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Effect of Stage of Development and Function on the Template Activity of Rat Mammary Gland Chromatin[1] (38006)

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(Introduced by W. R. Ruegamer)

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The mammary gland undergoes both structural and functional changes during pregnancy and lactation. The amounts of DNA and RNA in the glands of rats increase gradually during pregnancy followed by a rapid increase at the onset of lactation (1–3). The levels of RNA continue to increase through midlactation and closely parallel the changes in levels of many mammary gland enzymes (3, 4). Prolactin is thought to be responsible for the initiation of lactation at parturition (5). This relationship is further indicated in studies employing mammary gland explants in which prolactin is able to initiate the synthesis of casein and whey proteins in explants which have been permitted to undergo cell division in the presence of insulin and hydrocortisone (6). The increased rate of RNA synthesis and the initiation of milk protein synthesis suggest that there is marked activation of mammary gland genes at parturition. The purpose of this brief study is to evaluate the effects of stage of mammary gland development and function on the capacity of mammary gland chromatin to serve as a template for DNA-dependent RNA polymerase.

Materials and Methods. Mature, albino rats (Small Animal Supply Company, Omaha) which had weaned their first litter 2–4 weeks previously were used in this study. Groups of 10 rats were mated at various intervals and the date of copulation was noted by the presence of sperm in the vagina. Matings were planned such that groups of 6 animals representative of early and late stages of pregnancy and early and late stages of lactation could be sacrificed at the same time. At autopsy, the animals were killed by cervical dislocation and both abdominal mammary glands were quickly removed, frozen between two blocks of dry ice, and stored at −40°. Within 1 week samples of individual glands (0.5–1.0 g) were minced and chromatin was prepared as described previously for uterine chromatin (7). Template activity of the chromatin was determined by measuring the rate of incorporation of ATP-8-^{14}C into RNA by DNA-dependent RNA polymerase (purified from *E. coli* strain B). DNA, RNA, histone and nonhistone protein content of the chromatin and template activity assays were also as previously described (7).

Results. The dependency of the RNA synthesizing system on added mammary gland chromatin for the synthesis of RNA is given in Fig. 1. The rate of incorporation of ^{14}C-ATP into RNA (pmoles AMP incorporated/5 min) increases linearly with the addition of chromatin to the level of 18 μg/assay (0.25 ml) for chromatin derived from either nonpregnant or lactating (14 days) mammary glands. All subsequent

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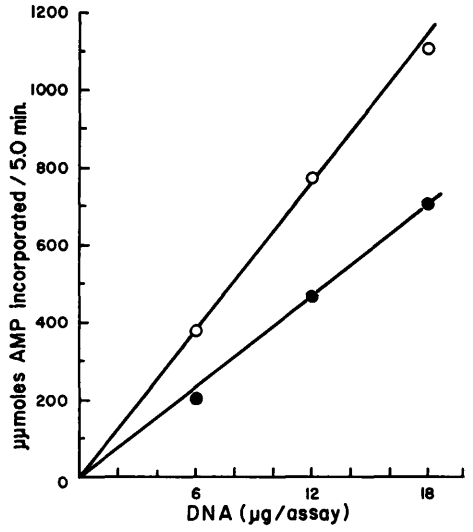

FIG. 1. Dependency of the *in vitro* RNA synthesizing system on the addition of mammary gland chromatin. Chromatin from a nonlactating, nonpregnant rat (closed circles) and a lactating rat (14 days) (open circles) was added to the reaction in the indicated amounts. In addition to chromatin, the reaction mixture contained 10 μmoles Tris–HCl, pH 8.0; 1.0 μmole $MgCl_2$; 0.25 μmole $MnCl_2$, 3.0 μmoles β-mercaptoethanol; 0.1 μmole each of CTP, UTP, GTP, and ATP-8-^{14}C (1000 cpm/nmole), and an amount of purified *E. coli* DNA-dependent RNA polymerase capable of incorporating 7,500 pmoles of AMP into RNA/5 min when 50 μg of purified salmon sperm DNA was used as template. Final volume was 0.25 ml. Each assay was performed in triplicate.

comparisons were made using 12 μg of chromatin DNA per assay.

The effect of stage of mammary gland development and function on the template activity of mammary gland chromatin is given in Table I. The template activities of mammary gland chromatin during early gestation (7–14 days) and late gestation (16–21 days) are the same and are increased by 20% above that seen in the glands of nonpregnant, nonlactating rats. Shortly after the onset of lactation (4 days), the template activity of mammary gland chromatin increases to 179% of the template activity seen in late gestation (16–21 days) and this elevated activity is maintained through the later stages (14–16 days) of lactation.

The chemical composition of mammary gland chromatin as a function of stage of development and function of the organ is given in Table II. With the exception of the slight decrease in the amount of nonhistone protein relative to DNA, the overall composition of the chromatin does not change during gestation. After the onset of lactation there is an increase in the amount of RNA (227 to 354%), histone (123 to 132%) and nonhistone protein (114 to 166%) relative to the amounts of these components in chromatin prepared from nonpregnant, nonlactating rats.

Discussion. The template activity of chromatin in directing the synthesis of RNA by *E. coli* DNA-dependent RNA polymerase has been shown to increase in relation to gene activation (8). The process of gene activation as measured by this assay is a complex one and the results must be interpreted accordingly. An increase in the template activity of chromatin reflects a composite of chromatin associated events including the binding of RNA polymerase, the rate of initiation of polynucleotide chains, the rate and extent of elongation of chains, the rate of detachment and reinitiation of RNA synthesis, and the rate of RNA hydrolysis associated with RNA processing systems which might be in close association with the isolated chromatin (9). Differences in gene activity can presumably involve activation of new genes or an alteration in the "ease" or "rate" of transcription of the same gene set. The type of template activity assays employed in this brief study do not differentiate between these possibilities.

The results of this study indicate that the template activity of mammary gland chromatin is increased at parturition. Within the limitations discussed above, these results indicate that a direct activation of mammary gland genes (chromatin) occurs at this stage of physiological transition. As the synthesis of milk proteins begins at this time, it would seem reasonable that the increased template activity results, at least in part, from the activation of a new set of genes. This activation is presumably the result of prolactin action (5). In addition, the mammary gland undergoes extensive hypertrophy after parturition and it is likely that

TABLE I. Effect of Stage of Development and Function on the Template Activity of Mammary Gland Chromatin in the DNA-Dependent RNA Polymerase Reaction.

Stage of development	n	Chromatin template activity[c] (pmoles AMP/5 min/12 μg DNA)
Nonpregnant	6	561 ± 5
Pregnant[a]		677 ± 47
7–14 days	6	
16–21 days	6	677 ± 31
Lactating[b]		1,211 ± 33
4 days	6	
14–16 days	6	1,167 ± 21

[a] Days after mating (sperm positive).

[b] Days after parturition.

[c] Template activity is expressed as pmoles of ATP incorporated into RNA as AMP by *E. coli* DNA-dependent RNA polymerase during a 5-min incubation with chromatin containing 12 μg of DNA as the sole source of template. The added polymerase was capable of incorporating 10,000 pmoles AMP into RNA/5 min when 50 μg of purified salmon sperm DNA was used as template, and under these conditions, purified rat mammary gland and liver DNA has a template activity of 7620 pmoles AMP/5 min/12 μg DNA. Incorporation by the polymerase in the absence of added template (67 pmoles) has been subtracted from the values reported. Each value represents the average ± SE. Each chromatin sample was assayed in triplicate.

the increased chromatin template activity might be associated with the activation of genes required for synthesis of new proteins involved in DNA synthesis and cell division. Numerous examples are known in which an increase in chromatin template activity precedes DNA synthesis. These include the regenerating rat liver (10, 11), the isoproterenol stimulated salivary gland (12), WI-38 human diploid fibroblasts in tissue culture (13, 14), the estrogen stimulated rat uterus (7), and many others. The very slight increase in template activity of mammary gland chromatin during pregnancy is probably related to the growth of the gland during this interval.

It is difficult to predict the cause and effect relationship between the compositional changes in chromatin prepared from mammary tissue at various stages of development and function, but there is a rather striking increase in the amount of RNA in chromatin after parturition. This increase probably reflects an increase in the amount of nascent RNA remaining attached to the chromatin during this interval when the rate

TABLE II. Effect of Stage of Development and Function of the Mammary Gland on the Composition of Purified Chromatin.

	Composition (mass ratios)			
	DNA	RNA	Histone	Nonhistone protein
Nonpregnant	1	0.11	1.31	0.74
Pregnant[a]				
7–14 days	1	—	—	0.66
16–21 days	1	0.13	1.32	0.55
Lactating[b]				
4 days	1	0.25	1.62	0.84
14–16 days	1	0.39	1.73	1.23

[a] Days after mating (sperm positive).

[b] Days after parturition.

of RNA synthesis and presumably the number of activated genes has increased.

Summary. The ability of purified mammary gland chromatin to serve as a template for DNA-dependent RNA polymerase increases slightly during gestation and increases nearly twofold after the onset of lactation. The composition of the purified chromatin changes at parturition and is characterized by a 2–3-fold increase in RNA, a 1.2–1.3-fold increase in histone, and a 1.1–1.7-fold increase in nonhistone chromatin proteins. Since the synthesis of milk proteins begins coincidentally with the increase in chromatin template activity, the response probably results from the expression of a new set of genes although the possibility of an increase in the rate of expression of previously active genes cannot be excluded.

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