ACTIVITY OF DNA-DEPENDENT RNA POLYMERASES IN RABBIT MAMMARY GLAND DURING LACTOGENESIS

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1. Introduction

Eukaryotic cells contain several DNA-dependent RNA polymerases (EC 2.7.7.6.) [1]. In the rat mammary gland, similarly as in other tissues, the major two RNA polymerases A and B have been separated by DEAE-Sephadex chromatography [2]. These enzymes have been distinguished on the basis of their sensitivity to α-amanitin, which acts as a highly selective inhibitor of polymerase B responsible for the synthesis of heterogeneous nuclear RNA, without affecting polymerase A transcribing ribosomal cistrons [1,3].

We have previously reported that rabbit mammary gland nuclei exhibit a higher RNA synthesis ability early in lactation than late in pregnancy [4]. At the same time the concentration of RNA/g wet wt of rabbit mammary gland has been found to be twice increased [5]. In the present study we determined the contribution of polymerases A and B to the total RNA synthesis in nuclei isolated about the time of parturition. In addition, the activity of RNA polymerases in nuclei during the mammary gland development were compared with that of solubilized preparations of the same enzymes assayed with calf thymus DNA as template.

2. Materials and methods

α-Amanitin was purchased from Boehringer, Mannheim, FRG. [5,6-^{3}H]Uridine-5′-triphosphate (ammonium salt), specific activity 12 Ci/mmol, obtained from the Radiochemical Centre, Amersham, England, was diluted with cold UTP to a final concentration of 500 Ci/mol and made ethanol-free.

Great Popielno-White rabbits in their first pregnancy or lactation were used.

The procedure of isolation and purification of nuclei was as described previously [6]. Rabbit mammary glands were homogenized in 3 vols buffer (0.25 M sucrose, 20 mM Tris–HCl, pH 7.8, 2 mM $MgCl_2$, 1 mM dithiothreitol). Nuclei were purified by sedimentation through 1.8 M sucrose.

RNA polymerases from mammary glands were solubilized and purified by the procedure of Roeder [7], involving sonication of chromatin at high ionic strength, ultracentrifugation, selective precipitation by $(NH_4)_2SO_4$ and ultracentrifugation, but without the step of DEAE-Sephadex chromatography. In the enzyme preparations polymerases A and B were not yet separated but they could be distinguished by their response to low doses of α-amanitin.

RNA polymerase activity was determined by measuring the incorporation of [^{3}H]UTP into acid-precipitable RNA in the absence or presence of α-amanitin (3.6 μg/ml). Assays with nuclei were performed as described previously [8]. Incubations, usually in duplicate, were in final vol. 0.15 ml and contained: 40 mM Tris–HCl, pH 8.0, 15% v/v glycerol, 4 mM $MgCl_2$, 3 mM $MnCl_2$, 100 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 0.4 mM each of ATP, GTP and CTP, 0.04 mM [^{3}H]UTP and nuclear suspension (15–45 μg DNA).

The assay mixtures with the solubilized RNA polymerases were in final vol. 75 μl and contained according to Roeder [7]: 70 mM Tris–HCl, pH 7.9, 12.5% glycerol, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 100 mM

$(NH_4)_2SO_4$, 0.2 mM dithiothreitol, 0.6 mM each of ATP, GTP and CTP, 0.02 mM [^{3}H]UTP, 0.04 mM EDTA, 7.5 μg native calf thymus DNA and 30–60 μg protein in enzyme preparation plus 30 μg bovine serum albumin.

Samples were incubated either for 15 min at 37°C (nuclei) or for 30 min at 30°C (solubilized enzymes) and then processed as described previously [8].

Polymerase B activity was quantitated by subtracting α-amanitin-insensitive RNA synthesis from total RNA synthesis. Polymerase A activity was determined by subtracting the value of a blank run with actinomycin D (10 μg/ml) from that of the sample with α-amanitin.

We found that polymerase A and B were responsible for the major proportion of the total RNA polymerase activity in nuclei. Polymerase C accounted for less than 10% (data not shown). Therefore, at low doses of α-amanitin, polymerases A and C were determined jointly.

Protein was assayed by the method of Lowry et al [9]. DNA was determined by the diphenylamine reaction [10].

3. Results and discussion

Figure 1a illustrates the rate of RNA synthesis in mammary gland nuclei late in pregnancy and early in lactation. Isolated nuclei were shown to exhibit a nearly two-times higher RNA synthetic activity on day-7 of lactation than on day 24 of pregnancy.

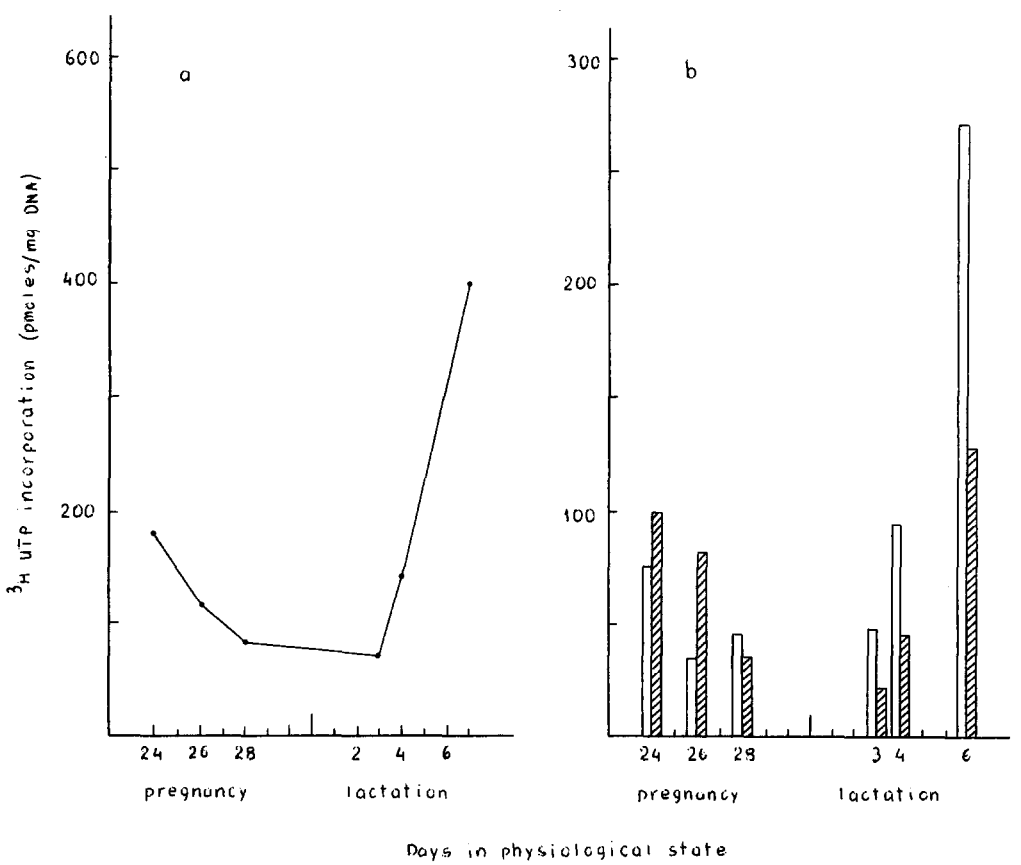

Fig.1. RNA synthesis in rabbit mammary gland nuclei. (a) Total RNA synthesis. (b) Polymerase A-directed RNA synthesis (▭), polymerase B-directed RNA synthesis (▨). The rate of RNA synthesis was expressed as pmol [^{3}H]UTP incorporated/mg DNA.

Between these days, however, no gradual increase in the RNA synthesis capacity occurred. On the contrary, at about the time of parturition a transient decrease in the rate of the [^{3}H]UTP incorporation into RNA was observed.

In order to define the contribution of polymerases A and B to the total RNA synthesis, studies with α-amanitin were performed. Experiments reported in fig.1b indicated that the intensity of the polymerase A-directed RNA synthesis decreased on day-28 of pregnancy and remained at a similar low level on day-3 of lactation. Subsequently it was greatly increased, and on day-7 of lactation the [^{3}H]UTP incorporation in the presence of α-amanitin was about two-times higher than that observed on day-24 of pregnancy.

The rate of polymerase B-directed RNA synthesis decreased continuously till day-3 of lactation. Thereafter it increased, though to smaller extent, as compared with the rate of the α-amanitin-insensitive reaction. Moreover, on day-7 of lactation the rate of the polymerase B-directed RNA synthesis did not pronouncedly exceed that observed on day-24 of pregnancy.

The contribution of polymerase A to the total RNA synthesis in isolated nuclei, expressed as percentage of the total [^{3}H]UTP incorporation, was 44% and 31% on days 24 and 26 of pregnancy, respectively, and 68% on day-7 of lactation. Thus, the enhancement of the RNA synthesis in nuclei early in lactation was greatly influenced by RNA polymerase A.

The rate of the RNA synthesis could be altered by changes in the template availability of chromatin and/or in the enzyme activities. In order to study the latter possibility, experiments using partially purified mammary gland RNA polymerases were performed with calf thymus DNA as template (table 1). It was shown that the polymerase B activity did not change markedly, while the polymerase A activity was on day-5 of lactation 4.3-times higher than on day-21 of pregnancy. Variations in the contribution of RNA polymerases to the total RNA synthesis resembled those observed upon the use of nuclei.

Our results are in agreement with the autoradiographic data [11] which indicate that in the mouse mammary gland during transition from the non-secretory to secretory state the incorporation of [^{3}H]uridine preferentially increases in the nucleolus, i.e., the site of the polymerase A action. High activity of RNA polymerase A was also been found in lactating rats [12].

The increased contribution of RNA polymerase A to the total RNA synthesis in the rabbit mammary gland early in lactation corresponds to the preferential rise of rRNA content at lactation, reported in this species by Deutsch and Norgren [13]. These observations suggest that changes in the RNA polymerase A activity reflect the in vivo ability of the mammary gland to transcribe ribosomal genes. The elevated activity of RNA polymerase A in lactating mammary cells may be related to the increased requirement of rRNA molecules during lactation.

Variations in physiological conditions have also

Table 1
Activity of rabbit mammary gland RNA polymerases during pregnancy and lactation, assayed with calf thymus DNA as template

Physiological state	Polymerase activity (pmol UMP incorporated)		Polymerase contribution (% total incorporation)	
	A	B	A	B
Pregnancy (day-21)	21	51	29	71
Pregnancy (day-25)	14	41	25	75
Lactation (day-4)	34	56	37	62
Lactation (day-5)	91	50	65	35

RNA polymerases A and B were solubilized from total cellular homogenate and assayed for activity with calf thymus DNA in the presence or absence of α-amanitin. The enzyme activity was expressed as pmol/mg mammary gland DNA.

been shown to influence greatly, cellular levels of RNA polymerase A activity in other tissues characterized by a rapid growth-rate and/or high levels of protein synthesis [14].

By contrast the activity of RNA polymerase B was relatively invariant during mammary gland development (table 1). RNA polymerase B transcribes a heterogenous group of genes [15–17]. Therefore, it has been suggested that the levels of this enzyme alone cannot differentially regulate the rates of synthesis of specific gene products and that additional components may direct enzyme specificity, or activity in vivo, or both [14].

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