I report on the isolation, structural analysis, and in vivo expression patterns of a fungal calmodulin gene. The gene is intronless and encodes a protein of 148 amino acid residues that is 92% homologous with vertebrate calmodulins. Through S1 nuclease transcript mapping, it was determined that the cloned gene (a) is transcribed in vivo, (b) has a 5'-untranslated region of about 400 nucleotides, and (c) has a 3'-untranslated end of about 300 nucleotides. Southern blot hybridization analysis of the genomic DNA and the cloned gene provide evidence for the existence of only one type of calmodulin gene in the organism. The amino acid sequence deduced from the DNA sequence shows that *Achlya klebsiana* calmodulin has amino acid substitutions that are a mix of those seen in calmodulins from invertebrates such as *Drosophila* and trypanosome when compared to mammalian calmodulins. Not surprisingly, it has less resemblance to calmodulins from *Saccharomyces* and *Dictyostelium*.

Calcium mediates numerous cellular processes and plays a pivotal role in the regulation of cellular homeostasis (1). These diverse cellular activities of Ca\(^{2+}\) are mediated by cytoplasmic Ca\(^{2+}\) receptors of which calmodulin is the major one in non-muscle cells (2). Calmodulin is a highly conserved acidic monomeric polypeptide ($M_\text{r}$ 16,700) that is present in eukaryotic cells as diverse as mammals (3-6), invertebrates (7-9), green plants (10, 11), and fungi (12, 13). The calmodulin gene has been studied primarily in the cDNA form in human (14), chicken (15), electric eel (16), rat (17), toad (18), trypanosome (19), and slime mold (20). The genomic form of the calmodulin gene of trypanosome are identical and intronless; the gene has been studied primarily in the cDNA form in human (14), chicken (15), electric eel (16), rat (17), toad (18), trypanosome (19), and slime mold (20). The genomic form of the gene has been well analyzed in chicken (15), *Drosophila* (21), rat (22), and trypanosome (19). Chicken and rat calmodulin genes have 5 introns in the coding region whereas the *Drosophila* gene has 3 introns. The three tandemly repeated calmodulin genes of trypanosome are identical and intronless; they are characterized by a 35-nucleotide 5' end leader that is not contiguous with the genes (19). Based entirely on restriction endonuclease DNA fragmentation and hybridization analyses, it was concluded that *Xenopus* (18) and *Dictyostelium* (22) calmodulin genes have introns. Thus several eukaryotic calmodulin genes studied so far have introns and the absence of intron in the *Achlya* gene may be unusual.

We have shown in a series of studies that *Achlya klebsiana* has a strong physiological dependence on calcium. Growth (23), energy-linked transport of amino acids (24), nucleosides (25), and sugars (26), and the process of sporulation (27) were all shown to be dependent on the availability of Ca\(^{2+}\). Ca\(^{2+}\) therefore, must play a pivotal role in cellular homeostasis of *Achlya*. In support of the notion that Ca\(^{2+}\) is important for sporulation, Suryanarayana et al. (28) isolated calmodulin from *Achlya amb isi caulis* and showed that its induction was associated with cell wall lysis during sporulation (29). I show here that *A. klebsiana* has a functional intronless calmodulin gene which is induced to produce an abundant quantity of calmodulin transcripts when it is undergoing sporulation.

**MATERIALS AND METHODS**

**Organisms**—A coenocytic freshwater mold, *A. klebsiana*, was used as the source of the calmodulin gene studied.

**Induction of Sporulation**—Hyphal cells derived from sporangiospores germinated and grown vegetatively for 20 h at 24 °C in defined medium, were harvested and resuspended in sporulation induction medium as described (30).

**Isolation of High Molecular Weight DNA**—About 20 g (fresh weight) of suction-dried mycelia was frozen with liquid nitrogen and ground to a fine powder with mortar and pestle. The powder was suspended in 50 ml of buffer of composition 0.1 M NaCl, 0.1 M Na\(_2\)EDTA, 50 mM Tris-Cl, pH 8, and 1% Sarkosyl. Proteinase K was added to a final concentration of 50 μg/ml and incubated for 1 h at 37 °C. An equal volume (70 ml) of buffer (0.1 M NaCl, 0.1 M Na\(_2\)EDTA, 50 mM Tris-Cl, pH 8) saturated “phenol” (redistilled phenol mixed with 0.1% 8-hydroxyquinoline and 0.5% 2-mercaptoethanol) was added. The solution was gently mixed by hand for 10 min. The mixture was centrifuged at 10,000 × g for 10 min at room temperature to separate the phases. The upper aqueous phase was recovered and phenol extraction of proteins repeated three times or until the interface was free of precipitate. The aqueous fraction was extracted twice with an equal volume of CHCl\(_3\). One-tenth volume of 3 M NaOAc, pH 5.2, was added to the aqueous phase and nucleic acids precipitated by layering 2.2 volumes of chilled absolute ethanol over the nucleic acid solution and DNA recovered by spooling with a clean glass rod. The DNA was resuspended in TE (10 mM Tris-Cl, pH 7.5, 1 mM EDTA) buffer and respooled. This process was repeated three times and the final resuspended DNA was air-dried for 10 min before dissolving in a small volume of TE buffer to a concentration of 0.5-1.0 mg/ml.

**Construction of Genomic Library and Screening for Calmodulin Gene**—DNA isolated as described above was consistently greater than 80 kb. The DNA was partially digested with restriction endonuclease MboI and size-fractioned in sucrose density gradients. DNA fragments of 15-25 kb were selected and ligated into the BamHI site of *B. subtilis* EMBL3. Ligated products were packaged in *Escherichia coli* P2.392. Ligation and packaging were done according to Stratagene, San Diego, CA and propagated in *E. coli* P2.392. The library was screened with \(^{32}P\)-labeled electric eel calmodulin cDNA (pCM1116) provided by John Putkey and Anthony Means of Baylor College, Houston, TX by the method of Benton and Davis (31).

**Preparation of cDNA**—Poly(A)\(^+\) RNA was isolated from vegetatively growing and sporulation induced *Achlya* cells (32) and converted to double stranded cDNA by fast protein liquid chromatography.
phy pure avian myeloblastosis virus reverse transcriptase, RNase H, E. coli DNA ligase, and DNA polymerase I combinations as recommended by Pharmacia (Uppsala, Sweden). \( ^{32}P \)dCTP was used during synthesis of the second strand to monitor the reaction and estimate yield.

Genomic Southern Blot Hybridization—High molecular weight DNA was digested to completion with a variety of restriction enzymes, and the DNA fragments were separated in 0.7% agarose gel slabs by electrophoresis and then transferred to Hybond (Amer sham Corp.) nylon sheets by the alkaline method of Reed and Mann (33). Hybridization was carried out using, as probes, the 2.0- and 1.7-kb DNA fragments representing the \( 5' \) and \( 3' \) ends, respectively, of Achlya calmodulin gene. A 0.7-kb DNA probes were labeled with \( ^{32}P \) by the random primer method (34).

RNA Blot Hybridization—Poly(A) \(^+\) RNA was isolated as described (32) and electrophoresed in 1.2% agarose denaturing gel containing 6% formaldehyde and transferred to Hybond. RNA on Hybond was probed with a 3.8-kb Sau3A DNA fragment containing the full Achlya calmodulin gene. The probe was \( ^{32}P \)-labeled by nick translation (35).

\( S1 \) Nuclease Mapping—The \( S1 \) nuclease transcript mapping procedure of Berk and Sharp (36) was used to evaluate whether the calmodulin gene was transcriptionally active in vivo and to determine the approximate initiation and termination sites of transcription of the gene. Total RNA was isolated from calmodulin-induced cells and used to hybridize against the 2.0- and 1.7-kb Clal DNA fragments representing the \( 5' \) and \( 3' \) ends of the fungal calmodulin gene. These two DNA fragments contain several hundred nucleotides of untranslated borders of the gene. A single Clal site situated about 90 nucleotides from the \( 5' \) end of the coding sequence of the gene was used as the reference point in determining transcription initiation and termination sites. \( S1 \) nuclease-insensitive DNA-RNA hybrids as well as standards were analyzed in non-denaturing 1.2% agarose gels according to Maniatis et al. (37).

Preparation of Phagemid M13/Calmodulin DNA Recombinants—Phagemids M13\(^+\) and M13\(^-\) (Stratagene, San Diego, CA) were linearized with Clal and ligated independently to 2.0- and 1.7-kb Clal fragments recovered from a recombinant clone isolated from the genome, and known to contain the fungal calmodulin gene. The phagemid recombinants were used to transform E. coli JM107.

Isolation of DNAs from Gels—DNA in agarose gels were recovered by the "Gene Clean" technique (Bio 101 Lab., La Jolla, CA).

Exonuclease III and Mung Bean Nuclease Deletions—Phagemid recombinants containing the 2.0- and 1.7-kb DNA fragments were linearized with Apal to yield 3' extended ends; this was followed by Sau3A digestion which created a 5' extension at one of the two Apal ends in both phagemids. In case of the 2.0-kb-containing M13 recombinant, a 0.2-kb fragment was removed from the insert by Sau3A resulting in a 1.8-kb/M13 recombinant phagemid. The 1.8-kb/M13 DNA fragment (representing the \( 5' \) end of the gene) and the 0.75-kb PstI fragment (representing the \( 3' \) end of the gene) were doubly digested with a combination of ClaI and Apal, and used for transformation E. coli JM107. Phagemids from transformants were screened and nesr of deletion recombinants covering the length of the inserts selected. The phagemids were purified by CaCl (39) and used for DNA sequencing.

DNA Sequencing—DNA sequencing was carried out by the dideoxy chain termination method (40). Prior to sequencing, the phagemid was denatured by treatment with alkali as described by Chen and Seeburg (41) and the primer annealed according to Korneluk et al. (42). \( ^{32}P \)dATP was used as the radioactive label in DNA sequencing reactions.

Computer Analysis—The DNA sequence was analyzed by the Beckman MicroGenie program (Beckman Instruments Inc., Palo Alto, CA).

Biohazard Precautions—All cloning experiments and disposal of recombinant cells were performed in compliance with the Medical Research Council of Canada guidelines.

 Autoradiography—DNA sequencing gels were exposed, after drying, to Kodak X-Omat AR film for 1-3 days. Other autoradiograms were prepared with Kodak X-Omat RP films.

RESULTS

Isolation of Fungal Calmodulin Gene—About 50,000 recombinant bacteriophages constructed as described under "Materials and Methods" and representing more than five genome equivalents of the freshwater mold A. klebsiana were screened with \( ^{32}P \)-labeled electric eel calmodulin cDNA as probe. Two positive clones were isolated. Both clones had DNA inserts of about 15 kb and identical restriction endonuclease digestion patterns (not shown). Consequently, one was selected for further study.

Restriction Endonuclease Map of Calmodulin Gene—DNA from the recombinant bacteriophage harboring the calmodulin gene was digested with several restriction endonucleases including BamHI, Clal, DraI, HindIII, HpaI, and Sau3A. Two major hybridizing bands appearing in the ChIIApaI digest products were selected for subcloning and structural analysis of the gene. The rationale was that the two Clai DNA fragments probably represented the \( 5' \) and \( 3' \) ends of the gene, as proved to be the case.

A detailed restriction endonuclease map of the cloned 15-kb genomic fragment containing the calmodulin gene was obtained using the 2- and 1.7-kb subfragments as probes (Fig. 1, lower region). Location and orientation of the coding region in the 2- and 1.7-kb fragments was achieved by using as probes the 0.19-kb PstI DNA fragment from electric eel calmodulin which represents the \( 5' \) end of the gene and the 0.75-kb PstI DNA fragment representing the \( 3' \) end of the gene (16) (Fig. 1, upper region).

Evidence for Existence of Single Calmodulin Gene—Total genomic DNA and 15-kb DNA fragment containing the calmodulin gene were doubly digested with a combination of Clai and either Apal, DraI, EcoRV, or Smal restriction endonucleases. Southern blots of these digests were probed with 2-kb Clai DNA fragment (representing the \( 5' \) end of the gene) and 1.7-kb Clal DNA fragment (representing the \( 3' \) end of the gene). The results (Fig. 2) show that the genomic DNA and the cloned 15-kb DNA have the same hybridization patterns. Two minor hybridizing bands appearing in the Clai/Apal digest of the cloned DNA probe with the \( 5' \) end of the gene are artifacts because no Apal site exists in the 2- or 1.7-kb fragment; a feature that was exploited in preparing progressive unidirectional deletions for sequencing of the gene (see Fig. 1. Restriction endonuclease map of a cloned 15-kb DNA fragment containing Achlya calmodulin gene. The upper part is an exploded view of the gene and its border sequences. The lower part is the genomic fragment cloned in EMBL3 arms. A, Apal; B, BglII; C, ClaI; D, DraI; E, EcoRI; Ev, EcoRV; H, HindIII; K, KpnI; P, PstI; S, Sau3A, X, XbaI; Bam, BamHI. (Note: The short arm of EMBL3 is on the left and the long arm is on the right.)
random primer-labeled DNA was digested with ClaI (in all cases) and either of the linearized plasmids as described under "Materials and Methods". Sequencing of fungal calmodulin was performed by the dideoxynucleotide chain termination method (35). The sequences obtained were assembled and analyzed by the Beckman Microgenie program, and a portion of it displaying the coding sequence of the gene and its 5'- and 3'-noncoding borders is presented in Fig. 2. The amino acid sequence of the fungal calmodulin gene was deduced from the DNA sequence and the information is incorporated into Fig. 4.

The assumption is made that like other calmodulins, the first amino acid of the mature protein succeeds the initiation codon AUG. This means that the protein is 148 amino acid residues long with alanine at the amino end and lysine at the carboxyl end. The protein sequence obtained is 92% homologous to electric eel calmodulin. A comparison of the amino acid sequence of calmodulins from human, electric eel, trypanosome and Achlya (Fig. 5) shows that compared to human, electric eel calmodulin has a single amino acid difference. Achlya calmodulin has 11 amino acid replacements and trypanosome has 11 also. Assuming that Achlya calmodulin has two AAATAA-like homologies at the 3' end. Whether these are several TATA box homologies and many CAAT-like consensus sequences at the 5' end and 3' end flanks of the coding region presented in Fig. 3. DNA blot hybridization analyses of cloned and genomic DNAs were doubly digested with ClaI (in all cases) and either ApoI (Ap), DraI (Dr), EcoRV (Ec), or Smal (Sm). a, DNA blots were probed with random primer-labeled 5' end of the gene (2-kb ClaI DNA). b, DNA blots were probed with random primer-labeled 3' end of the gene (1.7-kb ClaI DNA).

**FIG. 2.** Southern blot hybridization analyses of Achlya genomic DNA and a cloned 15-kb DNA fragment from Achlya containing a calmodulin gene. Cloned and genomic DNAs were doubly digested with ClaI (in all cases) and either ApoI (Ap), DraI (Dr), EcoRV (Ec), or Smal (Sm). a, DNA blots were probed with random primer-labeled 5' end of the gene (2-kb ClaI DNA). b, DNA blots were probed with random primer-labeled 3' end of the gene (1.7-kb ClaI DNA).

**FIG. 3.** Nested deletions of A. klebsiiana calmodulin genomic gene bisected by ClaI into 2-kb (a) and 1.7-kb (b) DNA fragments that were ligated and cloned separately in phagemid M13+ . Clone A, in both cases, represents undeleted fragments. Clone B in a is SalI truncation of the 2-kb DNA insert. All other clones are exonuclease III/mung bean nuclease deletions. Arrow signifies region of each clone that was sequenced from either the T3 (O) or T7 (●) promoters. The rectangular boxes specify the coding regions of the gene.

**FIG. 4.** Accumulation of calmodulin mRNA during Development—Suryanarayana and Thomas (29) have shown that during sporulation in A. ambisexualis, the cells accumulated high levels of calmodulin which were localized by immunofluorescence to sporangial discharge papillae and the spores themselves. Achlya cells induced to develop sporangia and sporulate were analyzed for calmodulin mRNA content during the induction phase (starvation). Poly(A)+ RNA was isolated from starving cells at different times, electrophoresed, transferred to nylon filter and probed with a 3.8-kb SalI cleaved DNA fragment containing the full length of the calmodulin gene and hundreds of nucleotides of its 5' and 3' end flanks. The results (Fig. 7a) show that there was a single poly(A)+ RNA hybridizing band whose intensity increased with time. The most dramatic increase occurred within the first 2 h. When
**DISCUSSION**

The amino acid sequence of *Achlya* calmodulin gene was deduced from the DNA sequence shown in Fig. 4. When it is compared to the amino acid sequence of calmodulins from trypanosome (19), electric eel (16), and the uniform sequence found in mammals such as human (14), chicken (15), and rat (17), and the amphibian *Xenopus* (18), it is seen that the fungal protein is 92% identical to the mammalian calmodulin and 93% identical to that of trypanosome (Fig. 4). Whereas *Achlya* and trypanosome calmodulins have 11 amino acid replacements, in each case, compared to the mammalian protein at only 3 residue positions: 99, 143, and 147.

L-glutamine, which arrests sporangial development (30), was added to starving cells the level of calmodulin poly(A)* RNA recovered from the cells diminished markedly (Fig. 7b). This implies that enhanced transcription of the calmodulin gene may be linked to asexual differentiation in this organism.

**Isolated Calmodulin Gene Is Transcribed**—Although the cloned calmodulin gene hybridized to blotted poly(A)* RNA, it was necessary to show that it represents a gene that is transcribed in vivo. Indirect evidence for this was obtained by S1 nuclease transcript mapping. Total cell RNA was hybridized to a 2-kb ClaI DNA fragment labeled at the 5' end and the represented the 5' end of the gene and (b) the 1.7-kb ClaI DNA fragment labeled at the 3' end and represented the 3' end of the gene. Following S1 nuclease hydrolysis of unprotected regions of the hybrids, the products were sized with neutral 1.2% agarose gel (Fig. 8). The approximate size of the fragment defining the transcriptional start site to the sole ClaI site in the coding sequence was 790 nucleotides, while the size of the fragment from the same ClaI site to the transcriptional termination point was about 370 nucleotides. These results are compatible with single transcriptional start and stop sites for the gene being expressed under nutrient starvation conditions.

The amino acid sequence of *Achlya* calmodulin gene was deduced from the DNA sequence shown in Fig. 4. When it is compared to the amino acid sequence of calmodulins from...
FIG. 5. Comparison of amino acid sequences of calmodulins from *A. klebsiana*, trypanosome, electric eel, and human. Sites where residue differences occur are boxed. * identifies boxed residues that have changed between the species but remain identical in *Achlya* and vertebrate calmodulins. * identifies boxed residues that have changed between the species but in a similar fashion in *Achlya* and trypanosome.

FIG. 6. Possible four Ca\(^{2+}\) binding domains of *A. klebsiana* calmodulin. The amino acid sequence in the calcium binding regions of the loops are identical to those of vertebrate calmodulin except for Phe-99 in domain II and Ile-130 in domain IV. * signifies amino acid residues that can participate in calcium binding. * signifies amino acid replacements in *Achlya* protein when compared to mammalian calmodulin.

binding region only) at the nucleic acid and protein levels is summarized in Table I. Domains I and III are nearly 70% homologous whereas domains II and IV are less than 56% homologous at the nucleotide level. At the amino acid level, domains I and III are 58.3% homologous while domains II and IV are only 50% homologous. In most comparisons of the various domains, there is a higher homology between the various domains at the nucleotide than amino acid level. This is the general pattern that has been observed for calcium binding domain homologies in other calmodulin genes (16, 21, 43, 45). Although 2 of the amino acid residue replacements, Phe-99 and Ile-143 fall into the calcium binding domains (Fig. 6), their effect on the binding function and loop structure is negligible because phenylalanine can replace tyrosine in bind-
Fungal Calmodulin Gene

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TABLE I

Homology between nucleotide and amino acid sequences of putative calcium binding domains I through IV of Achlya calmodulin

| Domains | Nucleotide | Amino acid |
|---------|------------|------------|
| I x II  | 52.8       | 58.3       |
| I x III | 69.4       | 58.3       |
| I x IV  | 58.3       | 58.3       |
| II x III| 47.2       | 58.3       |
| II x IV | 55.6       | 50         |
| III x IV| 55.6       | 50         |

% homology

TABLE II

Amino acid composition of calmodulins from Achlya, trypanosoma, eel, and human

| M, values: A. klebsiana, 16,567; trypanosoma, 16,709; eel, 16,880; human, 16,760. |
|-------------------------------------------------------------|
| Amino acid | A. ambisexualis* | A. klebsiana* | Trypanosoma Eel Human |
|------------|------------------|---------------|-----------------------|
| no. of residues/molecule | 13 | 10 | 8 | 11 | 11 |
| Alanine   | 4                | 5             | 5                      |
| Asparagine| 26               | 6             | 6                      |
| Aspartic acid| 6               | 6             | 6                      |
| Arginine  | 17               | 17            | 17                     |
| Cysteine  | 0                | 0             | 0                      |
| Glutamic acid| 21              | 20            | 21                     |
| Glutamine | 32               | 6             | 6                      |
| Glycine   | 17               | 13            | 11                     |
| Histidine | 11               | 9             | 8                      |
| Isoleucine | 11              | 9             | 8                      |
| Leucine   | 12               | 9             | 9                      |
| Lysine    | 7                | 9             | 9                      |
| Methionine| 8                | 11            | 9                      |
| Phenylalanine| 11             | 8             | 8                      |
| Proline   | 4                | 2             | 2                      |
| Serine    | 9                | 9             | 4                      |
| Threonine | 13               | 11            | 12                     |
| Tyrosine  | 2                | 1             | 2                      |
| Tryptophan| 0                | 0             | 0                      |
| Valine    | 7                | 5             | 6                      |

*Based on amino acid analysis of protein.
Based on deduced protein sequence obtained from gene sequence.

FIG. 7. RNA blots of poly(A)+ RNA from A. klebsiana and hybridization to electric eel calmodulin cDNA. a, poly(A)+ RNA from cells that were incubated in sporulation medium for 1, 2, and 3 h, and from vegetatively (V) growing cells that were sampled at the time of transfer of the cells from growth to induction medium. b, same as for a, but the induction medium contained 5 mM L-glutamine. Five micrograms of RNA was applied in every lane. Arrowheads signify hybridizing transcripts.

FIG. 8. S1 nuclease protection analysis of Achlya transcripts. S1 nuclease protection reactions were carried out with approximately 20 µg of total RNA and either 5'-end-labeled 2-kb ClaI DNA fragment representing the 5' end of the calmodulin gene or 3'-end-labeled 1.7-kb ClaI DNA fragment representing the 3' end of the calmodulin gene. Lane 1, Bethesda Research Laboratories 1-kb DNA size markers electrophoresed in 1.2% neutral agarose gel, Southern blotted, and probed with 32P-labeled 1-kb ladder DNA. Lane 2, 2-kb ClaI DNA fragment. Lane 3, S1 nuclease protected hybrid of total RNA and 2-kb ClaI DNA. Lane 4, 1.7-kb ClaI DNA fragment. Lane 5, S1 nuclease protected hybrid of total RNA and 1.7-kb ClaI DNA. Lane 6, same as for lane 4. Samples in lanes 2-6 were electrophoresed in 1.2% neutral agarose gel, Southern blotted, and probed with [α-32P]dATP nick-translated 3.8-kb SalI DNA fragment containing the entire coding sequence of the gene and hundreds of nucleotides at the 5' and 3'-untranslated ends. Small arrows indicate the positions of the resulting hybrids.

I have described here the nucleotide structure of a locus encoding calmodulin within the genome of A. klebsiana. Several lines of evidence support the idea that the isolate is the only gene for calmodulin in Achlya. First, restriction endonucleases digest the cloned gene and genomic DNA yielding fragments of identical sizes that hybridize to the 5' and 3' ends of the sequenced gene used as probes (Fig. 2). Hybridization patterns of these same DNA fragments to the 0.19 kb (5' end) and 0.75 kb (3' end) of electric eel calmodulin cDNA used as probes gave identical results, albeit slightly different from those presented in Fig. 2 (data not shown). Second, S1 nuclease mapping results (Fig. 8) show that the cloned gene is probably transcribed in vivo, and there is no indication that there exists another calmodulin transcript with significant homology to the one detected in S1 nuclease mapping. Third, the two calmodulin clones isolated from five haploid genome equivalents Achlya library were identical. The occurrence of a single calmodulin gene in the genomes of widely disparate organisms such as Drosophila (21), chicken (15), and Dictyostelium (20) has been reported. As well, multiple tandem copies and two non-allelic calmodulin genes have been reported for trypanosome (19) and Xenopus (18), respectively.

TABLE I

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|-------------------------------------------------------------|
| Homology implies presence of identical nucleotides and amino acids at corresponding positions in the domains. |
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| Glutamine | 32               | 6             | 6                      |
| Glycine   | 17               | 13            | 11                     |
| Histidine | 11               | 9             | 8                      |
| Isoleucine | 11              | 9             | 8                      |
| Leucine   | 12               | 9             | 9                      |
| Lysine    | 7                | 9             | 9                      |
| Methionine| 8                | 11            | 9                      |
| Phenylalanine| 11             | 8             | 8                      |
| Proline   | 4                | 2             | 2                      |
| Serine    | 9                | 9             | 4                      |
| Threonine | 13               | 11            | 12                     |
| Tyrosine  | 2                | 1             | 2                      |
| Tryptophan| 0                | 0             | 0                      |
| Valine    | 7                | 5             | 6                      |

*Based on amino acid analysis of protein.
Based on deduced protein sequence obtained from gene sequence.

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But with the exception of trypanosome and Achlya, the gene appears to be segmented into introns and exons in every other case, including humans. The DNA sequence results presented include about 500 nucleotides of presumed 5' -untranslated region, 447 nucleotides encoding the calmodulin protein and about 320 nucleotides of presumed 3' -untranslated region (Fig. 4). The 5' -untranslated region has several TATA- and CAAT-like sequence homologies, but it is not known which (if any) function in transcription. An interesting arrangement of two CAAT-like sequence homologies in direct repeat mode is present between -441 and -457. Within the 5' -untranslated region are several in-frame stop codons, one of which is only 6 nucleotides upstream from the assigned translation start codon. Such structural organization implies that (a) the protein is unlikely to be synthesized as a secretable entity, (b) Achlya calmodulin is not synthesized as a precursor protein, and (c) the translation start codon designated is probably correct. The 3' -untranslated end is replete with in-frame translation signals sequence homologies similar to those of eukaryotic transcripts identified in Fig. 7. The 3' untranslated end is replete with in-frame translation signals about 300 nucleotides downstream of the translation stop codon. Two possible closely set signals are about 300 nucleotides downstream of the last translation stop codon (Fig. 4). This gene, therefore, may encode the calmodulin protein in general. 

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