Protein-coding Capacities of Polyadenylated RNAs from Normal and Neoplastic Rat Mammary Tissues

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ABSTRACT

Patterns of gene expression in normal and neoplastic rat mammary tissues were compared by cell-free translation of their total polyadenylated RNAs and by dot blot hybridization of the RNA to cloned complementary DNA probes for six of the major milk proteins, including: M, 42,000 and 25,000 caseins, X-casein, whey phosphoproteins, α-proteins, and α-lactalbumin. Distinct but overlapping messenger RNA populations were evident from the translation patterns of normal virgin, pregnant, and lactating mammary glands. Dot blot analysis showed that each milk protein RNA had a different characteristic accumulation profile during pregnancy and lactation. The MTW9 and MCCCLX mammary tumor lines, which are transplantable, prolactin dependent for growth, and produce α-lactalbumin, both showed high α-lactalbumin and M, 42,000 casein messenger RNA activity. The tumors also had other milk protein RNA sequences, although in different proportions than at any stage during functional differentiation of normal adult mammary gland. Our results indicate that normal pregnant mammary gland expresses all of the abundant milk protein genes prior to detectable milk secretion. The patterns of gene expression in the two mammary tumors do not appear to correspond to any particular stage of functional differentiation of the normal mammary gland.

INTRODUCTION

Comparisons of normal and neoplastic mammary tissues are important for understanding the neoplastic process and for providing a rational basis for cancer treatment. In the studies reported here, we have examined changes in the patterns of gene expression in normal rat mammary gland during functional differentiation. Patterns of gene expression in 2 hormone-dependent mammary tumor lines, MCCCLX (8) and MTW9 (22), were then compared.

The poly(A)+ RNA of the normal and neoplastic mammary tissues were examined for their cell-free protein translation activities and for milk protein sequence content. Accumulation of α-LA (15) and casein (15,26) mRNA sequences at various stages of pregnancy had previously been followed by molecular hybridization using radioactive cDNAs to the purified RNAs or by translation assay of the mRNAs (16). Recently, recombinant cDNA clones for several of the major rat milk proteins have been isolated and characterized in our (4, 5, 21) and another laboratory (23). We selected 6 different milk protein cDNA clones, representing the gene sequences for M, 42,000 casein, M, 25,000 casein, Wp-proteins, α-LA, and 2 as yet unidentified proteins, "X" and k, to follow the accumulation of their corresponding RNA sequences in the mammary gland. By these techniques, we found that each milk protein mRNA had a characteristic pattern of appearance during functional differentiation. Both mammary tumors contained all of the milk protein RNAs that we could probe but in proportions that differed from the normal mammary gland.

MATERIALS AND METHODS

Materials. Materials were obtained as follows.

Sephadex G-25 and poly(U)-Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.); guanidine-HCl and molecular weight marker kit (Bethesda Research Laboratories, Rockville, Md.); creatine phosphokinase (Sigma Chemical Co., St. Louis, Mo.); [35S]methionine (carrier free; specific activity, 600 to 1000 Ci/mmoll) and [32P]-labeled nucleotides (Amersham, Arlington Heights, Ill.); [35S]cysteine (carrier free; specific activity 400 to 1000 Ci/mmoll) and En3Hance (New England Nuclear, New Bedford, Mass.); nitrocellulose filter papers (Schleicher & Schuell, Inc., Keene, N. H.); SDS (British Drug House); acrylamide (Bio-Rad Laboratories, Richmond, Calif.); bis-acrylamide (Eastman Kodak Co., Rochester, N. Y.); Pansorbin (fixed Staphylococcus aureus cells) (Calbiochem-Behring Corp., La Jolla, Calif.); 2,5-diphenyloxazole (Research Products International, Inc., Elk Grove Village, Ill.).

Animals. Mature virgin or primiparous Sprague-Dawley rats were used for preparation of RNA from normal tissues. The pregnant rats were examined by laparotomy to ensure that they were at the proper stage of pregnancy. The MTW9 mammary tumor was maintained by serial passage in W/Fu virgin females coimplanted with the MTW10 mammarytropic hormone-secreting pituitary tumor (22). MCCCLX mammary tumor tissue was generously provided by Drs. T. Kano-Sueoka and J. E. Errick of the University of Colorado, Boulder, Colo. It had been maintained by serial passage in 4- to 6-week-old male A x C rats coimplanted with a long-acting 25-mg estradiol pellet (10).

Poly(A)+ RNA Extraction. RNA was extracted from mammary tissues and livers that had been quickly frozen in liquid nitrogen and stored at -80°. The guanidine HCl/sodium acetate procedure described by Deeley et al. (6) was used, omitting the chloroform/butanol extraction. Total RNA was then applied to a poly(U)-Sepharose column, and the poly(A)+ RNA was eluted using 70% formamide buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid/EDTA (31). For maximal recovery, 50% formamide is used. The RNA was precipitated 2 times from ethanol in 0.2 M NaCl before using for cell-free translation or dot blot hybridization. The RNA was stored at -20° in water or 20 m 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.6. The A260 was typically 1.8.

Cell-free Translation. Wheat germ extract was prepared according to Roberts and Paterson (25) and Deeley et al. (6). Just prior to use, the extract was treated with micrococcal nuclease for 10 min at 25° (18).

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1 Part of this work was reported in abstract form (No. 2165) at the American Society Biological Chemistry, June 1 to 5, 1980 (9).

2 To whom requests for reprints should be addressed, at Building 10, Room 5B86, NIH, Bethesda, Md. 20205.

3 The abbreviations used are: poly(A)+ RNA, polyadenylated ribonucleic acid; α-LA, α-lactalbumin; cDNA, complementary deoxyribonucleic acid; SDS, sodium dodecyl sulfate; SSC, 1x = 0.15 m sodium chloride/0.015 m sodium citrate, pH 7.2.

4 E. Devinoy, A. M. Dandekar, and P. K. Qasba. cDNA clones for rat caseins, submitted for publication.
Wheat germ tRNA was purified by the procedure described by Zubay (38).

Cell-free translation was assayed by the procedure of Roberts and Paterson (25).

Incorporation of \(^{35}S\)-methionine or \(^{35}S\)-cysteine into protein was monitored by a hot trichloroacetic acid method (6).

Immunoprecipitations were carried out as described elsewhere (5) using Pansorbin.

Hybrid-selected mRNA translations were performed as described (5).

**Gel Electrophoresis.** SDS/polyacrylamide slab gels (0.75 mm thick) were prepared as described by Laemmli (11). Molecular weight standards were \(^{14}C\)-labeled bacteriophage T\(_4\) (34), kindly provided by Dr. L. L. Silver, or Bethesda Research Laboratories high-molecular-weight marker mix. Gels were fixed for 0.5 hr in 50% trichloroacetic acid, fluorographed using Enhance, dried, and then exposed to Kodak XR-5 film at -70°.

**Dot Blots.** The procedure used was as described by Thomas (30). Using a Pipetman pipettor, the RNA (100 ng) in a 1-μl aliquot was applied to nitrocellulose filters that had been treated by soaking in water, washing twice with 20× SSC, then drying under a heat lamp. Filters were prehydrized overnight and typically hybridized to \(^{32}P\)-labeled cloned cDNA probes at 42° for 2.5 days, then washed in decreasing concentrations of SSC/0.5% SDS (from 2× SSC to 0.2× SSC) at 88°. The final 2 washes were in 0.1× SSC/0.1% SDS for 15 min each at 68°. The filters were air dried and then exposed to Kodak XAR-5 film at -70°.

**Translation Patterns of Mammary Gland Poly(A)+ RNAs.** Individual mRNAs are known to have varying requirements for optimal translation (32, 36). Since our interest was to examine a mixed population of mRNAs, we sought conditions of cell-free protein synthesis that maximized the number of different mRNAs detected. SDS/polyacrylamide gel electrophoresis was used to monitor the diversity of translation products. With lactating RNA as the standard, optimum conditions used 0.8 μg of poly(A)+ RNA, 100 mM K+ and incubation at 25° for 2.5 hr.

**Fig. 1** shows a representative autoradiogram of translation products of virgin, pregnant, and lactating mammary gland poly(A)+ RNAs after SDS/polyacrylamide gel electrophoresis. On these gels, only the most active or abundant RNA products can be visualized; however, dramatic changes in the patterns of mRNA activity during functional differentiation were evident, while translation activity increased 3-fold.

Five-day lactating mammary gland RNA encoded a high proportion of abundant proteins (e.g., Bands 1 through 7). Protein Bands 7, 5, 4, and 1 contain M, 42,000, 29,000, and 25,000 caseins and \(\alpha\)-LA, respectively (Fig. 1C). In the region of protein Bands 2 through 4, there are several abundant proteins, which include X-casein, Wp-protein (4), and an unidentified \(\kappa\)-protein.\(^6\) Analysis using 2-dimensional gels clarifies this region (see below). Translation products larger than the M, 35,000 band (which corresponds to M, 42,000 casein) were not found under our assay conditions even when the gels were exposed for longer times.

In contrast, virgin poly(A)+ RNA generated a wide variety of polypeptides, some of which (Bands 1, 3, 5, 6) migrated to the same position as in the lactating translation pattern. The most abundant protein of the virgin RNA translation pattern was Band 8. Its RNA was either not translatable or absent from lactating RNA.

The translation pattern characteristic of lactation emerged during pregnancy. M, 25,000 and 42,000 caseins (Bands 4 and 7) became prominent as early as Day 5 of pregnancy; \(\kappa\)-protein, Wp-protein, and M, 29,000 casein (Bands 2, 3, and 5) began to increase somewhat later (between Days 8 and 10). Band 1 increased slowly during pregnancy. Band 6 appeared to maintain its relative proportion at all stages of pregnancy and was prominent in virgin and lactating translation products as well. Activities of mRNAs for the higher-molecular-weight proteins (Bands 8 to 11) appeared to decrease as pregnancy progressed. However, if we applied radioactivity to the gels in proportion to the poly(A)+ RNA concentration of the mammary gland at each stage, we found that the products of these mRNAs in the translation assay actually increased during pregnancy (gel not shown). This suggests that activities of the mRNAs for the large proteins increased at a slower pace than the mRNAs encoding the abundant proteins of lactating mammary gland.

These patterns indicate the presence of at least 3 temporally overlapping abundant mRNA populations which can be classified by the reproductive stages in which we can detect their translation activity (Table 1).

**Comparison of the Translation Patterns of Normal and Neoplastic Mammary Tissue.** MTW9 of the W/Fuo rat strain and MCCCLX of the A × C strain are adenocarcinomas that produce significant amounts of the milk protein, \(\alpha\)-LA (8,22). Both tumors had very high poly(A)+ RNA contents, averaging 20 and 31 μg/g wet weight of tissue, respectively; however, translation activities of their RNAs averaged only 30 to 50% of the RNA from lactating mammary gland.

As seen in Fig. 1B, MTW9 and MCCCLX poly(A)+ RNAs generated translation patterns that contained elements of both early pregnancy and lactation in the normal gland. Both tumors express the mRNAs for the high-molecular-weight proteins (Bands

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8 to 11). Bands 1, 4, 5, and 7 (containing α-LA and M, 25,000, 29,000, and 42,000 casein, respectively) are also prominent, indicating the presence of active mRNAs for milk proteins other than α-LA. However, as in early pregnant (e.g., 5 to 10 days) mammary glands, both tumors showed reduced activities of mRNAs encoding Bands 2 and 3 (containing κ-, Wp-, and X-proteins). In fact, there was a disproportionately large amount of Band 1 (α-LA; see also Fig. 3) produced in the tumors compared to any stage of pregnant mammary gland. It thus appeared from the cell-free translation patterns that both MTW9 and MCCLX tumors had aberrant patterns of poly(A)+ RNA expression.

**Analysis of α-LA and κ-Protein Translation Products.** Two molecular forms of α-LA, which in rats is a glycoprotein, have been isolated from W/Fu rat milk (20, 21). By radioimmunoassay, rabbit antiserum to rat milk α-LA1 recognized α-LA2 equally well (21).

Immunoprecipitates of [35S]labeled translation products are illustrated in Fig. 2A. When [35S]methionine was used as tracer here, 3 bands are precipitated, but when [35S]cysteine is used, only the lowest band is recovered. The 2 upper bands, which do not contain cysteine, have now been identified as κ-proteins by hybrid selection translation assay, while the lower band corresponds to α-LA (5). Thus, RNA activities for both α-LA and κ-protein could be monitored by using the α-LA antiserum and the appropriate tracer.

Figure 2B shows the comparison of [35S]methionine-labeled translation products and α-LA and κ-protein immunoprecipitates coded by the poly(A)+ RNA of the MCCLX and MTW9 tumors and the mammary glands of MTW9-bearing rats. Both tumors have significant mRNA activity for α-LA but little activity for κ-protein. On the other hand, MTW9 host mammary gland has relatively high mRNA activity for κ-protein and less for α-LA. The MTW9 host mammary gland translation pattern is very similar to late pregnant gland.

**Analysis of Translation Products by 2-Dimensional Gel Electrophoresis.** To further examine the translation products, we performed 2-dimensional gel electrophoresis. This was useful because 3 distinct cDNA clones containing coding sequences for X-caseins and Wp- and κ-proteins (included in the broad diffuse Bands 2 and 3 on SDS gels; Fig. 1C) had been isolated.6

To simplify the identification of the primary translation product(s) corresponding to the abundant milk protein RNAs, each mRNA was purified by hybrid selection to the 6 cDNA clones (5) and translated using [35S]methionine as tracer (Fig. 3A). mRNAs for α-LA, Wp-protein, and M, 25,000 and 42,000 casein each programmed only one translation product. X-Casein RNA generated a spectrum of translation products, which could be due to multiple RNAs or to early termination. κ-Protein RNA generated 2 spots of equal intensity that differed both in size and in charge. Fortuitously, the M, 18,400 molecular weight marker migrated close to the α-LA translation product.

Two-dimensional translation patterns of total poly(A)+ RNAs from normal lactating mammary glands of Sprague-Dawley rats and the MTW9 tumor (of W/Fu rats) are shown in Fig. 3B. Since we were especially interested in comparing the α-LAs of the 2 tissues, we used [35S]cysteine as tracer, so κ-protein in the lactating mammary gland translation products would not obscure the α-LA region. Both the normal and neoplastic RNAs generated one predominant spot with a trace amount of a more acidic spot in the α-LA region. This was also seen in 2-dimensional gels of α-LA immunoprecipitates (gels not shown). This suggests conservation of overall charge in the primary translation products for α-LA in the Sprague-Dawley and W/Fu strains, even though α-LAs isolated from the milk of various rat strains show different charge forms (13, 20, 21).

Tumor and lactating mammary gland translation patterns on 2-dimensional gels differ in the relative proportions of other, as yet unidentified, spots aside from the high-molecular-weight ones (Fig. 3B). Some of these spots are present to the same extent in the MTW9 tumor as in 16-day pregnant mammary gland poly(A)+ RNA translation products (Fig. 3B, p).6 One of the p-proteins, which is the same size as the α-LA translation product, is present at much higher levels than α-LA in 16-day pregnant translation assays (Horn et al., Fig. 2, Spot e).6 This probably contributes to the increase in Band 1 during pregnancy (Fig. 14).

**RNA Dot Blot Analysis.** In order to examine the large number of poly(A)+ RNA samples for their content of specific milk protein RNA sequences, we utilized the dot blot hybridization technique (30).

Filters containing dots of equal masses of total poly(A)+ RNAs from virgin, pregnant, and lactating mammary glands and livers were hybridized individually to 6 lactating mammary gland cDNA clone probes. Fig. 4 shows the autoradiograms. These were analyzed by microdensitometry. Chart 1 shows plots of the spot intensities at each stage after normalizing to the values of 12-day lactating mammary gland RNA. We could not detect any hybridization signal to liver RNA.

RNA sequences for M, 42,000 and 25,000 casein were already present in virgin mammary gland. X-Casein sequences could not be detected until about Day 10 of pregnancy. During pregnancy, M, 42,000 casein RNA reached a steady state level of about 50% of the sequence content in 12-day lactating mammary gland, while M, 25,000 casein content increased more rapidly and attained approximately lactating levels by Day 8 of pregnancy. By dot blot analysis, we could first detect the mRNA sequence for κ-protein at Day 5 of pregnancy (Fig. 4; Chart 1). At this time, the RNA was also translationally active, since small amounts of the 2 κ-protein translation products of 5-day pregnant mammary gland RNA could be immunoprecipitated with α-LA antisera in translation assays using [35S]methionine (not shown). κ-Protein RNA sequences increased continuously during pregnancy, attaining relatively higher levels than during lactation.

Wp-Protein RNA accumulated in a manner suggesting it is unstable during pregnancy. It could not be detected until Day 5 but then increased rapidly until Day 14. By Day 16, its level dropped to less than one-half; then it increased rapidly again during the last week of pregnancy.

α-LA RNA showed even more dramatic variation during pregnancy. Its sequences could be detected in one of the pools of virgin poly(A)+ RNA, and very low levels of the protein were also found in virgin mammary glands by radioimmunoassay.6 Significant amounts of α-LA RNA were present in one of the pregnant mammary gland poly(A)+ RNA pools from Days 10 and 12. This was reproducibly found, since filters washed free of α-LA and M, 42,000 casein probes both generated the same α-LA pattern when hybridized to fresh α-LA probe.

MTW9 and MCCLX poly(A)+ RNA contained higher proportions of the mRNA sequences for M, 42,000 casein and α-LA...
to the ones obtained earlier, using molecular hybridization to

uncloned cDNA probes to purified M, 42,000 casein and α-LA
to attain a steady state. Shortly after the 14th day of pregnancy. These results are similar
even in virgin mammary gland, but it increases transiently be-
etween Days 8 and 12 of pregnancy, reappearing in a uniform
differentiation of secretory cells and the detection of significant
mRNAs for X-casein and Wp-protein become detectable by
Day 8 of gestation, and α-protein RNA is present, although at
low levels, as early as Day 5. Appearance of these sequences
might result from the emergence of new cell types or changes in
gene expression of the existing cells triggered by changes in the
hormonal milieu of pregnancy.

In the present study, only the accumulated mRNA sequences
were measured. Rates of transcription or degradation of the
individual mRNA sequences may also change during different
reproductive stages, which could result in the transient appear-
ance of α-LA and Wp-protein RNAs during pregnancy.

Comparison of MTW9 and MCCLX tumors indicated that the
tumors had quite similar gene expression patterns. In both of
the tumors, the relative proportions of RNA sequences for M,
42,000 casein and α-LA were much higher than the other milk
protein mRNA sequences examined. By translation assay, the
tumors contained the mRNA sequences coding for proteins of
mass greater than 35,000 which were detectable only in virgin
and pregnant gland. The tumor RNAs also generated the Band
6 protein (Fig. 1) common to all the normal mRNA populations.
On the other hand, the tumors had comparatively very low levels
of X-casein, α-protein, and Wp-protein RNAs. MCCLX appeared
to have higher translation activity encoding Band 8 and less
encoding M, 29,000 casein than MTW9 (Fig. 1B). However, both
of their patterns of expression of nonmilk and milk protein
mRNAs did not precisely conform to any stage of pregnancy.

The translation patterns characteristic of MCCLX and MTW9
tumors could simply be due to the peculiar hormone environ-
ments in which they are maintained (nonpregnant rats having
abnormally high prolactin levels). However, RNA from the
mammary glands of MTW9-bearing rats generates a translation pat-
ttern closely resembling late pregnant mammary gland (Fig. 2).
Thus, the differences found in the tumors are intrinsic to them,
and not due to the unusual hormonal status of their hosts.

The very low levels of X-casein, α-protein, and Wp-protein
mRNAs in MTW9 and MCCLX tumors may be the result of one
or more of the following possibilities: (a) α- and Wp-proteins are
produced by the adipose cells, a constituent of normal mammary
gland not present in the adenocarcinomas; (b) adenocarcinomas
originated from a specific, though as yet unidentified, subpopu-
alation of mammary epithelial cells; (c) expression of the α- and
Wp-protein genes at high levels requires interaction with cell
types no longer present in the tumors; or (d) alterations in the
coding or the regulatory gene sequences of α- and Wp-proteins
are associated with neoplasia. Such possibilities are presently
being investigated in our laboratory.

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the absence or presence of 5 μg of unlabeled α-LA. All T-lanes contain 50,000 cpm of hot-trichloroacetic acid-precipitable material. Hosf, translation assays of poly(A)+ germ extract using 35S-methionine (Met) or 35S-cysteine (Cys) as amino acid tracer. Equal amounts of incorporated radioactivity were applied to each well of an 11.25% polyacrylamide gel. To visualize the 35S-methionine-labeled translation products, the processed dried gel was exposed to X-ray film overnight. At this exposure time, only the M, 47,000 α-LA protein is visible in the T-lanes. The translation assay was performed as described, using 35S-methionine as tracer in the standard assay. Approximately equal amounts of 35S-methionine as incorporated into protein were applied to each well of an 11% polyacrylamide gel. V, virgin, 34,000 cpm. Days of pregnancy: 5, 26,000 cpm; 8, 50,000 cpm; 10, 36,000 cpm; T1, T4; mol wt, molecular weight. B, MTW9 and MCCLX tumors (of the W/Fu and AxC inbred strains, respectively), 50,000 cpm. For details see Table 1.

Fig. 1. Profiles of translation products of poly(A)+ RNAs of normal and neoplastic rat mammary tissues. Poly(A)+ RNAs were translated using wheat germ extract and 35S-methionine as tracer in the standard assay. Approximately equal amounts of 35S-methionine as incorporated into protein were applied to each well of an 11% Laemmli gel so that the relative distributions of individual translation products could be monitored. A, translation profiles during pregnancy. 5L, 5-day lactating Sprague-Dawley mammary gland, 50,000 cpm. V, virgin, 34,000 cpm. Days of pregnancy: 5, 50,000 cpm; 12, 50,000 cpm; 14, 46,500 cpm; 16 to 20, 50,000 cpm. TC14-labeled bacteriophage T4, mol wt, molecular weight. B, MTW9 and MCCLX tumors (of the W/Fu and AxC inbred strains, respectively), 50,000 cpm/slot.

To visualize the [35S]methionine-labeled translation products, the processed dried gel was exposed to X-ray film overnight. At this exposure time, only the M, 47,000 head protein of the TC4-labeled T4 was detectable. To visualize the other T proteins, the gel was reexposed for 5 days several months later, by which time the [35S]methionine had undergone several half-lives of decay.

C. Assignment of cDNA clones and corresponding translation products of 5-day lactating mammary gland RNA. Caseins (cas): M, 42,000, 29,000, 25,000, and X. Whey proteins: α-LA, Wp-protein (wp-pro). α-LA Protein has not as yet been identified in rat milk. Correspondence of cDNA and translation product was obtained by hybrid selection purification of mRNA and cell-free translation (S, k, molecular weight, in thousands; p, plasmid number).

Fig. 2. A. Immunoprecipitates of cell-free translation products labeled with [35S]methionine or [35S]cysteine. Aliquots of poly(A)+ RNA were translated in the wheat germ extract using 35S-methionine (Met) or 35S-cysteine (Cys) as amino acid tracer. Equal amounts of incorporated radioactivity were applied to each well of an 11% Laemmli gel. Approximately 1 X 10^6 cpm of total translation products (7) were then immunoprecipitated with anti-α-LA antiserum at 1/200 final dilution using Pansorbin (5). Immunoprecipitation in the presence (IP) or absence (IP) of 5 μg of unlabeled α-LA (Arrows, α, α-LA translation product). The 2 upper bands (present only in methionine-labeled immunoprecipitates) correspond to α-protein. Note that some bands in the T-lanes are less intensely labeled when [35S]cysteine is used as tracer. B, comparison of α-LA and κ-protein translation products of normal and neoplastic poly(A)+ RNAs. The translation assay was performed as described, using 35S-methionine as tracer. Equal amounts of radioactivity (1 X 10^6 cpm) of total translation products were immunoprecipitated with anti-α-LA antiserum and Pansorbin in the absence or presence of 5 μg of unlabeled α-LA. All T-lanes contain 50,000 cpm of hot-trichloroacetic acid-precipitable material. Host, translation assays of poly(A)+ RNA from MTW9-bearing rats; MTW9, MTW9 mammary tumor; MCCLX, MCCLX mammary tumor.

Normal versus Neoplastic Rat Mammary RNAs


A. 

Day of pregnancy MTW9

B. 

MTW9 MCCLX

C. 

Clone

<table>
<thead>
<tr>
<th>Protein</th>
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Mol wt \( \times 10^{-3} \)
daltons

MET CYS

\( x \rightarrow \alpha \)

2A
Fig. 3. Two-dimensional gel electrophoresis patterns of cell-free translation products. A. [35S]methionine-labeled translation products of mRNAs purified by hybrid selection to cloned milk protein cDNA plasmids. The individual mRNAs were translated separately, and their protein product(s) were fractionated on separate 2-dimensional gels. Each sample contained about 20,000 cpm of translation product(s) and an equal amount of Bethesda Research Laboratories 14C-labeled molecular weight marker mix (see "Materials and Methods"). The individual autoradiograms were aligned using the molecular weight markers as landmarks and then photographed. 18.4, 43, and 68 indicate the molecular weight markers (×10^3 k). Fig. 1C identifies the particular plasmids used to hybrid select the mRNAs. wp-prot, Wp-protein; x-prot, X-protein. B. [35S]cysteine-labeled total translation products of lactating mammary gland and MTW9 tumor poly(A)+ RNAs. Gels were exposed so that α-LA spots were of equal intensity in the 2 samples. The lactating mammary gland sample also contained the Bethesda Research Laboratories molecular weight markers (18.4, 43, and 68 locations are indicated). p, translation products coded by both the MTW9 tumor and 16-day pregnant Sprague-Dawley mammary gland poly(A)+ RNAs (not shown). t, spot markedly elevated in the MTW9 tumor but not 16-day pregnant or lactating mammary gland. Sprague-Dawley and W/Fu indicate spots that may result from genetic differences between the 2 strains.
Fig. 4. Changes in RNA during functional differentiation of mammary glands. Replicate dot blots were made containing 100 ng in 1 μl of each poly(A)+ RNA preparation from mammary glands of mature virgin, pregnant, and lactating rats (M). Liver poly(A)+ RNAs from castrated, virgin, 5- to 12-day pregnant, and 12-day lactating rats are also spotted (L). The dots on the extreme right (a, b, and c) indicate poly(A)+ RNA (100 ng each) from pooled tumors MCCLX, MTW9, dimethylbenz[a]anthracene, respectively. The blots were hybridized separately for 2.5 days to radioactive probes of 6 cloned cDNAs of lactating mammary gland poly(A)+ RNA, washed to high stringency (0.1 x SSC, 0.1% SDS at 68°), then air dried, and autoradiographed. These are 4.5-hr exposures using 2 Cronex intensifier screens.

Abacissa (left to right), mature virgin; pregnant (5, 8, 10, 12, 14, 16, 18, 20 days); lactating (5, 12, 19 days). Ordinate vertical axis: each spot is a separate preparation of poly(A)+ RNA.
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