RNA polymerase I and II during estrogen treatment. During primary stimulation the activities of RNA polymerase forms I and II strongly increase while after withdrawal the activities of the two RNA polymerases decline to values present in nuclei of unstimulated animals. The secondary stimulation with diethylstilbestrol produces again an increase of the two activities; the increase of RNA polymerase II is more pronounced.

Enzyme	Enzyme concentration per ml	Acid precipitable dpm
None	—	5,300
DNase I	50 µg	5,150
RNase A	50 µg	5,350
Phosphodiesterase I	50 µg	430
Micrococcal nuclease	50 µg	5,400

Table 1: Effect of various enzymes on the acid precipitable material synthesized by the poly(ADP-Rib) polymerase. After incubation (see under Methods) the enzyme reaction was stopped by heating the mixture 30 sec at 100°C. Then the material was incubated with the different enzymes at 37°C for 60 min; the amount of acid precipitable material was determined as described under methods. Nuclei from animals, treated five days with diethylstilbestrol (primary stimulation), were used as source for the enzyme.

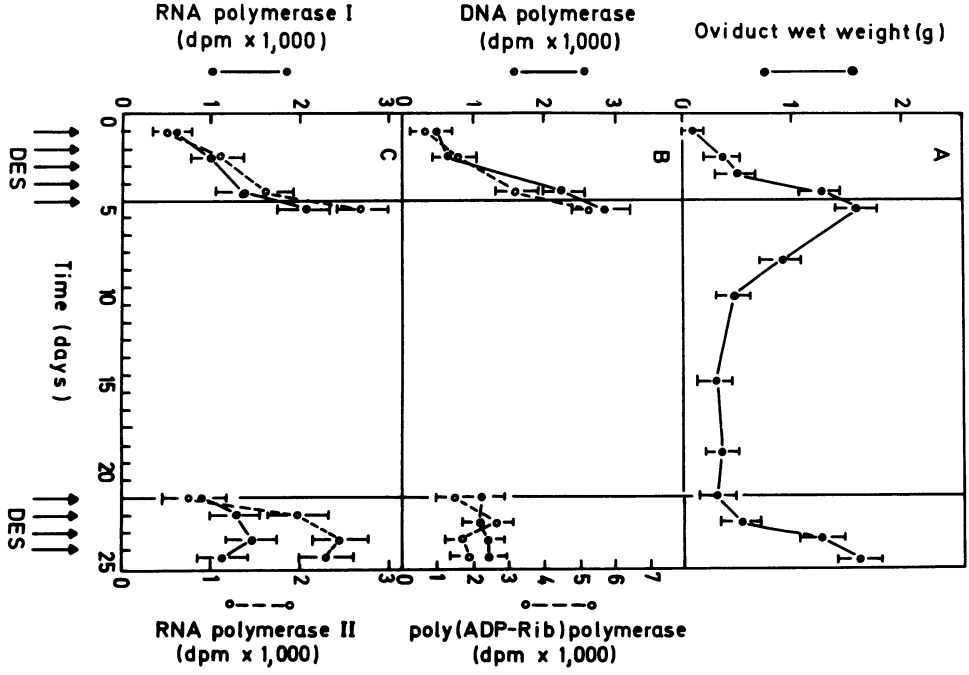

Fig. 1: Effect of primary and secondary stimulation by diethylstilbestrol (DES) on oviduct wet weight and different DNA dependent polymerase activities.

Change of poly(ADP-Rib) polymerase activity during progesterone induction. After the rapid growth of the oviduct during the primary stimulation period of five days, the rate of increase in oviduct wet weight declines. In the experiments summarized in Fig. 2, the quails, previously stimulated for five days with diethylstilbestrol, were treated three days longer with this estrogen hormone without (Fig. 2, left row) or in combination with progesterone (Fig. 2, right row). During this prolonged primary stimulation period the increase of oviduct wet weight is relatively low (Fig. 2, A). A simultaneous administration of progesterone for three days does not change the growth curve of the oviduct significantly.

During a prolonged primary stimulation period with diethylstilbestrol alone (Fig. 2, B left) as well as during a further treatment with this estrogen hormone in combination with progesterone (Fig. 2, B right) the activity of DNA polymerase does not change. However the activity pattern of this enzyme is significantly different from the one of the poly(ADP-Rib) polymerase during the administration with progesterone (Fig. 2, B right). During three progesterone applications the activity of the poly(ADP-Rib) polymerase decreases from 6,000 dpm to 1,300 dpm, while the DNA polymerase activity remains almost constant (2,800 to 2,300 dpm).

The prolonged primary stimulation with diethylstilbestrol does not markedly change (Fig. 2, C left) the activity of RNA polymerase of as well form I as of form II whereas a simultaneous administration of progesterone causes an increase of RNA polymerase II only (Fig. 2, C right).

DISCUSSION

The product synthesized from NAD^+ by the poly(ADP-Rib) polymerase, isolated from the oviducts of quails, has a similar enzyme sensitivity as the ones known from other eukaryotic systems (3,5,6), in which only phosphodiesterase I produces a significant degradation. The phosphodiesterase product had been identified as 2'-[5''-phosphoribosyl]-5'-adenosine monophosphate (3).

After exogenous primary stimulation of immature quails with estrogen hormones an onset of proliferation of the oviduct mucosa cells occurs. In the course of this event the epithelial cells differentiate from homogeneous-appearing, primitive cells of the mucosa, to three distinct types of cells (tubular gland cells, goblet cells and ciliated cells) (11). This primary hormonal stimulation is accompanied by a strong DNA syn-

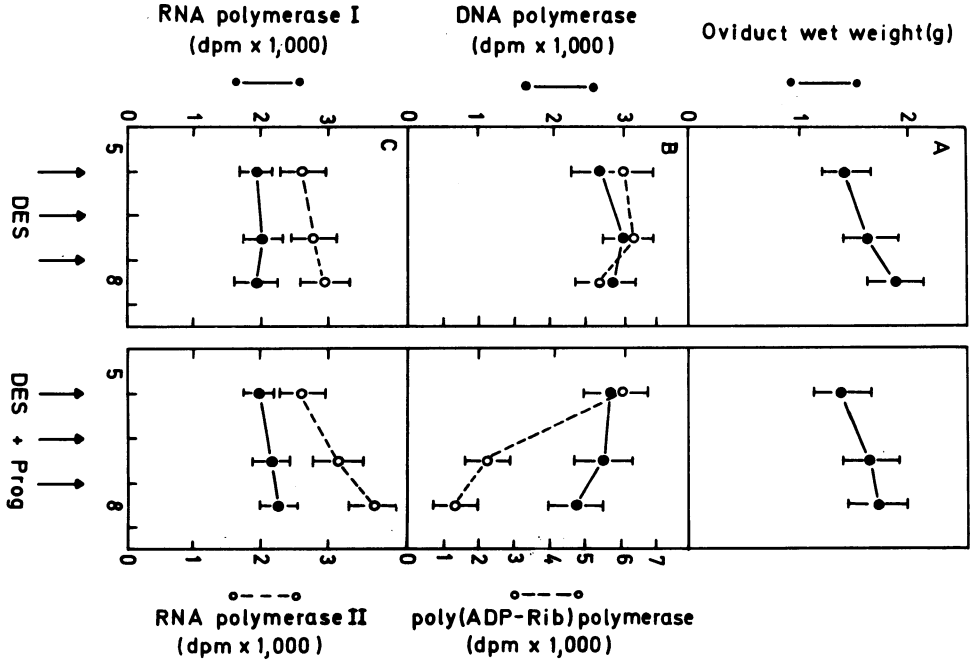

Fig. 2: Effect of progesterone (Prog) on oviduct wet weight and different DNA dependent polymerase activities.

thesis probably caused by an increase in DNA dependent DNA polymerase activity in the oviduct cells. The activity of the DNA polymerase in the nuclei increases in a linear fashion and follows the same pattern as the oviduct growth (Fig. 1). The finding that the activity of poly(ADP-Rib) polymerase seems to be strongly correlated with both the activity of DNA polymerase and the increase of oviduct wet weight is striking. The hormone-induced synthesis of cell-specific proteins (ovalbumin and lysozyme) is occuring in the tubular gland cells (11) as a second event besides the enlarged DNA synthesis during primary stimulation. The acceleration of cell-specific protein synthesis reflects an increase of r-RNA and m-RNA synthesis (20) mediated by DNA dependent RNA polymerases (11). The enzymes responsible for the synthesis of the two RNA species are present in higher activity in the nuclei (Fig. 1): The RNA polymerase I which is involved in the read-out of nucleolar (primarily ribosomal) RNA and the RNA polymerase II which transcribes primarily nucleoplasmic genes (synthesis of m-RNA) (21).

After withdrawal of estrogen hormones some processes induced during primary stimulation are reversible: Increase of oviduct wet weight and synthesis of cell-specific proteins (11,20). The differentiation stage attained and the amount of cells in the oviduct mucosa can however be not reversed (22). After administration of estrogen hormones (secondary stimulation), again RNA- and cell-specific protein synthesis are the main events. The level of DNA synthesis remains almost unaffected during secondary stimulation since the gland cells are already present (20). R-RNA synthesis is low during the early stages of this period (20); m-RNA synthesis is dominating. Thus, in the nuclei from oviducts of early stages during secondary stimulation only the activity of the RNA polymerase II is augmented; the increase of the RNA polymerase I activity is not significant, the DNA polymerase activity remains constant (Fig. 1). The activity of poly(ADP-Rib) polymerase in the nuclei does not change during the period of secondary stimulation.

These findings may indicated that a close correlation exists between poly(ADP-Rib) polymerase activity and DNA polymerase activity during primary exogenous estrogen stimulation, which is characterized by a rapid cell proliferation and a high rate of cytodifferentiation.

Administration of a single dose of progesterone to estrogen-stimulated birds results in an increase of RNA polymerase activity, in an increase of rapidly labeled nuclear

RNA and in a synthesis of a specific protein (avidin) (11). These processes occur in the goblet cells of the oviduct mucosa. The DNA synthesis is not influenced by progesterone (11). From the nuclear enzymes determined in this study, the activity of two polymerases are markedly affected after administration of progesterone to quails during the period of primary estrogen stimulation. The RNA polymerase II activity increases while the activity of poly(ADP-Rib) polymerase drops to values found in unstimulated oviducts and in oviducts during secondary stimulation. From the results obtained with the biological model of in vivo oviduct avidin induction, it seems to be reasonable to conclude that gene expression may occur at low poly(ADP-Rib) synthesis.

Summarizing the results of the estrogen-induced cell proliferation and cytodifferentiation system as well as the progesterone-stimulated avidin system of the quail oviduct a positive correlation has been found between the activities of DNA polymerase and poly(ADP-Rib).polymerase and a negative correlation between the activities of RNA polymerase II and poly(ADP-Rib) polymerase. Further experiments on the subcellular level are necessary to determine whether these correlations are accidental or not. Additionally, it has to be clarified whether the observed incorporation rate in the assays with different poly(ADP-Rib) polymerase preparations is parallel with the activity of this enzyme or whether the amount of the incorporated poly(ADP-Rib) into the in vitro assay is also dependent on poly(ADP-Rib) degrading enzymes (23), which might be present in different amounts in the polymerase preparations.

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