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PROLACTIN HOMOGENEOUSLY INDUCES THE tRNA POPULATION OF MOUSE MAMMARY EXPLANTS

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SUMMARY: Explants of mouse mammary glands were cultured with and without prolactin in the presence of inorganic [^{32}P] to estimate the effect of prolactin on tRNA synthesis. Labeled tRNA was extracted and characterized by two-dimensional gel electrophoresis. tRNA synthesis was 2-3 fold greater in the presence of prolactin; and the synthesis rate of each resolvable tRNA species was increased proportionally. tRNA populations from mouse mammary tissues at three stages of development were also examined. Alterations were noted between early pregnant and fully lactating tissues. The results of this study provide evidence that the tRNA population, which is known to be "specialized" for casein synthesis in the mammary gland, is determined as the gland develops and prepares for lactation.

Prominent among the events leading to the production of milk proteins in both the intact animal and explanted glands is the accumulation of RNA in the mammary gland. Several steroid and peptide hormones are required in concert to bring about the observed results. This subject has been reviewed (1,2). Prolactin has been found to be a major requirement for the increased RNA content of epithelial cells producing milk proteins (3). The role of glucocorticoids in the prolactin induced accumulation of caseins has been discussed (4-8). The increased RNA content is quantitatively accounted for by increases in the rates of synthesis of ribosomal RNA and transfer RNA. In explants of mammary glands (4,5) and in nuclei isolated from intact animals (9), prolactin is required for the increase to occur. The increased synthesis rates and the resulting accumulation have been found to be about the same for rRNA, tRNA, hnRNA, and mRNA coding for nonspecialized proteins (5,10). The much greater relative accumulation of mRNAs for the milk proteins has been ascribed not only to increased synthesis,

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but also to the prolactin-dependent acquisition of a 17-20 fold increase in the half-life of these mRNAs (10).

The intent of this study was to determine whether the increased amount of tRNA which is observed in response to prolactin in mammary gland explants is uniform for all tRNAs (as it is for RNA species in general) or whether the tRNA distribution is altered by selective changes in synthesis rates. We have used a modification of published two dimensional gel electrophoresis techniques (11,12) to compare the tRNA populations from mouse mammary explants cultured with and without prolactin. To determine if changes in tRNA patterns occur during development of the gland, tRNA populations from mouse mammary tissues at 7-8 days into pregnancy, 18 days into pregnancy and 10 days into lactation were examined.

MATERIALS AND METHODS

Reagents: Medium 199 with and without inorganic phosphate was obtained as described (13). Ovine prolactin, NIAMDDK PRL - 14, 31.0 I.U./mg was a gift from the Pituitary Hormone Distribution Program, NIAMDDK, NIH and [^{32}P]orthophosphoric acid in water at 1.0 Ci/ml was purchased from New England Nuclear Corporation. Other materials and their sources were as described (13).

Mice and mammary gland cultures: Abdominal mammary glands from two or three BALB/c AnN mice (obtained from NIH) 8-9 days in their first pregnancy were cultured as explants as described (13); the medium contained insulin, hydrocortisone and in addition prolactin at 0, 100 or 5000 ng/ml. In labeled explants, the medium was replaced on the final day with phosphate-free Medium 199 containing 25 Ci/ml of [^{32}P] and incubation was carried out for 20-22 hours. Other details were as described previously (13).

Preparation of tRNA and gel electrophoresis: tRNA was extracted from mouse tissues by a modification of the procedure of Roe (14). Tissue (40 to 400 mgs), buffer (0.14 M sodium acetate, pH 4.5 and 0.01 M magnesium chloride) and water saturated phenol (1:5.6:4; wt:v:v) were homogenized for two minutes in a glass homogenizer with a motor-driven teflon pestle, then shaken vigorously on a rotary shaker for 15 minutes at room temperature. The phases were separated and 3 volumes of cold 95% ethanol added to the upper phase. The resulting precipitate was collected, washed with cold 95% ethanol, and redissolved in 25 to 100 l of water. The redissolved material was centrifuged for 7.5 minutes in an Eppendorf centrifuge, transferred to a fresh tube, and held at 4°C for gel electrophoresis.

Two-dimensional polyacrylamide gel electrophoresis was carried out as follows: The first dimension was performed on a 17.5% gel (1.5 mm x 14 cm x 12.5 cm; 5% cross-linking) containing 4 M urea. Samples (10 to 30 l, containing tRNA in 8% sucrose with bromophenol blue and xylene cyanol-FF as markers) were loaded onto a 6.25% polyacrylamide stacking gel containing 1.5 x 8.0 x 25 mm wells. Gels were developed for 18 3/4 hours at 150 V and 6°C. Strips (1.2 x 7.0 cm) were cut out from the developed gels 2.5 cm below the top of the running gel and implanted horizontally in a 6.25% stacking gel such that 0.5 cm of stacking gel was present between the implant and the running gel for the second dimension. The second dimension

was performed on a 25% gel (1.5 mm x 14 cm x 13 cm; 5% cross-linking) containing 7M urea. Gels were developed an initial 15 to 18 hours at 150 V and 24°C followed by 46 to 47 hours at 300 V and 24°C. Developed gels containing [^{32}P] samples were exposed to X-ray film (Kodak X-OMAT, XAR-2) for varying time intervals and the film then developed by standard procedures. Developed gels which were not to be used for further electrophoresis or for autoradiograms were stained with Stains-all.

Computer analysis of autoradiograms: Autoradiograms were quantitatively analyzed on a computer system based on that described by Vo et. al. (15). This system is capable of finding virtually any spot resolvable in a two-dimensional gel and measuring the integrated intensity of each spot. (Integrated density is the sum of the machine density for every pixel found within a spot.) This intensity is normalized to the total density of the film and presented as a percentage value.

RESULTS

Explanted glands in organ culture: The synthesis rates of the various tRNAs in the presence and absence of prolactin were estimated by the addition of [^{32}P] to the medium on the last day of explant incubation. The isolated nucleic acids were further resolved by two dimensional electrophoresis, in which approximately equal amounts of radioactivity were loaded on each gel. Because the no-prolactin sample was derived from 17 mg tissue, and the prolactin sample from 6 mg tissue, the prolactin-supplemented culture incorporated 2-3 times as much label, indicating greater tRNA synthesis. The autoradiograms (Fig. 1) from each gel were quantitated by computer analysis (15). The results of this analysis were: 1) The program identified 39 spots common to both gels. In order to resolve as many of the spots in these gels as possible, parameters were set so that oversplitting of spots was allowed. Since no pixel contributes to the integrated intensity of more than one spot (15), the parts of the oversplit spots could be recombined with no loss of accuracy. In only two cases in one gel (prolactin-treated) was the computer unable to resolve spots. In both instances, the spots were in the very dense center of the gel where the optical density exceeded the range of the scanner; and 2) Within the error of the analysis, the distribution of radioactivity among the 39 spots was the same for both gels (see Fig. 2), indicating that the prolactin-dependent increase in tRNA synthesis was uniform in all the tRNA species resolved.

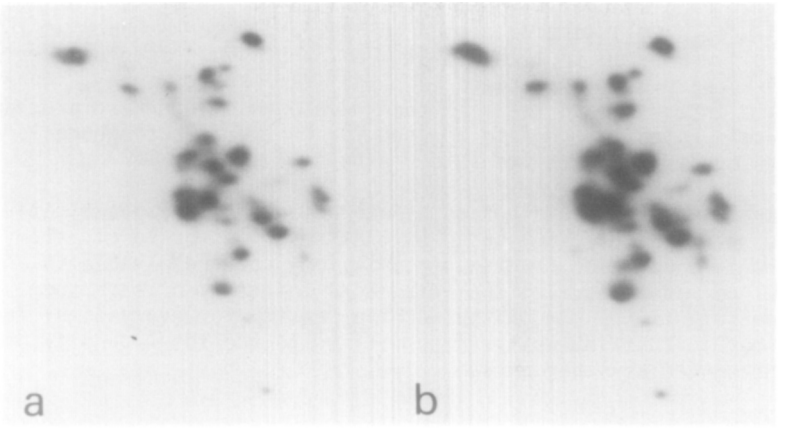

Fig. 1. Autoradiographs of mammary explant tRNAs labeled with [^{32}P] and separated by two dimensional gel electrophoresis on polyacrylamide gels. Panels show tRNA isolated from explants incubated for five days with insulin and hydrocortisone in (a) and for 3 days with insulin and hydrocortisone followed by 2 days with insulin, hydrocortisone and prolactin in (b). 0.3 A_{260} unit of nucleic acid and 3.06 x 10^5 cpm from 17 mgs of tissue (Panel a) and 0.2 A_{260} unit and 4.26 x 10^5 cpm from 6 mgs of tissue (Panel b) were applied to the gels. Direction of migration was from left to right in the first dimension and top to bottom in the second dimension.

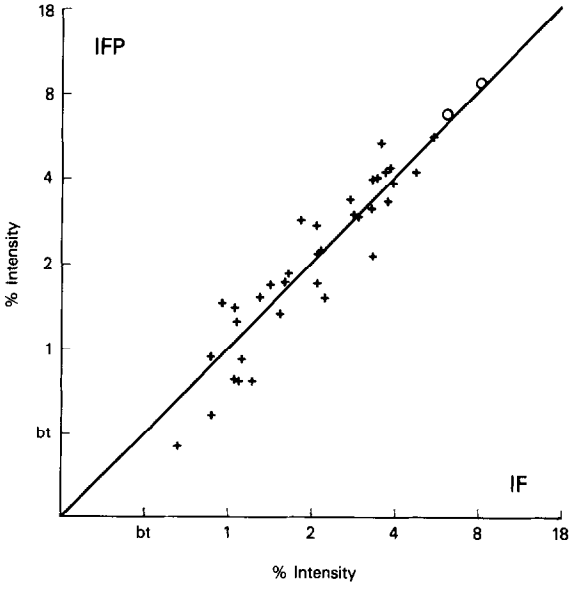

Fig. 2. Scatter plot of the integrated intensities of individual tRNA species. The percent intensity (defined in Material and Methods) of each matched spot in the non-prolactin sample (X-axis) is compared with the corresponding spot resolved in the prolactin induced sample (Y-axis), plotted on a log-log scale. Circles indicate matches to spots that were unresolved in the IFP gel (see text for details). IF = Insulin and hydrocortisone and IFP = Insulin, hydrocortisone and prolactin.

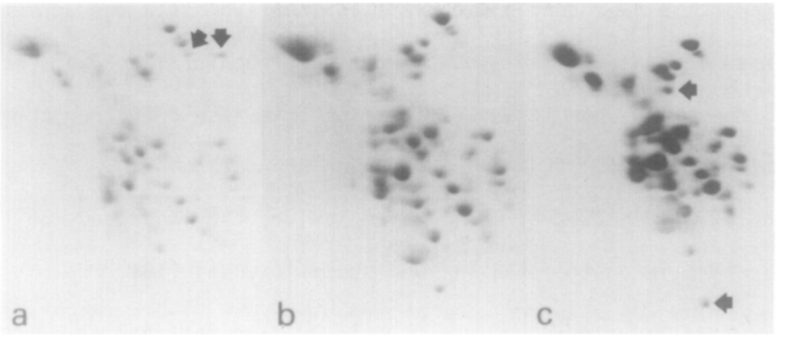

Fig. 3. Two-dimensional gel electrophoresis on polyacrylamide gels of tRNAs from mouse mammary glands. Panels show gland tRNAs from: a, mice 7-8 days into pregnancy; b, 17-18 days into pregnancy; and c, 10 days into lactation. 1.8 A_{260} units of nucleic acid from 232 mgs of tissue (Panel a), 3.2 A_{260} units from 125 mgs of tissue (Panel b) and 2.4 A_{260} units from 63 mgs tissue (Panel c) were applied to gels. Gels were stained with Stains-all. Arrows indicate some of the differences between early pregnancy and fully lactating tissues.

Intact glands: A two-dimensional electrophoretic examination was also made of the tRNA distribution in extracts of mammary glands from mice at three stages of development. Photographs of the stained gels from mammary glands 7-8 days into pregnancy, 18 days into pregnancy and 10 days into lactation are shown in Fig. 3, Panels A - C, respectively. The yield of nucleic acids from mammary gland increased with the progression of pregnancy (16 and the legend to Fig. 3). These differences in tRNA yield could be compensated for partially by adjusting the amount of tissue extract applied to the gel. Careful examination of the tRNA distribution in these three samples reveals several differences among them. As shown by the arrows in the figure some of the more apparent differences occurred between early pregnancy and fully lactating tissues.

DISCUSSION

The two-dimensional electrophoretic analysis of tRNA which we have developed for use in this work has permitted an examination of the relative synthesis rates of individual members of the tRNA population in the very small amounts of tissue which are available from mammary gland explants. Quantitation of the separated tRNAs was achieved by computer analysis of autoradiograms of the two-dimensional gels. The analysis revealed that a

2-3 fold prolactin-dependent enhancement in tRNA synthesis was achieved by a balanced increase in the synthesis rate of each of the tRNA species present in the gland when it was explanted. This result further confirms earlier observations (5) that a major action of prolactin is a general stimulation of all species of RNA, and emphasizes the importance of the effect of prolactin on the stability of specific mRNAs (10).

From quantitative measurements (see legends to figures) and from the overall staining of the gels, we confirm that there is a distinct increase in tRNA in the gland as it develops through pregnancy into lactation. Although numerous small alterations were observed during mammary gland development, for most of the tRNAs, the relative amount of each did not change appreciably as the total tRNA increased. In fact, very few differences were observed between the tRNA populations of late pregnancy and the fully lactating tissues. Therefore, we conclude that although there may be some selective synthesis of specific tRNA isoacceptors during early development of the gland, by the time of late pregnancy the tRNA population is determined. Any increase in tRNA synthesis during late pregnancy and lactation would appear to involve a net gain in tRNA and not an increase in specific isoacceptors.

Similar results as those observed in this study have been reported in a recent study. Weil et al. (17) analyzed the tRNA population in Friend leukemia cells before and after induction of erythroid differentiation. Although the tRNA population increased about two fold between days 4 and 6 after induction, the increase was uniform for all tRNAs examined. It is also of interest to note that the increase of tRNA in these cells did not result in a "specialization" of the tRNA population for hemoglobin synthesis, as is known to occur in the normal development of erythroid cells (18-21). Thus, induction of the tRNA population in Friend leukemia cells, as in the mammary gland, results in a net gain in tRNA. It does not result in selective enrichment of specific isoacceptors. Hentzen (22) has shown that the tRNA population of the mature mammary gland is "specialized"

for casein synthesis. Our results, which demonstrate that the mammary gland tRNA population is not altered following a 2 to 3 fold enrichment and concomitant induction of casein synthesis, may be interpreted as showing that the "specialization" of tRNA for casein synthesis (22) must occur as the gland develops and prepares for lactation.

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