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A cloned calmodulin structural gene probe is complementary to DNA sequences from diverse species

(mRNA/molecular cloning)

RAVI P. MUNJAAL*, T. CHANDRA, SAVIO L. C. WOO, JOHN R. DEDMAN*, AND ANTHONY R. MEANS

Department of Cell Biology and the Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030

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ABSTRACT **Calmodulin mRNA has been partially purified from a total nucleic acid extract of the electroplax of *Electrophorus electricus* by oligo(dT)-cellulose chromatography and sucrose gradient centrifugation. A 9- to 10S fraction was determined to contain 39% calmodulin mRNA by translation in a reticulocyte lysate followed by immunoprecipitation with antibodies to calmodulin. Double-stranded cDNA was synthesized from the RNA fraction by using reverse transcriptase from avian myeloblastosis virus. The double-stranded cDNA was joined to pBR322 linearized by restriction endonuclease *Pst* I and used to transform *Escherichia coli* RRI. DNAs from 60 tetracycline-resistant clones hybridized to [^{32}P]cDNA synthesized from the partially purified calmodulin mRNA fraction. By direct DNA sequence analysis, one of these clones, pCM109, was shown to contain calmodulin-specific sequences corresponding to amino acid residues 93–148 of calmodulin or approximately 38% of the peptide-coding region of the calmodulin structural gene sequence. pCM109 was hybridized to DNA isolated from three vertebrate and one plant species by the procedure of Southern. Positive hybridization bands were noted regardless of the DNA source. These data suggest that calmodulin gene sequences are evolutionarily conserved, as has been shown to be the case for the primary amino acid sequence.**

Calmodulin, an intracellular calcium receptor, has been revealed to be present in virtually every eukaryotic cell from ameba to man (1–8). The primary amino acid sequence of the protein has been highly conserved during evolution: only 7 conservative amino acid substitutions (out of 148 amino acids) have been noted between *Renilla reniformis* (sea pansy) (9), rat (10), and cow (11, 12). The multiple roles of calmodulin in regulation of cell function suggest it is unlikely that the levels of this protein would be markedly altered during normal cellular metabolism. Indeed, calmodulin appears to be constitutively expressed in a number of hormonally regulated systems, including chicken oviduct, rat testis, and rat pituitary. However, calmodulin levels are selectively elevated in a variety of cells transformed by oncogenic viruses (13–15).

We report herein the isolation and amplification of a gene probe for calmodulin. Because of the abundance of calmodulin in the electroplax of the electric eel (*Electrophorus electricus*) and the fact that this protein is immunologically identical to mammalian calmodulin (2, 16), this tissue was used as the source of mRNA. The gene probe (plasmid pCM109) contains 38% of the peptide coding sequence and should prove a valuable tool for unraveling the complexities of the calmodulin gene.

EXPERIMENTAL PROCEDURES

Materials. Electric eels were purchased from World Wide Animals, New York. The electroplax tissue was rapidly removed and frozen in dry ice. Reagent-grade phenol was obtained from Fisher and redistilled before use. Oligo(dT)-cellulose was from Collaborative Research (Waltham, MA) and the rabbit reticulocyte cell-free translation kit was obtained from New England Nuclear. Deoxynucleoside triphosphates were purchased from P-L Biochemicals and radioactive nucleotides from Amersham. Avian myeloblastosis virus (AMV) reverse transcriptase (RNA-dependent DNA polymerase) was a generous gift of J. W. Beard (Life Sciences, St. Petersburg, FL). Nitrocellulose filter discs were obtained from Millipore. Bacteriophage T4 polynucleotide kinase was purchased from Boehringer Mannheim and S1 nuclease from Miles. Restriction endonucleases were purchased from Bethesda Research Laboratories (Rockville, MD), and terminal deoxynucleotidyl transferase was a gift of F. Ratliff (University of California at Los Alamos, NM).

Preparation of Calmodulin-Enriched RNA. RNA was extracted from the frozen electroplax tissue as described by Rosen *et al.* (17). Total RNA was chromatographed on oligo(dT)-cellulose by the method of Aviv and Leder (18). Poly(A)-containing RNA was denatured at 68°C for 3 min, quickly cooled, and applied to 13–25% linear sucrose density gradients. The gradients contained 40 mM Tris·HCl at pH 7.4, 2 mM sodium acetate, 2 mM Na_2EDTA, and 1% sodium lauroyl sarcosinate and were centrifuged at 39,000 rpm in a Beckman SW 40 rotor for 20 hr at 4°C. Fractions (0.5 ml) of the gradients were collected and precipitated with ethanol. mRNA fractions (1 μg) were translated in a cell-free translation system from rabbit reticulocyte lysates, using [^{35}S]methionine as the tracer. Calmodulin mRNA activity in the total translation product was determined by immunoprecipitation using anti-calmodulin and *Staphylococcus aureus* protein A (2). The immunoprecipitates were analyzed by electrophoresis on 2-mm $NaDodSO_4$/14% polyacrylamide gels as described by Laemmli (19).

Preparation and Cloning of cDNA. Calmodulin mRNA (fraction 9 of Fig. 1) was copied with AMV reverse transcriptase (20). The reaction was stopped by the addition of EDTA and $NaDodSO_4$ to final concentrations of 10 mM and 0.5% respectively, followed by heating for 10 min at 68°C. tRNA was added to 80 μg/ml, and the mixture containing single-stranded cDNA was rechromatographed on an Ultrogel AcA 54 column (LKB). The first peak excluded from the column contained the cDNA, which was recovered by ethanol precipitation after 3.0 M sodium acetate, pH 5.5, had been added to the fractions to a final concentration of 0.3 M.

Enzymatic Synthesis of Double-Stranded cDNA (ds-cDNA). Single-stranded cDNA was used to synthesize the second strand

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Abbreviations: AMV, avian myeloblastosis virus; ds-cDNA, double-stranded complementary DNA; NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate; bp, base pair(s).

* Present address: Department of Medicine, Division of Endocrinology, The University of Texas Health Science Center, Houston, TX 77025.

of DNA, using AMV reverse transcriptase (20). The reaction was carried out at 47°C for 20 min and was stopped by adding EDTA (10 mM) and $NaDodSO_4$ (0.5%) followed by heating at 68°C for 10 min.

S1 Nuclease Treatment of ds-cDNA. The S1 nuclease nicking of ds-cDNA was accomplished in a final reaction mixture containing ds-cDNA at 4 μg/ml, S1 nuclease at 5000 units/ml, yeast RNA at 50 μg/ml in S1 buffer, which consists of 0.03 M sodium acetate at pH 4.5, 0.28 M NaCl, and 4.5 mM zinc acetate (21).

Poly(dC) Addition to ds-cDNA. dG residues were added to the 3′ end of linearized vector pBR322 and dC residues to the ds-cDNA molecules (22). Eight picomoles of [α-^{32}P]dCTP or [^{3}H]dGTP was added to the reaction mix to monitor synthesis. Terminal transferase was added to the solution at 400 units/ml, and the mixture was incubated at 37°C until 10–15 residues were added per terminus. The reaction was terminated by adding EDTA to a final concentration of 10 mM.

Annealing of ds-cDNA with the Vector Plasmid DNA pBR322. The two DNA preparations (ds-cDNA and linearized plasmid pBR322 DNA with homopolymer tails) were mixed at equimolar concentrations with the final plasmid DNA concentration at 2 μg/ml as described by McReynolds *et al.* (21). The DNA molecules were heated first at 70°C for 10 min, followed by 45°C for 2 hr, and finally room temperature overnight.

Transformation of *Escherichia coli* RRI. Bacterial transformation was performed as described by McReynolds *et al.* (21).

Screening of Recombinant Plasmids. Tetracycline-resistant transformants were selected and transferred onto fresh tetracycline-containing plates with sterile toothpicks. Plates were incubated at 37°C for 20 hr or longer, depending on the size of the colonies. Bacterial colonies were transferred to nitrocellulose filter discs and the DNA was released by lysis *in situ* as described by Grunstein and Hogness (23). After denaturation with Denhardt's solution (24), DNA on the filters was hybridized with a [^{32}P]cDNA probe synthesized from the partially purified calmodulin mRNA. The positive colonies were transferred from replicate plates and grown in M9 medium for isolation of plasmid DNA as described by Katz *et al.* (25).

Purification of cDNA Insert from the Plasmid DNA. The recombinant plasmid DNA was digested with restriction endonuclease *Pst* I at a concentration of 2 units/μg of DNA at 30°C for 2 hr and separated by electrophoresis on 4% polyacrylamide slab gels 557 mm long and 1.5 mm thick. The gels contained 5.8 g of acrylamide, 200 mg of *N,N′*-methylenebisacrylamide, 100 mg of ammonium persulfate, 100 μl of *N,N,N′,N′*-tetramethylenediamine in TEB buffer (10.8% Tris/0.95% Na_2EDTA/5.5% boric acid). The plasmid DNA and cDNA insert were visualized under UV light by ethidium bromide staining. The band containing the cDNA insert was cut from the gel and extracted in 0.1× NaCl/Cit [1× NaCl/Cit (standard saline citrate) is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0] for 12 hr. The DNA was passed through a glass fiber filter to remove excess acrylamide. DNA was precipitated with 2 volumes of ethanol and collected by centrifugation at 38,000 rpm in a Beckman SW 40 rotor for 1 hr.

Affinity Hybridization and Cell-Free Translation. This method is based on covalently linking cloned DNAs to aminobenzyloxymethylcellulose (26). DNA from various plasmids (500 μg) was digested with *Hha* I at a concentration of 2 enzyme units/μg of DNA for 2 hr and coupled to aminobenzyloxymethylcellulose. Total poly(A)-containing electroplax RNA (100 μg) was hybridized to the DNA-cellulose at 37°C for 2 hr in 50% (vol/vol) formamide. The mRNAs eluted from various DNA-cellulose columns were precipitated with ethanol and the pellets obtained after centifugation were dissolved in 10 μl of sterile water. The mRNA from different samples (0.5 μg) was translated *in vitro* in the presence of [^{35}S]methionine. The translation products were assayed on 15% polyacrylamide gels (19). The gel was dehydrated in dimethyl sulfoxide, soaked in 2,5-diphenyloxazole solution for 8 hr, dried in a gel drier, and exposed to an x-ray film for 6 hr to visualize the radiolabeled *in vitro* translation products.

Direct Determination of DNA Sequence. Plasmid DNA (50 μg) was cut with *Pst* I restriction endonuclease and passed through an Ultrogel AcA 34 column. The eluted DNA was precipitated in 2 vol of ethanol. The DNA pellet was dissolved in 30 μl of water and treated with 5 units of bacterial alkaline phosphatase at 60°C for 30 min in a 50-μl reaction mixture containing 20 mM Tris·HCl at pH 8.6 and 1 mM $MgCl_2$. The mixture was extracted with phenol and nucleic acid was precipitated with ethanol. The pellet was washed once with cold 70% (vol/vol) ethanol and dissolved in 1× kinase buffer [50 mM glycine–NaOH, pH 9.5/10 mM $MgCl_2$/5 mM dithiothreitol/0.1 mM spermidine/25% (vol/vol) glycerol]. The DNA solution was mixed with 200 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{32}P]ATP (3000 Ci/mmol), and T_4 polynucleotide kinase was added at 4000 units/ml. The reaction mixture was incubated at 37°C for 30 min. The products were analyzed on a 4% polyacrylamide gel to separate the 5′-end-labeled cDNA insert from the plasmid DNA. The cDNA insert was eluted from the gel in 0.1× NaCl/Cit as described earlier. The cDNA fragment was further cut with *Hae* III and the resulting fragments were separated by preparative polyacrylamide gel electrophoresis and nucleotide sequences were determined by the procedure of Maxam and Gilbert (27).

Southern Blotting of Genomic DNAs and Hybridization to [^{32}P]cDNA. The genomic DNAs were extracted from electric eel kidney, hen liver, human placenta, and wheat germ by the procedure of Marmur (28). The DNAs were cut separately with restriction endonucleases *Eco*RI and *Hin*dIII and electrophoresed in 1% agarose gels. The DNAs were denatured in the gel with NaOH before they were transferred onto the nitrocellulose filters as described by Southern (29). The nitrocellulose filter paper was soaked in Denhardt's solution for 10 hr before hybridization with the *Pst* I fragment of the recombinant plasmid labeled with ^{32}P to 1×10^8 cpm/μg of DNA by nick-translation (30). Hybridization was carried out with 20×10^6 cpm of the probe in 5 ml of hybridization buffer consisting of 1 mM EDTA/0.5% $NaDodSO_4$ in Denhardt's solution (0.04% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone in 6× NaCl/Cit. The hybridization was carried out at 60°C overnight. The nonspecifically associated radioactivity was removed by washing with 1× NaCl/Cit containing 0.5% $NaDodSO_4$ at 60°C. Finally, the filter paper was rinsed once with 1× NaCl/Cit, air dried, and exposed for 4 days to a Kodak x-ray film.

Biosafety Precautions. All cloning experiments were carried out in compliance with the National Institutes of Health guidelines for recombinant DNA research.

RESULTS

Partial Purification of Calmodulin mRNA. Translational analysis of a total RNA preparation from electric eel electroplax chromatographed on oligo(dT)-cellulose revealed that 9–10% of the newly synthesized protein was specifically precipitable by anti-calmodulin (2). Further purification was achieved by sucrose gradient centrifugation. As shown in Fig. 1, all fractions contained translatable mRNA. However, analysis of the translation products by anti-calmodulin precipitation revealed one major peak of calmodulin mRNA activity corresponding to 9–10 S. The peak fraction, which represented 39% calmodulin mRNA by translation, was chosen for the subsequent experiments.

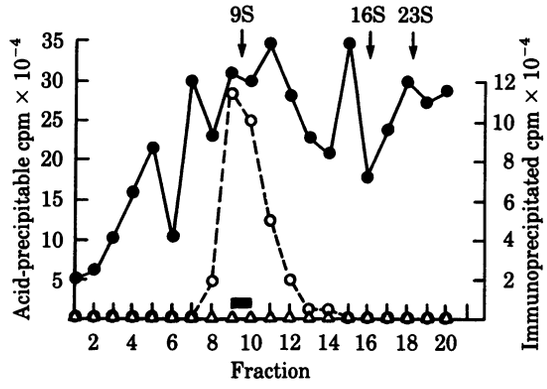

FIG. 1. Sucrose gradient centrifugation of poly(A)-containing RNA. Poly(A)-RNA (200 μg) was applied to a sucrose density gradient (13–25%) and centrifuged for 20 hr. Each fraction was precipitated with ethanol separately and translated *in vitro* in the presence of [^{35}S]methionine. Translation products were assayed for total peptides synthesized (●) and for calmodulin by immunoprecipitation in the absence (○) or presence (△) of 10 μg of exogenous calmodulin per tube.

Plasmid Construction and Selection by Hybridization and Translation. Ten micrograms of the calmodulin-enriched mRNA was employed to synthesize ds-cDNA by using reverse transcription. Greater than 90% of this ds-cDNA was resistant to degradation by S1 nuclease. The ds-cDNA was tailed with [α-^{32}P]dCTP to an average length of 12 dCs per terminus. Plasmid pBR322 was linearized with *Pst* I, dGTP was added to an average length of 14 dGs per terminus, and the tailed DNA was annealed with the ds-cDNA. This mixture, containing recombinant plasmids, was employed to transform *E. coli* RRI. From 30 ng of ds-cDNA, 180 tetracycline-resistant transformants were obtained. Thirty percent of these clones were shown to contain recombinant DNA sequences by *in situ* hybridization with calmodulin-enriched [^{32}P]cDNA. Several hybridization positive plasmids were selected for further analysis by translation after coupling to aminobenzyloxymethylcellulose and hybridization with electroplax poly(A)-RNA. The eluted RNAs were translated and analyzed by electrophoresis on 15% polyacrylamide gels followed by fluorography to detect radioactive bands. Fig. 2 illustrates the results of a representative experiment. The first slot shows the location of authentic rat testis calmodulin, which was included as a standard. Slot 2 represents proteins synthesized in the reticulocyte lysate in response to total electroplax poly(A)-RNA. Slots 3, 4, and 5 represent the products synthesized from RNA hybridized to DNA obtained from three recombinant clones. The final slot (6) represents RNA hybridized to pBR322 DNA. Mulitple bands representing newly synthesized proteins can be seen in each track. It is apparent that a band that comigrates with calmodulin is present in each preparation. The total poly(A)-RNA track (2) contains many peptides, as would be expected from the heterogeneity of the RNA utilized for translation. These bands are also present in the slots representing the clones (3–5) as well as that of the pBR322 DNA fragments (6). The major difference between pBR322 and two of the clones is the increase in relative intensity of the bands that comigrate with calmodulin (slots 3 and 5). Because an equal amount of acid-precipitable radioactivity was loaded on each slot it is likely that the increased intensity represented an enrichment in calmodulin mRNA.

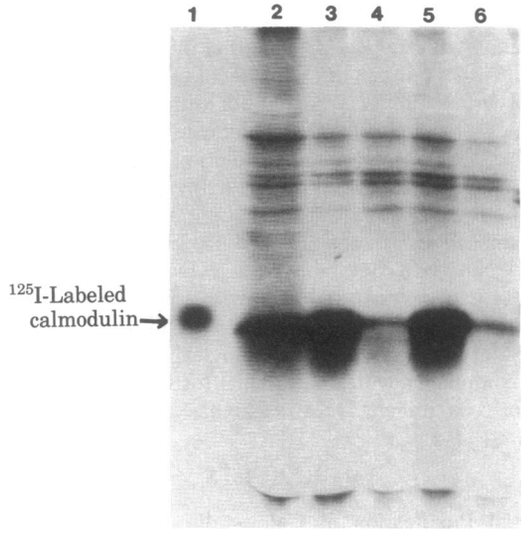

FIG. 2. Autoradiogram of [^{35}S]methionine-labeled *in vitro* translation products on a 15% polyacrylamide slab gel. mRNA was isolated from electroplax poly(A)-RNA by DNA hybridization. DNA was isolated from the sources listed below and covalently linked to aminobenzyloxymethyl-cellulose. The mRNA was then translated in the rabbit reticulocyte lysate. In each track, 20,000 trichloroacetic acid-precipitable cpm was applied. Track 1, ^{125}I-calmodulin; track 2, polypeptides whose synthesis was directed by electroplax total poly(A)-containing RNA; tracks 3, 4, and 5, peptides synthesized by mRNA bound to DNA from clones pCM103, pCM107, and pCM109, respectively; track 6, peptides synthesized from mRNA bound to pBR322 DNA.

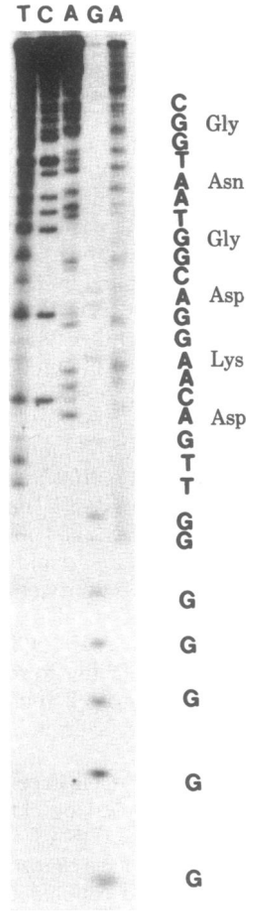

FIG. 3. Autoradiography of a DNA sequence gel. The sequence of the *Pst* I fragment was determined from the 5′ end towards the *Hae* III site and analyzed on a 20% polyacrylamide gel. The DNA sequence and the corresponding amino acid are shown on the side of the gel.

	93		95				100				105					110			113
Calmodulin	Asp	Lys	Asp	Gly	Asn	Gly	Tyr	Ile	Ser	Ala	Ala	Glu	Leu	Arg	His	Val	Met	Thr	Asn	Leu	Gly
DNA sequence	GAC	AAG	GAG	GGT	AAT	GGC	TAC	ATC	AGT	GCA	GCC	GAG	TTG	GGA	CAT	GTC	ATC	ACT	AAC	TTG	GGC
Amino acid	Asp	Lys	Asp	Gly	Asn	Gly	Tyr	Ile	Ser	Ala	Ala	Glu	Leu	Arg	His	Val	Met	Thr	Asn	Leu	Gly

FIG. 4. The sequence of pCM109 DNA was determined as described by Maxam and Gilbert (27). The calmodulin sequence (top line) was determined for the bovine brain protein by Watterson *et al.* (11). The DNA sequence shown is from the 170-bp *Hae* III fragment of the *Pst* I fragment obtained from pCM109. From the sequence gel (Fig. 3) the first codon unambiguously identified was GAC, which corresponds to amino acid 93, or Asp. This residue represents the codon in the pCM109 insert closest to the 5′ end. The bottom line shows the amino acids deduced from the nucleotide sequence.

Identification of pCM109 as a Calmodulin cDNA Recombinant by DNA Sequence Determination. The *Pst* I insert of pCM109 was 350 base pairs (bp) and was longer than the other recombinant plasmids shown in Fig. 2. The sequence of this fragment was determined by the method of Maxam and Gilbert (27). A representative 20% polyacrylamide gel showing a portion of the sequence of the 170-bp *Hae* III fragment beginning with the 5′ end is presented in Fig. 3. The nucleotide sequence corresponds to the codons for six amino acids that can be matched precisely to amino acids 93–98 in the primary sequence of calmodulin determined for the rat testis protein by Dedman *et al.* (8) and the bovine brain protein determined by Watterson *et al.* (9). The first codon to be unambiguously identified from the 5′ end of the 170-bp fragment is GAC, which codes for Asp and represents amino acid 93 in the previously determined calmodulin sequences. Fig. 4 shows a 63-nucleotide sequence that was obtained from the sequence gels. The amino acids represented by these nucleotides are shown below the nucleotides, whereas the corresponding amino acid sequence from bovine brain calmodulin is listed above. The nucleotide sequence corresponds to amino acids 93–113 of the bovine brain protein and shows that, in this region, the protein sequences of rat (8), cow (9), and fish (eel) calmodulins are identical. Finally, because the sequence illustrated in Fig. 4 corresponds to the 5′ end of the DNA insert, it is likely that pCM109 contains nucleotides that code for amino acids 93–148 or 38% of the peptide-coding portion of the gene. In addition, this recombinant plasmid contains some 3′ untranslated sequence and the poly(A) tail.

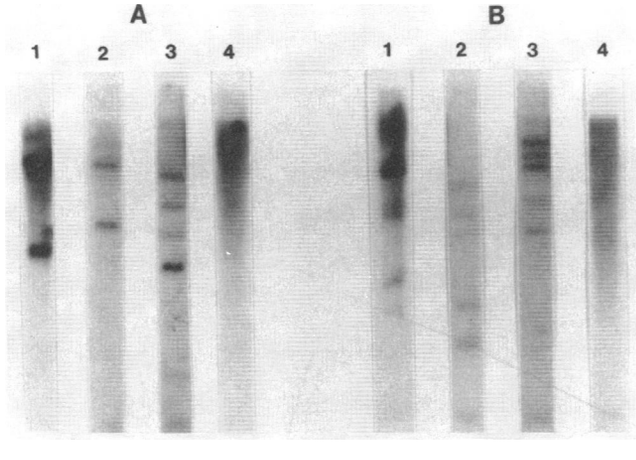

FIG. 5. Hybridization of cloned calmodulin cDNA and total genomic DNA from different species. Total genomic DNAs from electric eel kidney (tracks 1), hen liver (tracks 2), human placenta (tracks 3), and wheat germ (tracks 4) were cut with restriction enzymes *Eco*RI (*A*) or *Hin*dIII (*B*). The DNAs were precipitated with ethanol and dissolved in 10 mM Tris·HCl, pH 8.0/1 mM EDTA. Twelve micrograms of each DNA was subjected to electrophoresis on a 1% agarose horizontal slab gel. The gel was treated with alkali and DNAs were transferred to nitrocellulose paper. Filter-bound DNA was hybridized with 20×10^6 cpm of nick-translated calmodulin [^{32}P]cDNA (specific activity 100×10^6 cpm/μg) overnight at 60°C. The filters were washed with 1× NaCl/Cit at 60°C for 4–5 hr and exposed to an x-ray film for 48 hr.

Intraspecies Homology of Calmodulin Gene Sequences. Fig. 5 shows hybridization of the pCM109 cDNA insert, labeled with ^{32}P by nick-translation (30), to DNA isolated from several representative species (29). High molecular weight DNA was isolated from electric eel kidney, hen liver, human placenta, and wheat germ. Fig. 5*A* shows data obtained from *Eco*RI-cut DNA and Fig. 5*B* data from DNA cut with *Hin*dIII. Discrete hybridization bands are noted in all cases except wheat germ, for which the radioactivity appears as a broad smear in both panels. For the animal DNAs the number of bands present ranges from 2 to 6. These data suggest that the pCM109 calmodulin structural gene probe recognizes specific DNA sequences present in four diverse species.

DISCUSSION

We have isolated and characterized a calmodulin structural gene probe, beginning with a calmodulin-enriched mRNA fraction prepared from electroplax of the electric eel, *Electrophorus electricus*. Direct sequence analysis reveals that this probe, pCM109, contains 38% of the peptide-coding gene sequence. Calmodulin contains 148 amino acids and can be divided into four domains, each of which binds one Ca^{2+} (7–9). The amino acid sequences of the mammalian proteins investigated to date are identical. The only other sequence so far completed is that of the marine coelenterate *Renilla reniformis* (7). Seven conservative amino acid substitutions exist with respect to the mammalian proteins. One of these alterations is the substitution of Phe for Tyr at position 99. It is of interest that the eel amino acid sequence deduced from the DNA sequence contains Tyr at position 99. This implies that the substitution at position 99 occurred relatively early in the evolution of vertebrates, sometime between the appearances of coelenterates (invertebrates) and fish.

The recombinant plasmid pCM109 contains nucleotides that code for amino acids 93–148 of fish calmodulin. These amino acids represent most of the third and fourth functional domains of this protein (7–9). Calmodulin represents only one of a family of Ca^{2+}-binding proteins (11). Other include troponin C, parvalbumin, S-100, and a protein in *Chlamydomonas reinhardii* (12). Over 50% sequence homology exists between calmodulin and troponin C (7–9). Although the greatest degree of homology occurs within the first two domains (65%), 33% of the amino acids in the portion of the molecule represented by pCM109 still are identical. These facts make interpretation of the data in Fig. 5 less simple. It is clear that pCM109 recognizes specific sequences within the DNA from mammals (human), birds (hen), and fish (eel). In each case multiple hybridization bands were observed whether the DNAs were partially digested with *Eco*RI or *Hin*dIII. However, it should also be noted that the number of bands is limited, ranging between two and six. Because of homologies mentioned above and the hybridization conditions (60°C), some positive signals may represent fragments of DNA containing nucleotides that code for a portion of troponin C.

Because the Ca^{2+}-binding proteins contain functional protein domains, it is likely that their genes will contain intervening sequences (31). The question of the number of calmodulin gene copies present in the genomes of three animal classes examined cannot be answered until a genomic clone is available.

DNA from a plant (wheat germ) did not demonstrate discrete hybridization bands. Rather the positive signal generated upon reaction with the calmodulin probe was diffuse. The only certainty is that multiple plant DNA sequences are complementary to the probe. pCM109 contains the 3′ half of the calmodulin structural gene. Therefore, the probe also contains a poly (A·T) region (≈60 residues) corresponding to the poly(A)tail of the mRNA and a poly(G·C) tract at the extreme 3′ end generated by homopolymer addition. The data from plant DNA could be obtained by hybridization of any or all of these portions of the probe. Plants contain calmodulin, however, and this protein has been isolated from the peanut (32). Whereas it is immunologically identical to mammalian calmodulin (2), some differences in the amino acid composition exist (32). The data of Fig. 5 suggest, but do not prove, the presence of calmodulin gene sequences in plant DNA.

The calmodulin structural gene probe reported herein is of sufficient length and specificity to use to detect calmodulin mRNA sequences in a variety of tissues and cells. In preliminary experiments we have utilized the procedure of Alwine *et al.* (33) to detect the mRNA in tissues from rabbit, rat, and chicken. In all cases the mature mRNA appears to be approximately 820 nucleotides in length. pCM109 can also be used to select genomic calmodulin sequences from gene libraries. Again preliminary screening of a chicken library has revealed the presence of multiple cDNA segments. Our next task is to isolate and sequence a full-length ds-cDNA copy of calmodulin mRNA so that studies on the molecular organization of the calmodulin gene can proceed.

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