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DIFFERENTIAL CALMODULIN GENE EXPRESSION IN FETAL, ADULT, AND NEOPLASTIC TISSUES OF RODENTS

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Differential expression during rat development of three genes for calmodulin (CaM I-III) was examined in amnion, decidua, embryo, liver, placenta, parietal and visceral yolk sacs and uterus. CaMI expression was constant except for increasing activity in VYS during gestation. CaMII expression increased in all tissues except for a decrease in embryo. CaMIII did not change dramatically. Differential expression was also found in chemically or virally induced rat tumors, and in metastatic lung nodules of mouse mammary carcinoma. CaMII was the major gene expressed in all these neoplastic tissues.

Calmodulin (CaM) is a multifunctional protein which plays an important role in control of the cell growth division cycle and in signal transduction (1-3). There are many separate occurrences in vertebrates of the gene for this ubiquitous calcium-dependent modulator protein including several pseudogenes(4-9). Multiple mRNA species in rat tissues for CaM were detected in 1983 (10), and are now known to arise from at least three legitimate genes which all are translated into 148 residue CaMs of identical amino acid sequence (6-8). These are distinguished by the different sizes of the mRNA transcribed from the individual genes, i.e. 1.7kb for CaMI, 1.4kb for CaMII, and both 2.2 and 0.8kb for CaMIII. These three genes are known to be differentially expressed in mature rats, with CaMI in brain, liver and muscle, but CaMII and III predominating in brain (6,8).

CaM gene expression has been regarded as constituative, with no effects of hormones on levels of expression having been recorded. However CaM expression does appear to increase during the cell cycle (11-13), and following viral (12,14) or chemical neoplastic transformation (11,15,16). Changes in signal transduction of growth factors or their second messengers (eg CaM) may be partially responsible for neoplastic transformation. In order to obtain more information about CaM involvement in normal and neoplastic tissue development, we undertook the investigation of expression of the three CaM genes during prenatal development in the rat, in chemically and virally induced tumors, and in non-metastatic and metastatic mouse mammary carcinoma.

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MATERIALS AND METHODS

In vitro transcription kits, RNase-free DNase and the restriction endonuclease Xho1 were from Promega BioTec (Madison, WI). The Bam H1, and nucleic acid grade formamide used for hybridization were from Bethesda Research Laboratories (Gaithersburg, MD). T3 polymerase was from Stratagene (San Diego, CA), Hybond-N nylon transfer membranes from Amersham (Arlington Heights, IL), and [alpha-^{32}P]CTP used to label riboprobes from ICN Biomedicals (Irvin, CA). All other chemicals were of the highest available commercial quality.

Rat fibroblast cell lines have been described previously (17). Solid tumors were obtained by subcutaneous injection of 10^6 cells in athymic BALB/c nu/nu mice (6-10 weeks old and bred in this laboratory). Tumor tissue was removed 2-4 weeks later. The procedures involved in obtaining rat Morris hepatomas have been described (18). Sprague-Dawley rats bred in this laboratory (19) were used as a source of all prenatal and normal adult tissues. The SP1 cell line originated from a spontaneous mammary adenocarcinoma in a CBA/J mouse (20), but variants which metastasize to lung were produced by in vitro treatment of SP1 with hydoxyurea (S.1.4.1), calcium ionophore A23187 (3.3a & 3.1a), phorbol myristate acetate(PMA)(1.3a & 4.1a), and A23187 + PMA (1.1a & 1.2a) (21-23). Primary tumors were produced by subcutaneous injection of 10^5 cells and the tumors were excised 6 - 8 weeks later. The normal and neoplastic tissue samples were flash frozen in liquid nitrogen and stored at -80^{o}C until extraction.

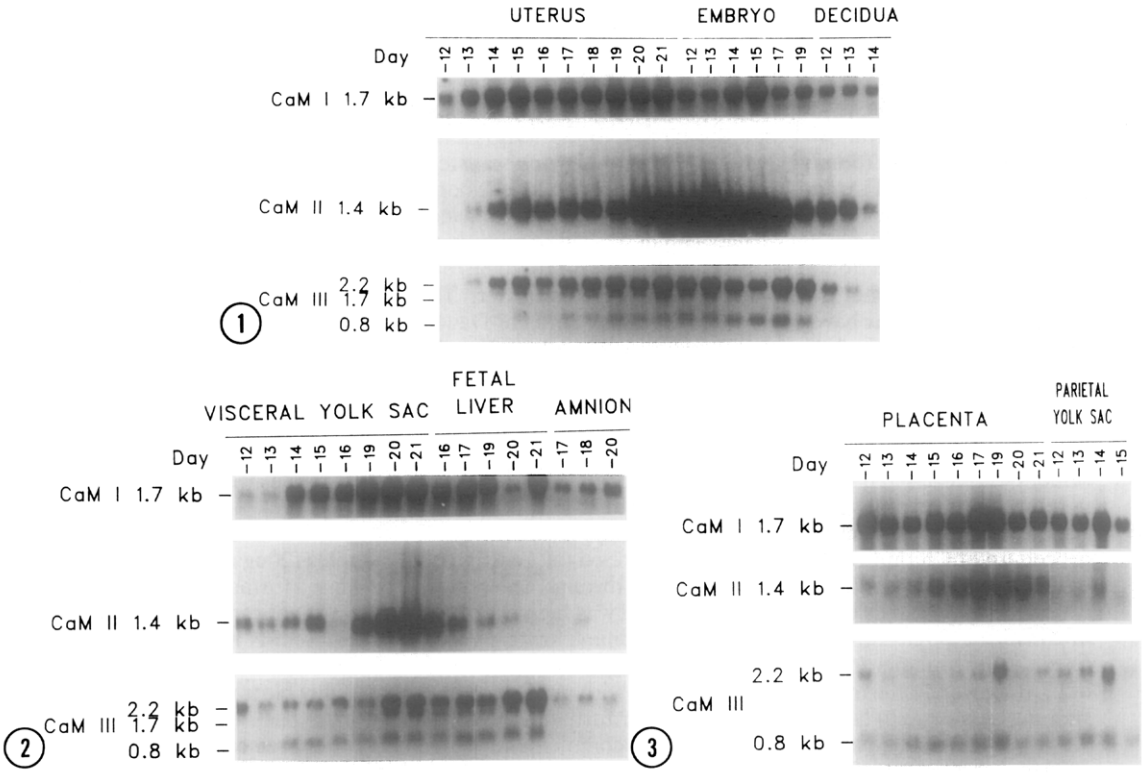
Total RNA from tumors and normal tissues was extracted using the isothiocyanate procedure of Chirgin et al as previously described (17,24,25) . RNA samples (20ug as measured by OD_{280}) were denatured and electrophoresed through agarose gels containing formaldehyde as described by Maniatis et al (26). Nucleic acid was then transferred by overnight capillary blotting in 20*SSC(1*SSC = 0.15M NaCl, 0.015M sodium citrate, pH 7.0) onto Hybond-N membranes and fixed to these membranes by baking (80^{o}C, 2 hours). The integrity of the RNA, and the efficiency of blotting were determined by staining blots with methylene blue (27). Only blots with identical staining from lane to lane were acceptable for further analysis.

Radiolabelled antisense RNA probes complementary to each of the three calmodulin mRNAs were prepared by run-on transcription. Template plasmids pR1-HT for CaMI (5) and pR3-HB for CaMII (6) were linearized by digestion with Xho1. The template pKK408H-1 for CaMIII was the generous gift of Dr J Brosius (8), and was linearized with Bam H1. T3 polmerase was used to prepare riboprobes from all three templates. Prehybridization and hybridization of the Northern blots were as described (17). Hybridization was for 16 hours at 60^{o}C with probe at a concentration of $5*10^6$ cpm/ml. Hybridized blots were exposed to Kodak SB-5 film.

RESULTS AND DISCUSSION

Differential CaM gene expression was evident during rat development (Fig 1-3). CaMI and III were expressed at individually constant levels in the uterus and decidua, in the extraembryonic membranes (parietal yolk sac, visceral yolk sac and amnion), and also in the placenta and embryo. In the fetal liver CaMIII expression appeared to increase during gestation (Fig 2). In contrast to the relative constant picture with CaMI and III, CaMII expression did change with an overall pattern of increased activity in uterus, in both parietal and visceral yolk sacs, and in amnion (Fig 2 & 3). Of note however, as the embryo developed, was the observation that the expression of CaMII decreased (Fig 1).

An increase in CaM expression has been previously shown to follow virally induced transformation of cells (12,14). We analyzed the pattern of CaM mRNAs in virally induced sarcomas (Fig 4). Whereas CaMIII appeared to be unaffected by transformation, both CaMI and II were increased compared to the NRK cell line or to normal kidney control. More interesting, when compared to one another a reciprocating pattern appeared for CaMI and II: for example, in PRC-ASV, tsK-NRK, and K-NRK induced tumors CaMII was > CaMI. However, in tsMSV, FBJ-208F, and SSV-NRK tumors the opposite -- CaMI > CaMII -- was apparent.



Figures 1-3 . Expression of three genes for CaM during gestation of the rat.

In another tumor model, the chemically induced rat Morris hepatomas, only CaMII was expressed to any great extent, and at a lower level than normal liver (Fig 5). This was in contrast to the increase in CaM protein reported in these tumors compared to normal liver (11,15,16). This suggests that some post-transcriptional regulatory mechanism such as a difference in the turnover of CaM mRNA in tumors compared to uninvolved tissue could be responsibe for the increased hepatoma CaM content, despite a decrease in CaM mRNA content. We have also been unable to show any increase in CaM mRNA from either of the three genes in the rat regenerating liver following partial hepatectomy (unpublished). This again was in contrast to the findings of increased CaM protein in such normal liver stimulated to proliferate (11,13).

The above analyses were restricted to comparison of CaM expression between normal tissues and primary tumors. We extend these studies to examine whether mouse metastatic tumors exhibit any differential CaM expression when compared to primary, non-metastatic mouse mammary carcinoma. All three CaM genes were expressed in these mouse tissues (Fig 6). This is the first time multigene expression for CaM has been noted for the mouse. Again CaMII was the major active gene. No indication of increased activity of either of the three genes in the metastatic lung nodules was observed when compared to primary mammary carcinomas (SP1 and H10.2.2a).

In conclusion, CaMII is the primary gene expressed in the tumors of rats and mice that we have screened. During rat prenatal development a complex differential expression pattern for

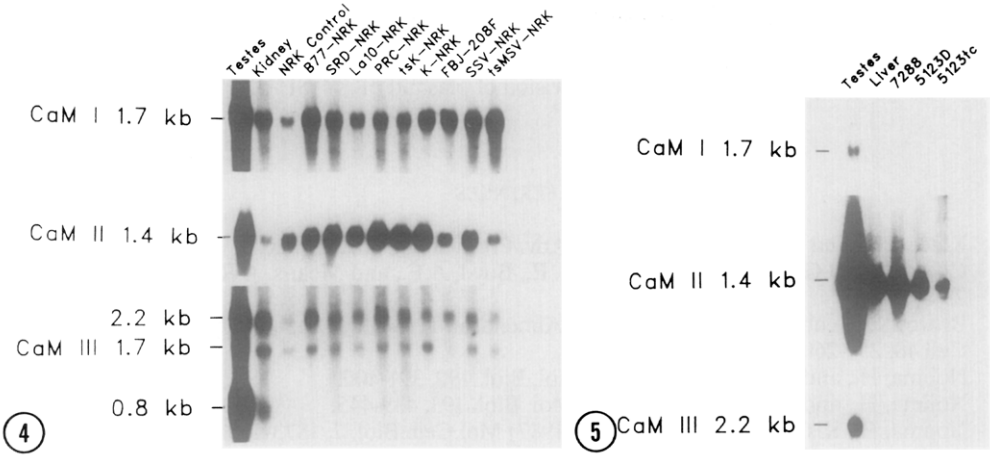


Figure 4. Expression of three genes CaM I-III in tumors arising from virally transformed rat fibroblasts NRK and 208F. B77, SRD, La10, PRC are different strains of avian sarcoma virus; tsK and K are Kirsten murine sarcoma virus; FBJ is Finkel-Biskis-Jinkins murine osteosarcoma virus; SSV is simian sarcoma virus; tsMSV is Moloney murine sarcoma virus.

Figure 5. CaMII is preferentially expressed in rat Morris hepatomas.

CaMII and CaMIII is evident in certain tissues which merits further attention. It seems reasonable to predict that CaMII is the gene which is increased during the transition from G1 to S phase of the cell cycle. No increase in CaM gene expression could be linked to neoplasia in rat hepatomas or in mouse adenocarcinoma whether primary or metastatic. However, a different profile of gene expression was observed in different virally induced rat sarcomas. The investigation of differences in the action of transcription factors following transformation by different viruses could profitably include the CaM genes.

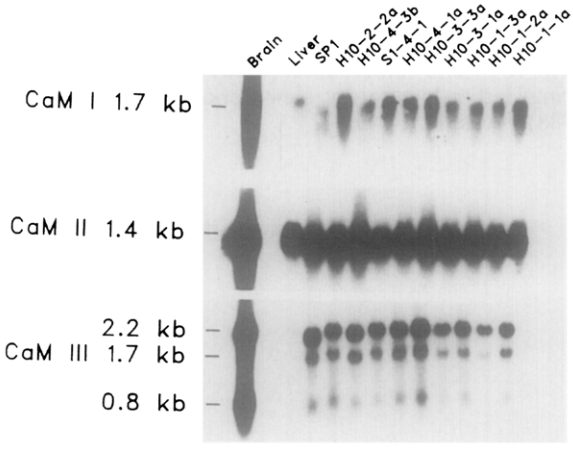


Figure 6. CaMII is the major gene expressed in mouse mammary carcinoma. This is the case in the primary tumors SP1 and H10.2.2a, and also in the metastatic tumors in lung arising from variants induced by PMA, by A23187, and hydroxyurea.

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