Characterization of a cDNA clone for a rare mRNA modulated by ovariectomy in mammary carcinomas

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A complementary DNA (cDNA) clone (p13) for a rare mRNA was isolated from a cDNA library generated from total polyA$^+$ RNA of 14-day lactating rat mammary gland. In vitro translation of the positively selected mRNA from p13 cDNA revealed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) a polypeptide of 24 kDa. The p13 cDNA clone hybridized on northern blots predominantly to ~1100 base size RNA and weakly to ~3800 base size RNA from lactating mammary gland. It hybridized only to ~3800 base size RNA from rat liver. Southern blot analysis of genomic DNA showed differences in gene organization in mammary gland and liver. The mRNA level for the 24 kDa polypeptide was higher in 7-12 DMBA-induced tumor and lower in the MTW_9 carcinoma as compared to lactating mammary gland. After ovariectomy, the mRNA level in mid pregnant gland increased but was reduced in the 7-12 DMBA tumors.

Rare mRNA *cDNA library* *Gene organization* *Tissue specificity* *Ovariectomy* *Mammary carcinoma*

1. INTRODUCTION

In most eucaryotic cells mRNA levels vary widely, depending upon their turnover and differential rates of transcription of their genes [1-5]. Considerable information is available on the role of abundant mRNAs. Comparatively little is known about rate mRNAs in eucaryotic cells. Previously, the abundant milk protein mRNAs have been successfully cloned and studied [6-13]. We report here the isolation of a rare cDNA clone which codes for a 24 kDA polypeptide. The level of mRNA specific to this cDNA clone is modulated by ovariectomy and in carcinogen-induced mammary tumors.

2. MATERIALS AND METHODS

2.1. *Animals and tumor source*

Mammary glands and livers from Sprague-Dawley rats were the source of RNA and high -M_r DNA. MTW_9 rat mammary tumors were grown as transplants [14] while DMBA-induced primary tumors were developed by standard procedures [15].

2.2. *Preparation of cDNA library*

Total polyA$^+$ RNA from 14-day lactating rat mammary glands was isolated [16,17] and fractionated on a 4-20% neutral sucrose gradient in an SW 27.1 rotor, spun at 22500 rpm for 16 h at 20°C. Fractions (0.3 ml each) were collected and pooled separately in the ranges 0-11 S, 12-23 S, 24 S and above. RNA from the pooled fractions was precipitated with ethanol at −20°C, lyophilized and translated in vitro using rabbit reticulocyte lysate [18]. L-[^{35}S]Methionine-labeled translation products were fractionated on 11.5% SDS-PAGE [19]. The 11-23 S RNA species pool was used for the synthesis of cDNA. Procedures for synthesis of cDNA and ds-cDNA by AMV reverse transcriptase, insertion of dC-tailed ds-cDNA into the *Pst*I site of dG-tailed pBR322, transformation of *Escherichia coli* strain RR_1 by chimeric plasmid, selection of Amp^{s}Tet^r transformants, and screening of cDNA clones were carried out as in [20].

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2.3. *Synthesis of labeled probes*

Single-stranded cDNA probes were synthesized by reverse transcription in the presence of [^{32}P]dNTPs (Amersham, 400 Ci/mmol), using polyA$^+$ RNA as template [20]. Inserts from the cloned cDNAs were excised using restriction endonuclease *Pst*I, purified on 1% low melting agarose gels, and labeled with [^{32}P]dNTPs by nick-translation [21].

2.4. *mRNA selection and translation*

DNA from clones was immobilized on nitrocellulose filters (Schleicher and Schuell BA85) and hybridized to sucrose-gradient-fractionated polyA$^+$ RNA. The filters were washed free of non-specific mRNA and the hybridized mRNA was eluted and translated in either wheat germ or rabbit reticulocyte lysate system [20]. Translation products were fractionated on SDS-PAGE, and the gel was fluorographed, dried and exposed to X-ray film [20].

2.5. *RNA analysis*

RNA analysis was done as in [22].

2.6. *Southern blot analysis*

High-M_r DNA was isolated from the tissues [23] and DNA blot analysis was carried out as in [24].

3. RESULTS AND DISCUSSION

Total polyA$^+$ RNA from 14-day lactating mammary gland was fractionated on a 4–20% neutral sucrose gradient. Three thousand Tet^r and Amp^s cDNA clones were generated from 11–23 S RNA as previously described [20]. The cDNA clones for rare mRNAs were selected by screening the recombinants by 'negative selection'. Briefly, the colonies were hybridized to ^{32}P-labeled cloned cDNA probes of adundant milk proteins, viz α-lactalbumin, whey phosphoprotein, $\varkappa$ protein and 3 types of caseins [6–8]. The colonies which did not hybridize were analyzed further. About 1000 recombinants thus selected were then hydridized to ^{32}P-labeled cDNAs synthesized from total polyA$^+$ RNA of 14-day lactating mammary gland. cDNA clones which hybridized weakly were selected. One such cDNA clone, designated as p13, was used for this study.

The *Pst*I insert of clone p13 showed hybridization only to one cDNA clone out of 3000 recombinants. Thus, p13 cDNA clone represents a rare mRNA constituting 0.03% of total polyA$^+$ RNA. On northern blots (fig.1) clone p13 hybridized predominantly to a ~1100 base size RNA and weakly to a ~3800 base size RNA (fig.1, lane 1) from mammary glands. In a wheat germ cell-free translation system, mRNA corresponding to clone p13 coded for a 24 kDa protein (fig.2, lane 3) designated hereafter as a mammary gland protein of 24 kDA (MGp24). In rabbit reticulocyte lysates, no translation products was detected (fig. 2, lane 7). The failure to obtain translation of the selected mRNA in the latter system is a property of this mRNA since discrete polypeptides were indeed synthesized when either total polyA$^+$ RNA from 14-day lactating gland (fig.2, lane 5) or selected RNA from $\varkappa$-casein cDNA clone were translated (lane 6). The failure to translate the p13 mRNA could be due to the presence of signal recognition

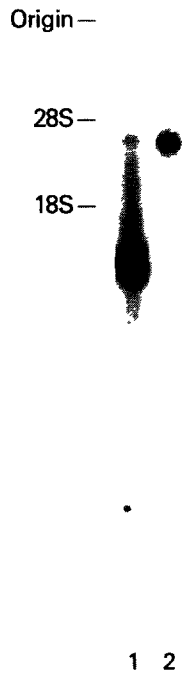

Fig. 1. Size classes of RNAs hybridized to p13 cDNA. Lane 1, total polyA$^+$ RNA from 14-day lactating rat mammary gland (10 μg); lane 2, rat liver polyA$^+$ RNA (10 μg). The 28 S and 18 S ribosomal RNAs were used as markers.

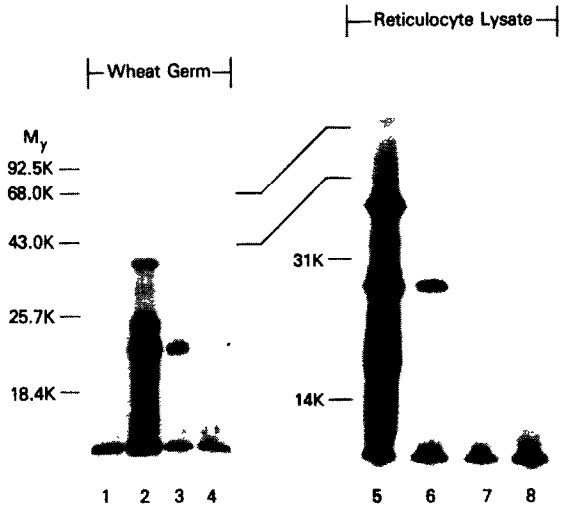

Fig. 2. In vitro translation of mRNA complementary to clone p13. Lanes 2 and 5, total polyA$^+$RNA of 14-day lactating rat mammary gland. Lane 1, plasmid pBR322-selected RNA. Lanes 3 and 7, mRNA complementary to p13 clone from rat mammary gland. Lanes 4 and 8, rat liver RNA complementary to clone p13. Lane 6, mammary gland RNA complementary to κ-casein cDNA. K, kDa.

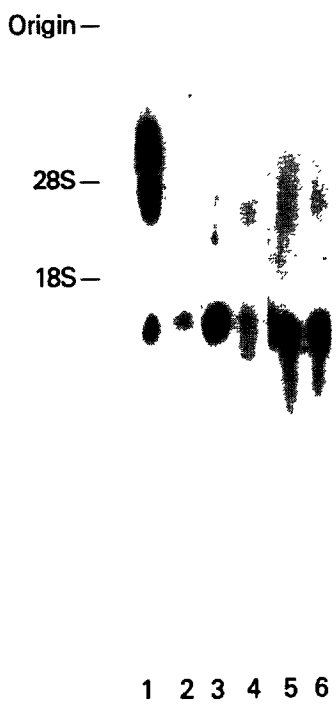

Fig. 3. Changes in the p13 specific mRNA levels in mammary tumors and in ovariectomized female rat. Lane 1, total polyA$^+$ RNA from 14-day lactating rat mammary gland; lane 2, polyA$^+$RNA from rat mammary tumor MTW_9, lane 3, polyA$^+$RNA from mammary tumor induced by 7-12 DMBA; lane 4, polyA$^+$RNA from 7-12 DMBA-induced mammary tumor after regression. Lane 5, polyA$^+$RNA from 10-day pregnant rat mammary gland; lane 6, polyA$^+$ from ovariectomized 10-day pregnant rat mammary gland. The 28 S and 18 S ribosomal RNAs were used as markers.

particles [25] and the absence of microsomal membranes in the rabbit reticulocyte lysate [26].

Using northern blot analysis, the level of mRNA for MGp24 was determined in mammary gland and two mammary carcinomas. Total polyA$^+$ RNA from 14-day lactating mammary gland and from primary 7-12 DMBA-induced and MTW_9 transplantable tumors were hybridized to ^{32}P-labeled p13 cDNA. As compared with lactating mammary gland (fig.3, lane 1) the content of mRNA for MGp24 was higher in 7-12 DMBA-induced tumor (fig.3, lane 3) and lower in MTW_9 carcinoma (lane 2). After ovariectomy, the RNA level increased in the normal gland at mid pregnancy (fig.3, lanes 5,6) but was reduced in the 7-12 DMBA tumors (fig.3, lane 4). The RNA level was determined in 4 samples removed 4 days after ovariectomy when the tumor started to regress. Thus expression of MGp24 appears to be under hormonal control as shown for the secretory class of milk proteins [8].

Tissue specificity of clone p13 was determined by hybridizing ^{32}P-labeled p13 cDNA to liver polyA$^+$ RNA of lactating rat. The cDNA hybridized only to ~3800 base size RNA and not to ~1100 base size RNA (fig.1, lane 2). This indicated that p13 clone is not specific to mammary gland. However, the ~3800 base size RNA could not be translated in vitro in either system (fig.2, lanes 4,8). It is possible that this RNA species is a precursor to ~1100 base size RNA and needs to be processed to become a functional mRNA.

It was of interest to determine if the differences in expression of MGp24 gene in the mammary gland and liver were related to a modification in gene structure. Hence, high-M_r DNA from the two tissues was digested with various restriction en-

donucleases and analysed on southern blots. The *Eco*RI restriction endonuclease generated two fragments, 23 kbp and 5.4 kbp, from DNA of virgin, 10-day pregnant and 10-day lactating rat mammary gland (fig. 4, lanes 1-6). Under similar conditions, liver DNA also generated two fragments complementary to clone p13, one of which was of a different size, 3.6 kbp (fig.4, lanes 7-8) than those generated from mammary gland DNA. Digestion of mammary gland DNA with *Xba*I endonuclease revealed four fragments, two of which, viz 4.2 kbp and 2.2 kbp, hybridized strongly while the remaining two, 6.2. kbp and 2.9 kbp, hybridized weakly (fig.4, lanes 17-22). These weakly hybridizable fragments were not detected on digestion of liver DNA (lanes 23-24). The restriction patterns for *Pvu*II (fig.4, lanes 9-16), *Pst*I and *Hin*dIII (not shown) of DNA from the two tissues were similar. Differences observed in the restriction patterns of genomic DNA for clone p13 may result from base substitution or modification at the restriction sites of *Eco*RI and *Xba*I. Similar modifications of restrictions sites in gene structure for rat hepatic protein hp22 [23] and male rat liver $\alpha_{2\mu}$ globulin [27] have been reported.

In conclusion, a rare mRNA from lactating rat mammary gland was cloned. RNA sequences complementary to the clone were also found in the liver of lactating rats, but of ~3800 base size only. Structural differences between the gene in rat liver and in mammary gland were demonstrated as well. The mRNA level for the 24 kDa protein is under hormonal control in both the mammary gland and

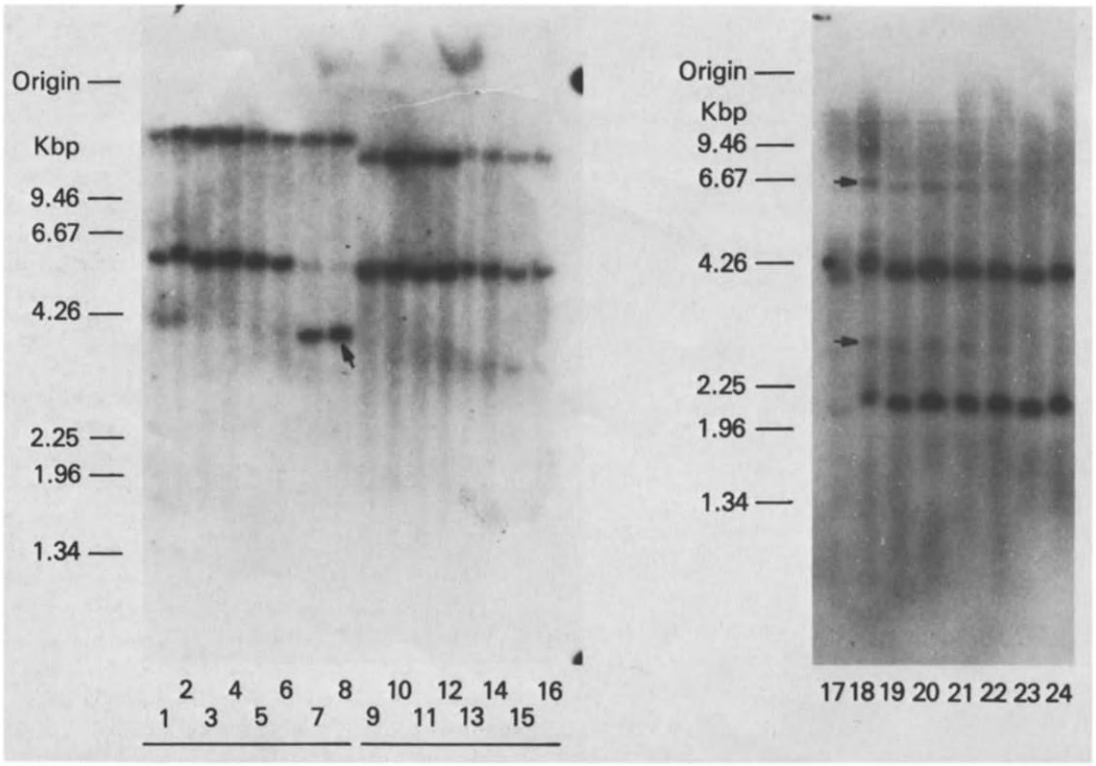

Fig. 4. Autoradiogram of a southern blot of high-M_r DNA from mammary gland during functional differentiation and from liver of 10-day lactating rat. Sixty μg DNA were digested with either *Eco*RI (lanes 1-8). *Pvu*II (lanes 9-16) or *Xba*I (lanes 17-24). DNA samples shown are: lanes 1-2, 9-10, and 17-18, virgin rat mammary gland; lanes 3-4, 11-12, and 19-20, 10-day pregnant rat mammary gland; lanes 5-6, 13-14, and 21-22, 10-day lactating rat mammary gland; lanes 7-8, 15-16, and 23-24, liver of 10-day lactating rat. M_r markers shown are *Hin*dIII digested λDNA and *Hae*III digested ΦX174RF DNA.

carcinogen-induced mammary tumors. The p13 clone described here appears to be a good model for understanding the role(s) of rare mRNAs in functional differentiation of mammary gland.

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