THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 265, No. 4, Issue of February 5, pp. 2244-2256, 1990
Printed in U.S.A.

Molecular Cloning of cDNA Encoding Human and Rabbit Forms of the Ca^{2+} Release Channel (Ryanodine Receptor) of Skeletal Muscle Sarcoplasmic Reticulum*

(Received for publication, August 18, 1989)

Francesco Zorzato‡§, Junichi Fujii‡¶, Kinya Otsu‡, Michael Phillips‡, N. Michael Green‖, F. Anthony Lai‡‡, Gerhard Meissner**, and David H. MacLennan‡**

*From the ‡Banting and Best Department of Medical Research, University of Toronto, C. H. Best Institute, Toronto, Ontario M5G 1L6, Canada, the ‖National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom, and the **Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27599*

We have cloned cDNAs encoding the rabbit and human forms of the Ca^{2+} release channel of sarcoplasmic reticulum. The human cDNA encodes a protein of 5032 amino acids, with a molecular weight of 563,584, which is made without an NH_2-terminal signal sequence. Amino acid substitutions between rabbit and human sequences were noted in 163 positions and deletions or insertions in eight regions accounted for additional sequence differences between the two proteins. Analysis of the sequence indicates that 10 potential transmembrane sequences in the COOH-terminal fifth of the molecule and two additional, potential transmembrane sequences nearer to the center of the molecule could contribute to the formation of the Ca^{2+} conducting pore. The remainder of the molecule is hydrophilic and presumably constitutes the cytoplasmic domain of the protein. A 114–120 amino acid motif is repeated four times in the protein, in residues 841–954, 955–1068, 2725–2844, and 2845–2958 and a 16 amino acid part of the motif is repeated twice more in residues 1344–1359 and 1371–1386. Although the channel is modulated by Ca^{2+}, ATP, and calmodulin, no clear high affinity Ca^{2+}-binding domain of the EF hand type and no clear high affinity ATP-binding domain were detected in the primary sequence. An acidic sequence in residues 1872–1923 contains 79% glutamate or aspartate residues and this sequence is a potential low affinity Ca^{2+}-binding site. Several potential calmodulin-binding sites were observed in the sequence, in the region 2800 to 3050.

Ca^{2+} release from fractions of the sarcoplasmic reticulum containing terminal cisternae has been characterized extensively during the past decade (Miyamoto and Racker, 1982; Morii and Tonomura, 1983; Seiler *et al.*, 1984; Meissner, 1984; Meissner, 1986; Meissner *et al.*, 1986). Rapid Ca^{2+} release from isolated vesicles is activated by micromolar Ca^{2+} and millimolar adenine nucleotides and inhibited by millimolar Mg^{2+}. Calmodulin at micromolar concentrations partially inhibits Ca^{2+} release, apparently by direct protein-protein interaction with the Ca^{2+} release channel. Single channel measurements in planar bilayers (Smith *et al.*, 1986) have shown that Ca^{2+} release from the sarcoplasmic reticulum is mediated by a ligand-gated channel with a conductance greater than 100 pS in 50 mM Ca^{2+}.

Identification and isolation of the Ca^{2+} release channel were facilitated through the use of the plant alkaloid, ryanodine (Jenden and Fairhurst, 1969), which was shown to bind to the protein with high affinity and to modulate its function (Seiler *et al.*, 1984; Fleischer *et al.*, 1985; Pessah *et al.*, 1985; Pessah *et al.*, 1986; Inui *et al.*, 1987a; Campbell *et al.*, 1987; Lai *et al.*, 1987; Lai *et al.*, 1988a). Single channel recordings showed that purified ryanodine receptor preparations, comprised of tetrameric complexes of a single major polypeptide of M_r 350,000–450,000, exhibited an intrinsic Ca^{2+} channel activity that was modulated by Ca^{2+}, ATP, and Mg^{2+} (Imagawa *et al.*, 1987; Hymel *et al.*, 1988; Smith *et al.*, 1988; Lai *et al.*, 1988a) in a manner similar to native Ca^{2+} release channels (Smith *et al.*, 1985). Analysis of the stoichiometry and subunit composition of the 30 S ryanodine receptor complex indicates that it is a cooperatively coupled, negatively charged homotetramer (Lai *et al.*, 1989).

Studies of the morphology of the ryanodine receptor (Inui *et al.*, 1987a; Lai *et al.*, 1988a; Saito *et al.*, 1988; Wagenknecht *et al.*, 1989) have shown it to have an exquisite quatrefoil structure, with hydrophobic segments of the four identical subunits forming a putative membrane-spanning baseplate structure, and hydrophilic segments forming a cytoplasmic domain that surrounds and decorates the central baseplate. Three-dimensional image reconstruction (Wagenknecht *et al.*, 1989) suggests the presence of four internal channels which branch from a common origin above the baseplate and open into vestibules in the four quarters of the tetramer. The morphology of the purified Ca^{2+} release channel shows that it makes up the "feet" structures (Franzini-Armstrong, 1970; Ferguson *et al.*, 1984) that bridge the gap between the sarcoplasmic reticulum and the transverse tubule (Inui *et al.*, 1987a; Saito *et al.*, 1988).

The next major advance in the study of the Ca^{2+} release channel is cloning of the cDNA encoding the protein, thereby opening up new avenues of investigation of the protein, including its primary and predicted secondary structure, its expression and mutagenesis, and its genomic structure, local-

* This research was supported by grants (to D. H. M.) from the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada (MDAC) and by a grant (to G. M.) from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Postdoctoral Fellow of the Canadian Heart Foundation.

¶ Postdoctoral Fellow of the Muscular Dystrophy Association of Canada.

‡‡ Postdoctoral Fellow of Muscular Dystrophy Association.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05200.

ization, and involvement in disease. Takeshima *et al.* (1989) have reported the cloning and expression of cDNA encoding the rabbit skeletal muscle ryanodine receptor and Marks *et al.* (1989) have reported the isolation of partial clones encoding the rabbit protein. We have reported the cloning of cDNA encoding full length rabbit and human skeletal muscle ryanodine receptors and the localization of the ryanodine receptor gene to the proximal long arm of human chromosome 19 (Zorzato *et al.*, 1989a; MacLennan *et al.*, 1989; MacKenzie *et al.*, 1989). We have shown that the ryanodine receptor gene is not involved in myotonic dystrophy but that it is a candidate for the defective gene in malignant hyperthermia. We now report the full nucleotide and deduced amino acid sequences for the human ryanodine receptor cDNA. These sequences will be of great interest as studies of the involvement of the ryanodine receptor gene in human muscle disease unfolds.

EXPERIMENTAL PROCEDURES

Materials—Hybond nylon membranes, [α-^{35}S]dATP, [α-^{32}P]dCTP, and [γ-^{32}P]ATP were from Amersham Corp; Zeta-Probe blotting membranes were from Bio-Rad; restriction endonucleases and modifying enzymes were from Boehringer Mannheim and Pharmacia LKB Biotechnology Inc.; acrylamide and *N-N'*-bis-acrylamide were from Bio-Rad; low melting point agarose was from FMC Bio Products; electrophoresis grade agarose was from Schwartz Biotechnology; alkaline phosphatase-conjugated anti-guinea pig IgG and the Riboclone *Eco*RI adaptor system were from Promega; nitrocellulose filters were from Millipore; oligo(dT)-cellulose and dextran sulfate were from Pharmacia LKB Biotechnology Inc.; the Bluescript vector, the *Exo*III/mung bean nuclease deletion kit, the λZAP II cloning vector, and the Gigapack Gold packaging extracts were from Stratagene; nucleic acid sequencing kits were from Pharmacia LKB Biotechnology Inc. and United State Chemical Corporation; oligonucleotides were from Allelix.

RNA and DNA Manipulations—mRNA from neonatal rabbit skeletal muscle and adult human muscle (kindly provided by Dr. R. G. Worton, Hospital for Sick Children, Toronto) was isolated as described by MacLennan and de Leon (1983). For Northern blotting, poly(A)$^+$ RNA from neonatal rabbit skeletal muscle was separated in formaldehyde-agarose gels and transferred to Hybond nylon membranes. Hybridization to restriction endonuclease fragments of cDNA or genomic DNA was carried out as described by Wahl *et al.* (1979). A human dystrophin cDNA probe (Ray *et al.*, 1985) used in Northern blot analysis was a kind gift from Drs. H. Klamut and R. G. Worton, Toronto. Other manipulations of mRNA and DNA were carried out using standard protocols (Maniatis *et al.*, 1982) with the exception of DNA transfers to nitrocellulose. In this case, DNA was transferred electrophoretically to Zeta-Probe nylon membranes for 60 min at 50 V using the transfer apparatus described by Gershoni *et al.* (1980), in a buffer consisting of 45 mM boric acid, 45 mM Tris, and 1 mM EDTA, pH 8.0. Labeling of cDNA probes was carried out with [α-^{32}P]ATP using the oligolabeling kit supplied by Pharmacia LKB Biotechnology Inc. Sequencing of cDNA was carried out using the dideoxy method of Sanger *et al.* (1977). Templates for sequencing were prepared in the Bluescript vector using the *Exo*III/mung bean nuclease deletion strategy.

Isolation of Rabbit cDNA Clones—The λgt11 cDNA expression library, constructed from poly(A)$^+$ RNA from rabbit fast-twitch *psoas* muscle (Ellis *et al.*, 1988) was a gift from Drs. S. B. Ellis and M. Harpold, Salk Institute, Biochemistry/Industrial Associates, San Diego, CA and Dr. A. Schwartz, Department of Pharmacology, University of Cincinnati, Cincinnati, OH. The library was screened with an affinity purified polyclonal antibody (Zorzato *et al.*, 1989b) specific for the Ca^{2+} release channel. Screening of the library was carried out by the method of Young and Davis (1983), essentially as described by Leberer *et al.* (1989a).

The screening of 3×10^6 recombinants led to the isolation of two cDNA clones in the region defined by nucleotides 14280–14629 and 13434–13758 in Fig. 1. Analysis of the sequences of these clones showed that both were rearranged when compared with the linear sequence of the human cDNA, which was by then available in our laboratory. Accordingly, restriction endonuclease fragments from these isolated cDNA clones were used as probes to isolate longer, unrearranged cDNA clones from the neonatal rabbit skeletal muscle cDNA library described by MacLennan *et al.* (1985). The longest clone that we obtained was 6.8 kb[1] and it is defined by nucleotides 8615–15241 in Fig. 1. This clone was subcloned into the Bluescript vector and sequenced.

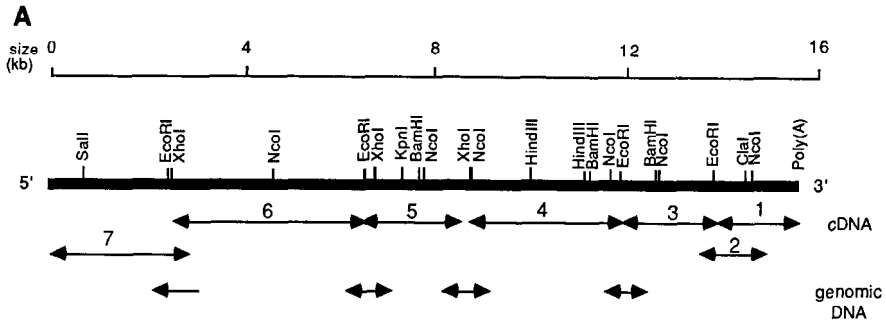
The clone was extended by construction of a primer extension library using 100 μg of neonatal rabbit muscle poly(A)$^+$ RNA. The primer site was defined with 0.25 nmol of an 18-mer oligonucleotide complementary to the rabbit equivalent of residues 9118–9135 in Fig. 1 (residues 9123–9140 in Takeshima *et al.* (1989)). Primer extension was carried out using a cDNA synthesis kit provided by Bethesda Research Laboratories. After second-strand synthesis, cDNAs were blunt ended by adding 2 units of T4 DNA polymerase/ug of DNA to the reaction mixture. The cDNA was phenol-chloroform extracted, ethanol precipitated, and then size fractionated by separation on 0.5% low melting point agarose gels in 90 mM Tris, 90 mM boric acid, 1 mM EDTA buffer, pH 8.0. A 20-fold molar excess of *Eco*RI adaptors were added to the size-fractionated cDNA, and the sample was ligated for 4 h at 20 °C with 2 units of T4 DNA ligase. The sample was then phosphorylated with 10 units of T4 polynucleotide kinase. Excess adaptor was removed by spin-column chromatography and aliquots containing an equimolar ratio of DNA to vector arms were ligated overnight at 15 °C, in 5 μl, to 1 μg of dephosphorylated arms of the λZAPII vector using 2 units of T4 DNA ligase. *In vitro* packaging was performed with 1–2-μl aliquots of each ligation mixture using the Gigapack Gold packaging extract. Subsequent screening was carried out with the unamplified library. In the first primer extension, the cDNA was extended up to nucleotide 4527. In the second extension, an 18-mer oligonucleotide complementary to the rabbit equivalent of residues 4892–4909 in Fig. 1 (residues 4900–4917 in Takeshima *et al.* (1989)) was used to extend the cDNA to nucleotide 3231. In the third and final primer extension, an 18 mer oligonucleotide complementary to the rabbit equivalent of residues 3499–3516 in Fig. 1 (residues 3502–3519 in Takeshima *et al.* (1989)) was used to extend the sequence into the 5′-untranslated region of the mRNA.

Isolation of Human cDNA Clones—Rabbit ryanodine receptor cDNA probes were used to screen a human fetal *psoas* muscle cDNA library in λgt10 (Koenig *et al.*, 1987), kindly provided to us by Drs. M. Koenig and L. M. Kunkel, Harvard University, Boston, MA. Cross-hybridization was strong for all of the clones isolated, and washing was carried out with relatively high stringency (0.1 × SSCP at 62 °C, Maniatis *et al.*, 1982). In the first screen, over 30 clones were isolated but only one, of about 2000 bp (clone 2 in Fig. 1), had an internal *Eco*RI restriction site. All others terminated at an *Eco*RI restriction site 1641 bp upstream of the poly(A) site (Fig. 1), suggesting that the cDNA used to make the library was undermethylated, allowing the full length cDNA to be cleaved at *Eco*RI sites prior to its ligation into the λgt10 vector. Accordingly, it was necessary to isolate rabbit cDNA clones first and then to use them as probes to identify and isolate new human cDNA clones. Eventually, a series of six linear cDNA clones were isolated from the library using rabbit cDNA probes (Fig. 1). As a further complication in the isolation of human cDNA clones, an adenine-rich region between residues 8501 and 8512 in Fig. 1 acted as a second priming site for cDNA synthesis. While this led to the synthesis of an enhanced number of clones upstream of this site, it also terminated transcription. Thus clones 4 and 5 in Fig. 1 were separated, not by an *Eco*RI cleavage site, but by an actual gap in the cDNA. Clone 6 was the last cDNA clone isolated from the library and it represented the 5′ end of cDNAs primed at the internal poly(A) site. The final clone, clone 7, was obtained from a primer extension library constructed from human skeletal mRNA using the protocols that were used for primer extension of rabbit skeletal muscle mRNA. In this case the primer was a 17-mer oligonucleotide complementary to residue 2620–2636 in Fig. 1.

Genomic DNA encoding sequences overlapping the various *Eco*RI restriction sites and the gap in the cDNA introduced by the second primer initiating site were isolated from a chromosome 19-specific library constructed by Dr. Pieter de Jong, Lawrence Livermore National Laboratories, Livermore, CA, and provided to us under the designation Lawrence Livermore LL19NL01 Human Chromosome 19 library in Charon 40 by the American Type Culture Collection, Rockville, MD.

Purification of Tryptic Peptides and Sequence Analysis—The Ca^{2+} release channel complex of rabbit skeletal muscle was isolated from heavy sarcoplasmic reticulum membranes enriched in [^{3}H]ryanodine

[1] The abbreviations used are: kb, kilobase; bp, basepair; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-propanesulfonic acid; H-PLC, high performance liquid chromatography.



B

```
                                                                                                                    GTCTCCGAGGTCTCCGACCCCGCCCGCGCCCAGCCCTCCCGCCCAGCCCGCAGCCCCCTCCCTCTGTTCCCCGACCTCAGACCCTGGGCTTCCGACCTCGACATC  -1
ATGGGTGACGCAGAAGGCGAAGACGAGGTCCAGTTCCTGCGGACGGACGATGAGGTGGTCCTGCAGTGCAGCGCTACCGTGCTCAAGGAGCAGCTCAAGCTCTGCCTGGCCGCCGAGGGC  120
MetGlyAspAlaGluGlyGluAspGluValGlnPheLeuArgThrAspAspGluValValLeuGlnCysSerAlaThrValLeuLysGluGlnLeuLysLeuCysLeuAlaAlaGluGly  40

TTCGGCAACCGCCTGTGCTTCCTGGAGCCCACTAGCAACGCGCAGAATGTGCCCCCCGATCTGGCCATCTGTTGCTTCGTCCTGGAGCAGTCCCTGTCTGTGCGAGCCCTGCAGGAGATG  240
PheGlyAsnArgLeuCysPheLeuGluProThrSerAsnAlaGlnAsnValProProAspLeuAlaIleCysCysPheValLeuGluGlnSerLeuSerValArgAlaLeuGlnGluMet  80

CTGGCTAACACGGTGGAGGCTGGCGTGGAGTCATCCCAGGGCGGGGGACACAGGACGCTCCTGTATGGCCATGCCATCCTGCTCCGGCATGCACACAGCCGCATGTATCTGAGCTGCCTC  360
LeuAlaAsnThrValGluAlaGlyValGluSerSerGlnGlyGlyGlyHisArgThrLeuLeuTyrGlyHisAlaIleLeuLeuArgHisAlaHisSerArgMetTyrLeuSerCysLeu  120

ACCACCTCCCGCTCCATGACTGACAAGCTGGCCTTCGATGTGGGACTGCAGGAGGACGCAACAGGAGAGGCTTGCTGCTGGACCATGCACCCACCCTCCAAGCACAGGTCTGAAGGAGAA  480
ThrThrSerArgSerMetThrAspLysLeuAlaPheAspValGlyLeuGlnGluAspAlaThrGlyGluAlaCysTrpTrpThrMetHisProAlaSerLysGlnArgSerGluGlyGlu  160

AAGGTCCGCGTTGGGGATGACATCATCCTTGTCAGTGTCTCCTCCGAGCGCTACCTGCACCTGTCGACCGCCAGTGGGGAGCTCCAGGTTGACGCTTCCTTCATGCAGACACTGTGGAAC  600
LysValArgValGlyAspAspIleIleLeuValSerValSerSerGluArgTyrLeuHisLeuSerThrAlaSerGlyGluLeuGlnValAspAlaSerPheMetGlnThrLeuTrpAsn  200

ATGAACCCCATCTGCTCCCGCTGCGAAGAGGGCTTCGTGACGGGAGGTCACGTCCTCCGCCTCTTTCATGGACATATGGATGAGTGTCTGACCATTTCCCCTGCTGACAGTGATGACCAG  720
MetAsnProIleCysSerArgCysGluGluGlyPheValThrGlyGlyHisValLeuArgLeuPheHisGlyHisMetAspGluCysLeuThrIleSerProAlaAspSerAspAspGln  240

CGCAGACTTGTCTACTATGAGGGGGGAGCTGTGTGCACTCATGCCCGCTCCCTCTGGAGGCTGGAGCCACTGAGAATCAGCTGGAGTGGGAGCCACCTGCGCTGGGGCCAGCCACTCCGA  840
ArgArgLeuValTyrTyrGluGlyGlyAlaValCysThrHisAlaArgSerLeuTrpArgLeuGluProLeuArgIleSerTrpSerGlySerHisLeuArgTrpGlyGlnProLeuArg  280

GTCCGGCATGTCACTACCGGGCAGTACCTAGCGCTCACCGAGGACCAGGGCCTGGTGGTGGTTGACGCCAGCAAGGCTCACACCAAGGCTACCTCCTTCTGCTTCCGCATCTCCAAGGAG  960
ValArgHisValThrThrGlyGlnTyrLeuAlaLeuThrGluAspGlnGlyLeuValValValAspAlaSerLysAlaHisThrLysAlaThrSerPheCysPheArgIleSerLysGlu  320

AAGCTGGATGTGGCCCCCAAGCGGGATGTGGAGGGCATGGGCCCCCCCTGAGATCAAGTACGGGGAGTCACTGTGCTTCGTGCAGCATGTGGCCTCAGGACTGTGGCTCACCTATGCCGCT  1080
LysLeuAspValAlaProLysArgAspValGluGlyMetGlyProProGluIleLysTyrGlyGluSerLeuCysPheValGlnHisValAlaSerGlyLeuTrpLeuThrTyrAlaAla  360

CCAGACCCCAAGGCCCTGCGGCTCGGCGTGCTCAAGAAGAAGGCCATGCTGCACCAGGAGGGCCACATGGACGACGCACTGTCGCTGACCCGCTGCCAGCAGGAGGAGTCCCAGGCCGCC  1200
ProAspProLysAlaLeuArgLeuGlyValLeuLysLysLysAlaMetLeuHisGlnGluGlyHisMetAspAspAlaLeuSerLeuThrArgCysGlnGlnGluGluSerGlnAlaAla  400

CGCATGATCCACAGCACCAATGGCCTATACAACCAGTTCATCAAGAGCCTGGACAGCTTCAGCGGGAAGCCACGGGGCTCGGGGCCACCCGCTGGCACGGCGCTGCCCATCGAGGGCGTT  1320
ArgMetIleHisSerThrAsnGlyLeuTyrAsnGlnPheIleLysSerLeuAspSerPheSerGlyLysProArgGlySerGlyProProAlaGlyThrAlaLeuProIleGluGlyVal  440

ATCCTGAGCCTGCAGGACCTCATCATCTACTTCGAGCCTCCCTCCGAGGACTTGCAGCACGAGGAGAAGCAGAGCAAGCTGCGAAGCCTGCGCAACCGCCAGAGCCTCTTCCAGGAGGAG  1440
IleLeuSerLeuGlnAspLeuIleIleTyrPheGluProProSerGluAspLeuGlnHisGluGluLysGlnSerLysLeuArgSerLeuArgAsnArgGlnSerLeuPheGlnGluGlu  480

GGGATGCTCTCCATGGTCCTGAATTGCATAGACCGCCTAAATGTCTACACCACTGCTGCCCACTTTGCTGAGTTTGCAGGGGAGGAGGCAGCCGAGTCCTGGAAAGAGATTGTGAATCTT  1560
GlyMetLeuSerMetValLeuAsnCysIleAspArgLeuAsnValTyrThrThrAlaAlaHisPheAlaGluPheAlaGlyGluGluAlaAlaGluSerTrpLysGluIleValAsnLeu  520

CTCTATGAACTCCTAGCTTCTCTAATCCGTGGCAATCGTAGCAACTGTGCCCTCTTCTCCACAAACTTGGACTGGCTGGTCAGCAAGCTGGATCGGCTGGAGGCCTCATCTGGCATCCTG  1680
LeuTyrGluLeuLeuAlaSerLeuIleArgGlyAsnArgSerAsnCysAlaLeuPheSerThrAsnLeuAspTrpLeuValSerLysLeuAspArgLeuGluAlaSerSerGlyIleLeu  560

GAGGTCCTGTACTGTGTCCTCATTGAGAGTCCAGAGGTTCTGAACATCATCCAGGAGAATCACATCAAGTCCATCATCTCCCTCCTGGACAAGCATGGGAGGAACCACAAGGTCCTGGAC  1800
GluValLeuTyrCysValLeuIleGluSerProGluValLeuAsnIleIleGlnGluAsnHisIleLysSerIleIleSerLeuLeuAspLysHisGlyArgAsnHisLysValLeuAsp  600

GTGCTATGCTCCCTGTGTGTGTGTAATGGTGTGGCTGTACGCTCCAACCAAGATCTTATTACTGAGAACTTGCTGCCTGGCCGTGAGCTTCTGCTGCAGACAAACCTCATCAACTATGTC  1920
ValLeuCysSerLeuCysValCysAsnGlyValAlaValArgSerAsnGlnAspLeuIleThrGluAsnLeuLeuProGlyArgGluLeuLeuLeuGlnThrAsnLeuIleAsnTyrVal  640

ACCAGCATCCGCCCCAACATCTTTGTGGGCCGAGCGGAAGGCACCACGCAGTACAGCAAATGGTACTTTGAGGTGATGGTGGACGAGGTGACTCCATTTCTGACAGCTCAGGCCACCCAC  2040
ThrSerIleArgProAsnIlePheValGlyArgAlaGluGlyThrThrGlnTyrSerLysTrpTyrPheGluValMetValAspGluValThrProPheLeuThrAlaGlnAlaThrHis  680

TTGCGGGTGGGCTGGGCCCTCACCGAGGGCTACACCCCCTACCCTGGGGCCGGCGAGGGCTGGGGCGGCAACGGGGTCGGCGATGACCTCTATTCCTACGGCTTTGATGGACTGCATCTC  2160
LeuArgValGlyTrpAlaLeuThrGluGlyTyrThrProTyrProGlyAlaGlyGluGlyTrpGlyGlyAsnGlyValGlyAspAspLeuTyrSerTyrGlyPheAspGlyLeuHisLeu  720

TGGACAGGACACGTGGCACGCCCAGTGACTTCCCCAGGGCAGCACCTCCTGGCCCCTGAAGACGTGATCAGCTGCTGCCTGGACCTCAGCGTGCCGTCCATCTCCTTCCGCATCAACGGC  2280
TrpThrGlyHisValAlaArgProValThrSerProGlyGlnHisLeuLeuAlaProGluAspValIleSerCysCysLeuAspLeuSerValProSerIleSerPheArgIleAsnGly  760

TGCCCTGTGCACGGTGTCTTTGAGTCCTTCAACCTGCACGGGCTCTTCTTCCCTGTTGTCAGCTTCTCGGCTGGTGTCAAGGTGCGGTTCCTCCTGGGTGGCCGCCATGGTGAATTCAAG  2400
CysProValGlnGlyValPheGluSerPheAsnLeuAspGlyLeuPhePheProValValSerPheSerAlaGlyValLysValArgPheLeuLeuGlyGlyArgHisGlyGluPheLys  800

TTCCTGCCCCCACCTGGCTATGCTCCATGCCATGAGGCTGTGCTCCCTCGAGAGCGACTCCATCTTGAACCCATCAAGGAGTATCGACGGGAGGGGCCCCGGGGGGCCTCACCTGGTGGGC  2520
PheLeuProProProGlyTyrAlaProCysHisGluAlaValLeuProArgGluArgLeuHisLeuGluProIleLysGluTyrArgArgGluGlyProArgGlyProHisLeuValGly  840
```

FIG. 1. **Characterization of cDNA encoding the human ryanodine receptor.** *A*, restriction endonuclease mapping and sequencing strategy. The first line shows the size in kb of the full length cDNA. The second shows a partial restriction endonuclease map of the 15.3-kb cDNA which encodes the human ryanodine receptor. The third and fourth lines define the six cDNA clones (*1–6*) that were isolated from the full cDNA library and the single clone (*7*) that was isolated from a primer-extended human cDNA library. *Arrows* inside the clones indicate that they were all sequenced in two directions. The fifth line designates the regions where genomic DNAs were sequenced to obtain overlapping sequences between the various clones. *B*, nucleotide and deduced amino acid sequence of the cDNA encoding the human ryanodine receptor. The nucleotides are *numbered positively* beginning at the first residue of the initiator methionine codon. The nucleotides comprising the 5′-non-translated region are numbered negatively in the 3′ to 5′ orientation. The deduced amino acid sequence of the open reading frame is *numbered* from 1 to 5032. Peptide sequences determined from the purified receptor are *underlined once*. The potential phosphorylation sites are *underlined twice*.

B

```
CCCAGTCGCTGCCTCTCACACACCGACTTCGTGCCCTGCCCTGTGGACACTGTCCAGATTGTCCTGCCGCCCCATCTGGAGCGCATTCGGGAGAAGCTGGCGGAGAACATCCACGAGCTC 2640
ProSerArgCysLeuSerHisThrAspPheValProCysProValAspThrValGlnIleValLeuProProHisLeuGluArgIleArgGluLysLeuAlaGluAsnIleHisGluLeu  880

TGGGCGCTAACCCGCATCGAGCAGGGCTGGACCTACGGCCCGGTTCGGGATGACAACAAGAGGCTGCACCCGTGTCTTGTGGACTTCCACAGCCTTCCAGAGCCTGAGAGGAACTACAAC 2760
TrpAlaLeuThrArgIleGluGlnGlyTrpThrTyrGlyProValArgAspAspAsnLysArgLeuHisProCysLeuValAspPheHisSerLeuProGluProGluArgAsnTyrAsn  920

CTGCAGATGTCTGGGGAGACGCTCAAGACTCTGCTGGCTCTGGGCTGCCACGTGGGCATGGCGGATGAGAAGGCGGAGGACAACCTGAAGAAGACAAAACTCCCCAAGACGTATATGATG 2880
LeuGlnMetSerGlyGluThrLeuLysThrLeuLeuAlaLeuGlyCysHisValGlyMetAlaAspGluLysAlaGluAspAsnLeuLysLysThrLysLeuProLysThrTyrMetMet  960

AGCAATGGGTACAAGCCGGCTCCGCTGGACCTGAGCCACGTGCGGCTGACGCCGGCGCAGACAACACTGGTGGACCGTCTGGCAGAAAATGGGCACAACGTGTGGGCCCGAGACCGCGTG 3000
SerAsnGlyTyrLysProAlaProLeuAspLeuSerHisValArgLeuThrProAlaGlnThrThrLeuValAspArgLeuAlaGluAsnGlyHisAsnValTrpAlaArgAspArgVal 1000

GGCCAGGGCTGGAGCTACAGCGCAGTGCAGGACATCCCAGCGCGCCGAAACCCTCGGCTGGTGCCCTACCGCCTGCTGGATGAAGCCACCAAGCGCAGCAACCGGGACAGCCTCTGCCAG 3120
GlyGlnGlyTrpSerTyrSerAlaValGlnAspIleProAlaArgArgAsnProArgLeuValProTyrArgLeuLeuAspGluAlaThrLysArgSerAsnArgAspSerLeuCysGln 1040

GCCGTGCGCACCCTCCTGGGCTACGGCTACAACATCGAGCCTCCTGACCAGGAGCCCAGTCAGGTGGAGAACCAGTCTCGTTGTGACCGGGTGCGCATCTTCCGGGCAGAGAAATCCTAT 3240
AlaValArgThrLeuLeuGlyTyrGlyTyrAsnIleGluProProAspGlnGluProSerGlnValGluAsnGlnSerArgCysAspArgValArgIlePheArgAlaGluLysSerTyr 1080

ACAGTGCAGAGCGGCCGCTGGTACTTCGAGTTTGAAGCAGTCACCACAGGCGAGATGCGCGTGGGCTGGGCGAGGCCCGAGCTGAGGCCTGATGTAGAGCTGGGAGCTGACGAGCTGGCC 3360
ThrValGlnSerGlyArgTrpTyrPheGluPheGluAlaValThrThrGlyGluMetArgValGlyTrpAlaArgProGluLeuArgProAspValGluLeuGlyAlaAspGluLeuAla 1120

TATGTCTTCAATGGGCACCGCGGCCAGCGCTGGCACTTGGGCAGTGAACCATTTGGGCGCCCCTGGCAGCCGGGCGATGTCGTTGGCTGTATGATCGACCTCACAGAGAACACCATTATC 3480
TyrValPheAsnGlyHisArgGlyGlnArgTrpHisLeuGlySerGluProPheGlyArgProTrpGlnProGlyAspValValGlyCysMetIleAspLeuThrGluAsnThrIleIle 1160

TTCACCCTCAATGGCGAGGTCCTCATGTCTGACTCAGGCTCCGAAACAGCCTTCCGGGAGATTGAGATTGGGGACGGCTTCCTGCCCGTCTGCAGCTTGGGACCTGGCCAGGTGGGTCAT 3600
PheThrLeuAsnGlyGluValLeuMetSerAspSerGlySerGluThrAlaPheArgGluIleGluIleGlyAspGlyPheLeuProValCysSerLeuGlyProGlyGlnValGlyHis 1200

CTGAACCTGGGCCAGGACGTGAGCTCTCTGAGGTTCTTTGCCATCTGTGGCCTCCAGGAAGGCTTCGAGCCATTTGCCATCAACATGCAGCGCCCAGTCACCACCTGGTTCAGCAAAGGC 3720
LeuAsnLeuGlyGlnAspValSerSerLeuArgPhePheAlaIleCysGlyLeuGlnGluGlyPheGluProPheAlaIleAsnMetGlnArgProValThrThrTrpPheSerLysGly 1240

CTGCCCCAGTTTGAGCCAGTGCCCCTTGAACACCCTCACTATGAGGTATCCCGAGTGGACGGCACTGTGGACACGCCCCCCTGCCTGCGCCTGACCCACCGCACCTGGGGCTCCCAGAAC 3840
LeuProGlnPheGluProValProLeuGluHisProHisTyrGluValSerArgValAspGlyThrValAspThrProProCysLeuArgLeuThrHisArgThrTrpGlySerGlnAsn 1280

AGCCTGGTGGAGATGCTTTTCCTGCGGCTGAGCCTCCCAGTCCAGTTCCACCAGCACTTCCGCTGCACTGCAGGGGCCACCCCGCTGGCACCTCCTGGCCTGCAGCCCCCCGCCGAGGAC 3960
SerLeuValGluMetLeuPheLeuArgLeuSerLeuProValGlnPheHisGlnHisPheArgCysThrAlaGlyAlaThrProLeuAlaProProGlyLeuGlnProProAlaGluAsp 1320

GAGGCCCGGGCGGCGGAACCCGACCCTGACTACGAAAACCTGCGCCGCTCAGCTGGGGGCTGGAGCGAGGCAGAGAACGGCAAAGAAGGGACTGCGAAGGAGGGCGCCCCCGGGGGCACC 4080
GluAlaArgAlaAlaGluProAspProAspTyrGluAsnLeuArgArgSerAlaGlyGlyTrpSerGluAlaGluAsnGlyLysGluGlyThrAlaLysGluGlyAlaProGlyGlyThr 1360

CCGCAGGCGGGGAGAGGCGCACCGGCCAGGGCGGAGAATGAGAAGGATGCCACCACCGAGAAGAACAAGAAGAGAGGCTTCTTATTCAAGGCCAAGAAGGTCGCCATGATGACCCAGCCA 4200
ProGlnAlaGlyArgGlyAlaProAlaArgAlaGluAsnGluLysAspAlaThrThrGluLysAsnLysLysArgGlyPheLeuPheLysAlaLysLysValAlaMetMetThrGlnPro 1400

CCGGCCACCCCCACGCTGCCCCGACTCCCTCACGACGTGGTGCCTGCAGACAACCGCGATGACCCCGAGATCATCCTCAACACCACCACGTACTATTACTCCGTGAGGGTCTTTGCTGGA 4320
ProAlaThrProThrLeuProArgLeuProHisAspValValProAlaAspAsnArgAspAspProGluIleIleLeuAsnThrThrThrTyrTyrTyrSerValArgValPheAlaGly 1440

CAGGAGCCCAGCTGCGTGTGGGCGGGCTGGGTCACCCCTGACTACCATCAGCACGACATGAGCTTCGACCTCAGCAAGGTCCGGGTCGTGACGGTGACCATGGGGGATGAACAAGGCAAC 4440
GlnGluProSerCysValTrpAlaGlyTrpValThrProAspTyrHisGlnHisAspMetSerPheAspLeuSerLysValArgValValThrValThrMetGlyAspGluGlnGlyAsn 1480

GTCCACAGCAGCCTCAAGTGTAGCAACTGCTACATGGTGTGGGGCGGAGACTTTGTGAGTCCCGGGCAGCAGGGCCGGATCAGCCACACGGACCTTGTCATTGGGTGCCTGGTGGACTTG 4560
ValHisSerSerLeuLysCysSerAsnCysTyrMetValTrpGlyGlyAspPheValSerProGlyGlnGlnGlyArgIleSerHisThrAspLeuValIleGlyCysLeuValAspLeu 1520

GCCACTGGCTTAATGACCTTTACAGCCAATGGCAAAGAGAGCAACACCTTTTTCCAGGTGGAACCCAACACTAAGCTATTTCCTGCCGTCTTCGTCCTGCCCACCCACCAGAACGTCATC 4680
AlaThrGlyLeuMetThrPheThrAlaAsnGlyLysGluSerAsnThrPhePheGlnValGluProAsnThrLysLeuPheProAlaValPheValLeuProThrHisGlnAsnValIle 1560

CAGTTTGAGCTGGGGAAGCAGAAGAACATCATGCCGTTGTCAGCCGCCATGTTCCAAAGCGAGCGCAAGAACCCGGCCCCGCAGTGCCCACCGCGGCTGGAGATGCAGATGCTGATGCCA 4800
GlnPheGluLeuGlyLysGlnLysAsnIleMetProLeuSerAlaAlaMetPheGlnSerGluArgLysAsnProAlaProGlnCysProProArgLeuGluMetGlnMetLeuMetPro 1600

GTGTCCTGGAGCCGCATGCCCAACCACTTCCTGCAGGTGGAGACGAGGCGTGCCGGGCGAGCGGCTGGGCTGGGCCGTGCAGTGCCAGGAGCCGCTGACCATGATGGCGCTGCACATCCCC 4920
ValSerTrpSerArgMetProAsnHisPheLeuGlnValGluThrArgArgAlaGlyGluArgLeuGlyTrpAlaValGlnCysGlnGluProLeuThrMetMetAlaLeuHisIlePro 1640

GAGGAGAACCGGTGCATGGACATCCTGGAGCTGTCGGAGCGCCTGGACCTGCAGCGCTTCCACTCGCACACCCTGCGCCTCTACCGCGCTGTGTGCGCCCTGGGCAACAATCGCGTGGCG 5040
GluGluAsnArgCysMetAspIleLeuGluLeuSerGluArgLeuAspLeuGlnArgPheHisSerHisThrLeuArgLeuTyrArgAlaValCysAlaLeuGlyAsnAsnArgValAla 1680

CACGCTCTGTGCAGCCACGTAGACCAAGCTCAGCTGCTGCACGCCCTGGAGGACGCGCACCTGCCAGGCCCACTGCGCGCAGGCTACTATGACCTCCTCATCAGCATCCACCTCGAAAGT 5160
HisAlaLeuCysSerHisValAspGlnAlaGlnLeuLeuHisAlaLeuGluAspAlaHisLeuProGlyProLeuArgAlaGlyTyrTyrAspLeuLeuIleSerIleHisLeuGluSer 1720

GCCTGCCGCAGCCGCCGCTCCATGCTCTCTGAATACATCGTGCCCCTCACGCCTGAGACCCGCGCCATCACGCTCTTCCCTCCTGGAAGGAGCACAGAAAATGGTCACCCCCGGCATGGC 5280
AlaCysArgSerArgArgSerMetLeuSerGluTyrIleValProLeuThrProGluThrArgAlaIleThrLeuPheProProGlyArgSerThrGluAsnGlyHisProArgHisGly 1760

CTGCCGGGAGTTGGAGTCACCACTTCGCTGAGGCCCCCGCATCATTTCTCGCCCCCCTGTTTCGTGGCCGCTCTGCCAGCTGCTGGGGCAGCAGAGGCCCCGGCCCGCCTCAGCCCTGCC 5400
LeuProGlyValGlyValThrThrSerLeuArgProProHisHisPheSerProProCysPheValAlaAlaLeuProAlaAlaGlyAlaAlaGluAlaProAlaArgLeuSerProAla 1800

ATCCCGCTGGAGGCCCTGCGGGACAAGGCACTGAGGATGCTGGGGGAGGCGGTGCGCGACGGTGGGCAGCACGCTCGCGACCCCGTCGGGGCCTCCGTGGAGTTCCAGTTTGTGCCTGTG 5520
IleProLeuGluAlaLeuArgAspLysAlaLeuArgMetLeuGlyGluAlaValArgAspGlyGlyGlnHisAlaArgAspProValGlyAlaSerValGluPheGlnPheValProVal 1840

CTCAAGCTCGTGTCCACCCTGCTGGTGATGGGCATCTTTGGCGATGAGGATGTGAAACAGATCTTGAAGATGATTGAGCCTGAGGTCTTCACTGAGGAAGAAGAGGAGGAGGACGAGGAG 5640
LeuLysLeuValSerThrLeuLeuValMetGlyIlePheGlyAspGluAspValLysGlnIleLeuLysMetIleGluProGluValPheThrGluGluGluGluGluGluAspGluGlu 1880

GAAGAGGGTGAAGAGGAAGATGAGGAGGAGAAGGAGGAGGATGAGGAGGAAACAGCACAGGAAAAGGAAGATGAGGAAAAAGAGGAAGAGGAGGCAGCAGAAGGGGAGAAAGAAGAAGGC 5760
GluGluGlyGluGluGluAspGluGluGluLysGluGluAspGluGluGluThrAlaGlnGluLysGluAspGluGluLysGluGluGluGluAlaAlaGluGlyGluLysGluGluGly 1920

TTGGAGGAAGGGCTGCTCCAGATGAAGTTGCCAGAGTCTGTGAAGTTACAGATGTGCCACCTGCTGGAGTATTTCTGTGACCAAGAGCTGCAGCACCGTGTGGAGTCCCTGGCAGCCTTT 5880
LeuGluGluGlyLeuLeuGlnMetLysLeuProGluSerValLysLeuGlnMetCysHisLeuLeuGluTyrPheCysAspGlnGluLeuGlnHisArgValGluSerLeuAlaAlaPhe 1960

GCGGAGCGCTATGTGGACAAGCTCCAGGCCAACCAGCGGAGCCGCTATGGCCTCCTCATAAAAGCCTTCAGCATGACCGCAGCAGAGACTGCAAGACGTACCCGCGAGTTCCGCTCCCCA 6000
AlaGluArgTyrValAspLysLeuGlnAlaAsnGlnArgSerArgTyrGlyLeuLeuIleLysAlaPheSerMetThrAlaAlaGluThrAlaArgArgThrArgGluPheArgSerPro 2000

CCCCAGGAACAGATCAATATGCTATTGCAATTCAAAGATGGTACAGATGAGGAAGACTGTCCTCTCCCTGAAGAGATTCGACAGGATTTGCTTGACTTTCATCAAGACCTGCTGGCACAC 6120
ProGlnGluGlnIleAsnMetLeuLeuGlnPheLysAspGlyThrAspGluGluAspCysProLeuProGluGluIleArgGlnAspLeuLeuAspPheHisGlnAspLeuLeuAlaHis 2040

TGTGGAATTCAGCTAGATGGAGAGGAGGAGGAACCAGAGGAAGAGACCACCCTGGGCAGCCGCCTCATGAGCCTGTTGGAGAAAGTGCGGCTGGTGAAGAAGAAGGAAGAGAAACCTGAG 6240
CysGlyIleGlnLeuAspGlyGluGluGluGluProGluGluGluThrThrLeuGlySerArgLeuMetSerLeuLeuGluLysValArgLeuValLysLysLysGluGluLysProGlu 2080

GAGGAGCGGTCAGCAGAGGAGAGCAAACCCCGGTCCCTGCAGGAGCTGGTGTCCCACATGGTGGTGCGCTGGGCCCAAGAGGACTTCGTGCAGAGCCCCGAGCTGGTGCGGGCCATGTTC 6360
GluGluArgSerAlaGluGluSerLysProArgSerLeuGlnGluLeuValSerHisMetValValArgTrpAlaGlnGluAspPheValGlnSerProGluLeuValArgAlaMetPhe 2120
```

FIG. 1—*continued*

B

```
AGCCTCCTGCACCGGCAGTACGACGGGCTGGGTGAGCTGCTGCGTGCCCTGCCGCGGGCGTACACCATCTCACCGTCCTCCGTGGAAGACACCATGAGCCTGCTCGAGTGCCTCGGCCAG  6480
SerLeuLeuHisArgGlnTyrAspGlyLeuGlyGluLeuLeuArgAlaLeuProArgAlaTyrThrIleSerProSerSerValGluAspThrMetSerLeuLeuGluCysLeuGlyGln  2160

ATCCGCTCGCTGCTCATCGTGCAGATGGGCCCCCAGGAGGAGAACCTCATGATCCAGAGCATCGGGAACATCATGAACAACAAAGTCTTCTACCAACACCCGAACCTGATGAGGGCGCTG  6600
IleArgSerLeuLeuIleValGlnMetGlyProGlnGluGluAsnLeuMetIleGlnSerIleGlyAsnIleMetAsnAsnLysValPheTyrGlnHisProAsnLeuMetArgAlaLeu  2200

GGCATGCACGAGACGGTCATGGAGGTCATGGTCAACGTCCTCGGGGGCGGCGAGTCCAAGGAGATCCGCTTCCCCAAGATGGTGACAAGCTGCTGCCGCTTCCTCTGCTATTTCTGCCGA  6720
GlyMetHisGluThrValMetGluValMetValAsnValLeuGlyGlyGlyGluSerLysGluIleArgPheProLysMetValThrSerCysCysArgPheLeuCysTyrPheCysArg  2240

ATCAGCCGGCAGAACCAGCGCTCCATGTTTGACCACCTGAGCTACCTGCTGGAGAACAGTGGCATCGGCCTGGGCATGCAGGGCTCCACGCCCCTGGACGTGGCTGCTGCCTCCGTCATT  6840
IleSerArgGlnAsnGlnArgSerMetPheAspHisLeuSerTyrLeuLeuGluAsnSerGlyIleGlyLeuGlyMetGlnGlySerThrProLeuAspValAlaAlaAlaSerValIle  2280

GACAACAATGAGCTGGCCTTGGCATTGCAGGAGCAGGACCTGGAAAAGGTTGTGTCCTACCTGGCAGGCTGTGGCCTCCAGAGCTGCCCCATGCTTGTGGCCAAAGGGTACCCAGACATT  6960
AspAsnAsnGluLeuAlaLeuAlaLeuGlnGluGlnAspLeuGluLysValValSerTyrLeuAlaGlyCysGlyLeuGlnSerCysProMetLeuValAlaLysGlyTyrProAspIle  2320

GGCTGGAAGCCCTGTGGTGGAGAGCGCTACCTGGACTTCCTGCGCTTTGCTGTCTTCGTCAACGGCGAGAGCGTGGAGGAGAACGCCAATGTGGTGGTGCGGCTGCTCATCCGGAAGCCT  7080
GlyTrpLysProCysGlyGlyGluArgTyrLeuAspPheLeuArgPheAlaValPheValAsnGlyGluSerValGluGluAsnAlaAsnValValValArgLeuLeuIleArgLysPro  2360

GAGTGCTTCGGACCCGCCCTGCGGGGTGAGGGTGGCTCAGGGCTGCTGGCTGCCATCGAAGAGGCCATCCGCATCTCCGAGGACCCTGCGAGGGATGGCCCAGGCATCCGCAGGGACCGG  7200
GluCysPheGlyProAlaLeuArgGlyGluGlyGlySerGlyLeuLeuAlaAlaIleGluGluAlaIleArgIleSerGluAspProAlaArgAspGlyProGlyIleArgArgAspArg  2400

CGGCGCGAGCACTTTGGTGAGGAACCGCCTGAAGAAAACCGGGTGCACCTGGGACACGCCATCATGTCCTTCTATGCCGCCTTGATCGACCTGCTCGGACGCTGTGCACCAGAGATGCAT  7320
ArgArgGluHisPheGlyGluGluProProGluGluAsnArgValHisLeuGlyHisAlaIleMetSerPheTyrAlaAlaLeuIleAspLeuLeuGlyArgCysAlaProGluMetHis  2440

CTAATCCAAGCCGGCAAGGGTGAGGCCCTGCGGATCCGCCGCCATCCTCCGCTCCCTTGTGCCCTTGGAGGACCTTGTGGGCATCATCAGCCTCCCACTGCAGATTCCCACCCTGGGCAAA  7440
LeuIleGlnAlaGlyLysGlyGluAlaLeuArgIleArgAlaIleLeuArgSerLeuValProLeuGluAspLeuValGlyIleIleSerLeuProLeuGlnIleProThrLeuGlyLys  2480

GATGGGGCTCTGGTGCAGCCAAAGATGTCAGCATCCTTCGTGCCGGACCACAAGGCGTCCATGGTGCTCTTCCTGGACCGTGTGTATGGCATCGAGAACCAGGACTTCTTGCTGCACGTG  7560
AspGlyAlaLeuValGlnProLysMetSerAlaSerPheValProAspHisLysAlaSerMetValLeuPheLeuAspArgValTyrGlyIleGluAsnGlnAspPheLeuLeuHisVal  2520

CTGGACGTGGGGTTCCTGCCCGACATGAGGGCAGCCGCCTCGCTGGACACGGCCACTTTCAGCACCACCGAGATGGCGCTGGCCGTGAACCGCTACCTGTGCCTGGCCGTGCTGCCGCTC  7680
LeuAspValGlyPheLeuProAspMetArgAlaAlaAlaSerLeuAspThrAlaThrPheSerThrThrGluMetAlaLeuAlaValAsnArgTyrLeuCysLeuAlaValLeuProLeu  2560

ATCACCAAGTGTGCGCCGCTCTTTGCGGGCACAGAACACCGCGCCATCATGGTGGACTCTATGCTGCATACCGTGTACCGCCTGTCTCGGGGTCGTTCGCTCACCAAGGCGCAGCGTGAC  7800
IleThrLysCysAlaProLeuPheAlaGlyThrGluHisArgAlaIleMetValAspSerMetLeuHisThrValTyrArgLeuSerArgGlyArgSerLeuThrLysAlaGlnArgAsp  2600

GTCATCGAGGACTGCCTCATGTCGCTCTGCAGGTACATCCGCCCGTCGATGCTGCAGCACCTGTTGCGCCGCCTGGTGTTCGACGTGCCCATCCTCAACGAGTTCGGCCAAGATGCCACTC  7920
ValIleGluAspCysLeuMetSerLeuCysArgTyrIleArgProSerMetLeuGlnHisLeuLeuArgArgLeuValPheAspValProIleLeuAsnGluPheAlaLysMetProLeu  2640

AAGCTCCTCACCAACCACTATGAGCGCTGTTGGAAGTACTACTGCCTACCCACGGGCTGGGCCAACTTCGGGGTCACCTCAGAGGAGGAGCTGCACCTCACACGGAAACTCTTCTGGGGC  8040
LysLeuLeuThrAsnHisTyrGluArgCysTrpLysTyrTyrCysLeuProThrGlyTrpAlaAsnPheGlyValThrSerGluGluGluLeuHisLeuThrArgLysLeuPheTrpGly  2680

ATCTTTGACTCTCTGGCCCATAAGAAATACGACCCGGAGCTGTACCGCATGGCCATGCCTTGTCTGTGCGCCATTGCCGGGGCTCTGCCCCCCGACTATGTGGATGCCTCATACTCATCT  8160
IlePheAspSerLeuAlaHisLysLysTyrAspProGluLeuTyrArgMetAlaMetProCysLeuCysAlaIleAlaGlyAlaLeuProProAspTyrValAspAlaSerTyrSerSer  2720

AAGGCAGAGAAAAAGGCCACAGTGGATGCTGAAGGCAACTTTGATCCCCGGCCTGTGGAGACCCTCAATGTGATCATCCCGGAGAAGCTGGACTCCTTCATTAACAAGTTTGCGGAGTAC  8280
LysAlaGluLysLysAlaThrValAspAlaGluGlyAsnPheAspProArgProValGluThrLeuAsnValIleIleProGluLysLeuAspSerPheIleAsnLysPheAlaGluTyr  2760

ACACACGAGAAGTGGGCCTTCGACAAGATCCAGAACAACTGGTCCTATGGAGAGAACATAGACGAGGAGCTGAAGACCCACCCCATGCTGAGGCCCTACAAGACCTTTTCAGAGAAGGAC  8400
ThrHisGluLysTrpAlaPheAspLysIleGlnAsnAsnTrpSerTyrGlyGluAsnIleAspGluGluLeuLysThrHisProMetLeuArgProTyrLysThrPheSerGluLysAsp  2800

AAAGAGATTTACCGCTGGCCCATCAAGGAGTCCCTGAAGGCCATGATTGCCTGGGAATGGACGATAGAGAAGGCCAGGGAGGGTGAGGAGGAGAAGACGGAAAAGAAAAAAACGGCGAAG  8520
LysGluIleTyrArgTrpProIleLysGluSerLeuLysAlaMetIleAlaTrpGluTrpThrIleGluLysAlaArgGluGlyGluGluGluLysThrGluLysLysLysThrAlaLys  2840

ATATCACAAAGTGCCCAGACCTATGATCCTCGAGAAGGCTACAACCCTCAGCCCCCCGACCTTAGTGCTGTTACCCTGTCCCGGGAGCTGCAGGCCATGGCAGAACAACTGGCAGAAAAT  8640
IleSerGlnSerAlaGlnThrTyrAspProArgGluGlyTyrAsnProGlnProProAspLeuSerAlaValThrLeuSerArgGluLeuGlnAlaMetAlaGluGlnLeuAlaGluAsn  2880

TACCACAACACGTGGGGACGGAAGAAGAAGCAGGAGCTGGAAGCCAAAGGCGGTGGGACCCACCCCCTGCTGGTCCCCTACGACACGCTCACGGCCAAGGAGAAGGCACGAGATCGAGAG  8760
TyrHisAsnThrTrpGlyArgLysLysLysGlnGluLeuGluAlaLysGlyGlyGlyThrHisProLeuLeuValProTyrAspThrLeuThrAlaLysGluLysAlaArgAspArgGlu  2920

AAGGCCCAGGAGCTACTGAAATTCCTGCAGATGAATGGCTACGCGGTTACAAGAGGCCTTAAGGACATGGAACTGGACTCGTCTTCCATTGAAAAGCGGTTTGCCTTTGGCTTCCTGCAG  8880
LysAlaGlnGluLeuLeuLysPheLeuGlnMetAsnGlyTyrAlaValThrArgGlyLeuLysAspMetGluLeuAspSerSerSerIleGluLysArgPheAlaPheGlyPheLeuGln  2960

CAGCTGCTGCGCTGGATGGACATTTCTCAGGAGTTCATTGCCCACCTGGAGGCTGTGGTCAGCAGTGGGCGAGTGGAAAAGTCCCCACATGAACAGGAGATTAAATTCTTTGCCAAGATC  9000
GlnLeuLeuArgTrpMetAspIleSerGlnGluPheIleAlaHisLeuGluAlaValValSerSerGlyArgValGluLysSerProHisGluGlnGluIleLysPhePheAlaLysIle  3000

CTGCTCCCTTTGATCAACCAGTACTTCACCAACCACTGCCTCTATTTCTTGTCCACTCCGGCTAAAGTGCTGGGCAGCGGTGGCCACGCCTCTAACAAGGAGAAGGAAATGATCACCAGC  9120
LeuLeuProLeuIleAsnGlnTyrPheThrAsnHisCysLeuTyrPheLeuSerThrProAlaLysValLeuGlySerGlyGlyHisAlaSerAsnLysGluLysGluMetIleThrSer  3040

CTCTTCTGCAAACTTGCTGCTCTCGTCCGCCACCGAGTCTCTCTCTTTGGGACAGACGCCCCAGCTGTGGTCAACTGTCTTCACATCCTGGCCCGCTCCCTGGATGCCAGGACAGTGATG  9240
LeuPheCysLysLeuAlaAlaLeuValArgHisArgValSerLeuPheGlyThrAspAlaProAlaValValAsnCysLeuHisIleLeuAlaArgSerLeuAspAlaArgThrValMet  3080

AAGTCAGGCCCTGAGATCGTGAAGGCTGGCCTCCGCTCCTTCTTCGAGAGTGCCTCGGAGGACATCGAGAAGATGGTGGAGAACCTGCGGCTGGGCAAGGTGTCGCAGGCGCGCACCCAG  9360
LysSerGlyProGluIleValLysAlaGlyLeuArgSerPhePheGluSerAlaSerGluAspIleGluLysMetValGluAsnLeuArgLeuGlyLysValSerGlnAlaArgThrGln  3120

GTGAAAGGCGTGGGCCAGAACCTCACCTACACCACTGTGGCACTGCTGCCGGTCCTCACCACCCTCTTCCAGCACATCGCCCAGCACCAGTTCGGAGATGACGTCATCCTGGACGACGTC  9480
ValLysGlyValGlyGlnAsnLeuThrTyrThrThrValAlaLeuLeuProValLeuThrThrLeuPheGlnHisIleAlaGlnHisGlnPheGlyAspAspValIleLeuAspAspVal  3160

CAGGTCTCTTGCTACCGAACGCTGTGCAGTATCTACTCCCTGGGAACCACCAAGAACACTTATGTGGAAAAGCTTCGGCCAGCCCTCGGGGAGTGCCTGGCCCGTCTGGCAGCAGCCATG  9600
GlnValSerCysTyrArgThrLeuCysSerIleTyrSerLeuGlyThrThrLysAsnThrTyrValGluLysLeuArgProAlaLeuGlyGluCysLeuAlaArgLeuAlaAlaAlaMet  3200

CCGGTGGCGTTCCTGGAGCCGCAGCTGAACGAGTACAACGCCTGCTCCGTGTACACCACCAAGTCTCCGCGGGAGCGGGCCATCCTGGGGCTCCCCAACAGTGTGGAGGAGATGTGTCCC  9720
ProValAlaPheLeuGluProGlnLeuAsnGluTyrAsnAlaCysSerValTyrThrThrLysSerProArgGluArgAlaIleLeuGlyLeuProAsnSerValGluGluMetCysPro  3240

GACATCCCGGTGCTGGAGCGGCTCATGGCAGACATTGGGGGGCTGGCCGAGTCAGGTGCCCGCTACACAGAGATGCCGCATGTCATCGAGATCACGCTGCCCATGCTATGCAGCTACCTG  9840
AspIleProValLeuGluArgLeuMetAlaAspIleGlyGlyLeuAlaGluSerGlyAlaArgTyrThrGluMetProHisValIleGluIleThrLeuProMetLeuCysSerTyrLeu  3280

CCCCGATGGTGGGAGCGCGGGCCCGAGGCACCCCCTTCCGCCCTGCCCGCCGGCGCCCCCCCACCCTGCACAGCTGTCACCTCTGACCACCTCAACTCCCTGCTGGGGAATATCCTGAGA  9960
ProArgTrpTrpGluArgGlyProGluAlaProProSerAlaLeuProAlaGlyAlaProProProCysThrAlaValThrSerAspHisLeuAsnSerLeuLeuGlyAsnIleLeuArg  3320

ATCATCGTCAACAACCTGGGCATTGACGAGGCCTCCTGGATGAAGCGGCTGGCTGTGTTCGCACAGCCCATTGTGAGCCGTGCACGGCCGGAGCTCCTGCAGTCCCACTTCATCCCAACT  10080
IleIleValAsnAsnLeuGlyIleAspGluAlaSerTrpMetLysArgLeuAlaValPheAlaGlnProIleValSerArgAlaArgProGluLeuLeuGlnSerHisPheIleProThr  3360

ATCGGGCGGCTGCGCAAGAGGGCAGGGAAGGTGGTGTCCGAGGAGGAGCAGCTGGCCCTGGAGGCCAAGGCGGAGGCCCAGGAGGGCGAGCTGCTGGTGCGGGACGAGTTCTCTGTGCTC  10200
IleGlyArgLeuArgLysArgAlaGlyLysValValSerGluGluGluGlnLeuAlaLeuGluAlaLysAlaGluAlaGlnGluGlyGluLeuLeuValArgAspGluPheSerValLeu  3400
```

FIG. 1—*continued*

B

```
TGCCGGGACCTCTACGCCCTGTATCCGCTGCTCATCCGCTACGTGGACAACAACAGGGCGCAGTGGCTGACGGAGCCGAATCCCAGCGCGGAGGAGCTGTTCAGGATGGTGGGCGAGATC 10320
CysArgAspLeuTyrAlaLeuTyrProLeuLeuIleArgTyrValAspAsnAsnArgAlaGlnTrpLeuThrGluProAsnProSerAlaGluGluLeuPheArgMetValGlyGluIle  3440

TTCATCTACTGGTCCAAGTCCCACAACTTCAAGCGCGAGGAGCAGAACTTTGTGGTCCAGAATGAGATCAACAACATGTCCTTCCTGACTGCTGACAACAAAAGCAAAATGGCTAAGTCC 10440
PheIleTyrTrpSerLysSerHisAsnPheLysArgGluGluGlnAsnPheValValGlnAsnGluIleAsnAsnMetSerPheLeuThrAlaAspAsnLysSerLysMetAlaLysSer  3480

GGTGGCTCGGACCAGGAACGCACCAAGAAGAAGCGCCGGGGGGACCGGTACTCTGTGCAGACGTCACTGATCGTGGCCACACTGAAGAAGATGCTGCCCATCGGCCTGAATATGTGTGCG 10560
GlyGlySerAspGlnGluArgThrLysLysLysArgArgGlyAspArgTyrSerValGlnThrSerLeuIleValAlaThrLeuLysLysMetLeuProIleGlyLeuAsnMetCysAla  3520

CCCACCGACCAAGACCTCATCACGCTGGCCAAGACCCGTTACGCCCTGAAAGACACAGATGAGGAGGTCCGGGAATTTCTGCACAACAACCTTCACCTTCAGGGAAAGGTCGAAGGCTCC 10680
ProThrAspGlnAspLeuIleThrLeuAlaLysThrArgTyrAlaLeuLysAspThrAspGluGluValArgGluPheLeuHisAsnAsnLeuHisLeuGlnGlyLysValGluGlySer  3560

CCGTCTCTGCGCTGGCAGATGGCTCTGTACCGGGGCGTCCCGGGTCGCGAGGAGGACGCCGATGACCCCGAGAAAATCGTGCGCAGAGTCCAGGAAGTGTCAGCCGTGCTCTACTACCTG 10800
ProSerLeuArgTrpGlnMetAlaLeuTyrArgGlyValProGlyArgGluGluAspAlaAspAspProGluLysIleValArgArgValGlnGluValSerAlaValLeuTyrTyrLeu  3600

GACCAGACCGAGCACCCTTACAAGTCTAAGAAGGCCGTGTGGCACAAGCTTTTGTCCAAACAGCGCCGGCGGGCAGTCGTGGCCTGTTTCCGTATGACGCCCCTGTACAACCTGCCCACG 10920
AspGlnThrGluHisProTyrLysSerLysLysAlaValTrpHisLysLeuLeuSerLysGlnArgArgArgAlaValValAlaCysPheArgMetThrProLeuTyrAsnLeuProThr  3640

CACCGGGCATGTAACATGTTCCTGGAGAGCTACAAGGCTGCATGGATCCTGACTGAAGACCACAGTTTTGAGGACCGCATGATAGATGACCTTTCAAAAGCTGGGGAGCAGGAGGAGGAG 11040
HisArgAlaCysAsnMetPheLeuGluSerTyrLysAlaAlaTrpIleLeuThrGluAspHisSerPheGluAspArgMetIleAspAspLeuSerLysAlaGlyGluGlnGluGluGlu  3680

GAGGAAGAGGTGGAAGAGAAGAAGCCAGACCCCCTGCACCAGTTGGTCCTGCACTTCAGCCGCACTGCCCTGACGGAAAAGAGCAAACTGGATGAGGATTACCTGTACATGGCCTATGCT 11160
GluGluGluValGluGluLysLysProAspProLeuHisGlnLeuValLeuHisPheSerArgThrAlaLeuThrGluLysSerLysLeuAspGluAspTyrLeuTyrMetAlaTyrAla  3720

GATATCATGGCAAAGAGCTGCCACCTGGAGGAGGGAGGGGAGAACGGTGAAGCTGAAGAGGAGGTTGAGGTCTCCTTTGAGGAGAAACAGATGGAGAAGCAGAGGCTCTTGTACCAGCAA 11280
AspIleMetAlaLysSerCysHisLeuGluGluGlyGlyGluAsnGlyGluAlaGluGluGluValGluValSerPheGluGluLysGlnMetGluLysGlnArgLeuLeuTyrGlnGln  3760

GCACGGCTGCACACCCGGGGGGCGGCCGAGATGGTGCTGCAGATGATCAGTGCCTGCAAAGGAGAGACAGGTGCCATGGTGTCCTCCACCCTGAAGCTGGGCATCTCCATCCTCAATGGA 11400
AlaArgLeuHisThrArgGlyAlaAlaGluMetValLeuGlnMetIleSerAlaCysLysGlyGluThrGlyAlaMetValSerSerThrLeuLysLeuGlyIleSerIleLeuAsnGly  3800

GGCAATGCTGAGGTCCAGCAGAAAATGCTGGATTATCTTAAGGACAAGAAGGAAGTTGGCTTCTTCCAGAGTATCCAGGCACTGATGCAAACATGCAGCGTCCTGGATCTCAATGCCTTT 11520
GlyAsnAlaGluValGlnGlnLysMetLeuAspTyrLeuLysAspLysLysGluValGlyPhePheGlnSerIleGlnAlaLeuMetGlnThrCysSerValLeuAspLeuAsnAlaPhe  3840

GAGAGACAGAACAAGGCCGAGGGGCTGGGCATGGTGAATGAGGATGGCACTGTCATCAATCGCCAGAACGGAGAGAAGGTCATGGCGGATGATGAATTCACACAAGACCTGTTCCGATTC 11640
GluArgGlnAsnLysAlaGluGlyLeuGlyMetValAsnGluAspGlyThrValIleAsnArgGlnAsnGlyGluLysValMetAlaAspAspGluPheThrGlnAspLeuPheArgPhe  3880

CTACAATTGCTCTGTGAGGGGCACAATAATGATTTCCAGAACTACCTACGGACACAGACAGGGAACACGACCACTATTAACATCATCATTTGCACTGTGGACTACCTCCTGCGGCTGCAG 11760
LeuGlnLeuLeuCysGluGlyHisAsnAsnAspPheGlnAsnTyrLeuArgThrGlnThrGlyAsnThrThrThrIleAsnIleIleIleCysThrValAspTyrLeuLeuArgLeuGln  3920

GAATCCATCAGCGACTTCTACTGGTACTACTCGGGCAAGGATGTCATTGAAGAGCAGGGCAAGAGGAACTTCTCCAAAGCCATGTCGGTGGCTAAGCAGGTGTTCAACAGCCTCACTGAG 11880
GluSerIleSerAspPheTyrTrpTyrTyrSerGlyLysAspValIleGluGluGlnGlyLysArgAsnPheSerLysAlaMetSerValAlaLysGlnValPheAsnSerLeuThrGlu  3960

TACATCCAGGGTCCCTGCACCGGGAACCAGCAGAGCCTGGCGCACAGTCGCCTATGGGACGCAGTGGTGGGATTCCTGCACGTGTTCGCCCACATGATGATGAAGCTCGCTCAGGACTCA 12000
TyrIleGlnGlyProCysThrGlyAsnGlnGlnSerLeuAlaHisSerArgLeuTrpAspAlaValValGlyPheLeuHisValPheAlaHisMetMetMetLysLeuAlaGlnAspSer  4000

AGCCAGATCGAGCTGCTGAAGGAGCTGCTGGATCTGCAGAAGGACATGGTGGTGATGTTGCTGTCGCTACTAGAAGGGAACGTGGTGAACGGCATGATCGCCCGGCAGATGGTGGACATG 12120
SerGlnIleGluLeuLeuLysGluLeuLeuAspLeuGlnLysAspMetValValMetLeuLeuSerLeuLeuGluGlyAsnValValAsnGlyMetIleAlaArgGlnMetValAspMet  4040

CTCGTGGAATCCTCATCCAATGTGGAGATGATCCTCAAGTTCTTCGACATGTTCCTGAAACTCAAGGACATTGTGGGCTCTGAAGCCTTCCAGGACTACGTAACGGATCCCCGTGGCCTC 12240
LeuValGluSerSerSerAsnValGluMetIleLeuLysPhePheAspMetPheLeuLysLeuLysAspIleValGlySerGluAlaPheGlnAspTyrValThrAspProArgGlyLeu  4080

ATCTCCAAGAAGGACTTCCAGAAGGCCATGGACAGCCAGAAGCAGTTCAGCGGTCCAGAAATCCAGTTCCTGCTTTCGTGCTCCGAAGCGGATGAGAACGAAATGATCAACTGCGAAGAG 12360
IleSerLysLysAspPheGlnLysAlaMetAspSerGlnLysGlnPheSerGlyProGluIleGlnPheLeuLeuSerCysSerGluAlaAspGluAsnGluMetIleAsnCysGluGlu  4120

TTCGCCAACCGCTTCCAGGAGCCAGCACGCGACATCGGCTTCAACGTGGCGGTGCTGCTGACCAACCTGTCGGAGCATGTGCCGCATGACCCTCGCCTGCACAACTTCCTGGAGCTGGCC 12480
PheAlaAsnArgPheGlnGluProAlaArgAspIleGlyPheAsnValAlaValLeuLeuThrAsnLeuSerGluHisValProHisAspProArgLeuHisAsnPheLeuGluLeuAla  4160

GAGAGCATCCTTGAGTACTTCCGCCCCTACCTGGGCCGCATCGAGATCATGGGCGCGTCACGCCGCATCGAGCGCATCTACTTCGAGATCTCAGAGACCAACCGCGCCCAGTGGGAGATG 12600
GluSerIleLeuGluTyrPheArgProTyrLeuGlyArgIleGluIleMetGlyAlaSerArgArgIleGluArgIleTyrPheGluIleSerGluThrAsnArgAlaGlnTrpGluMet  4200

CCCCAGGTGAAGGAGTCCAAGCGCCAGTTCATCTTCGACGTGGTGAACGAGGGCGGCGAGGCTGAGAAGATGGAGCTCTTCGTGAGTTTCTGCGAGGACACCATCTTCGAGATGCAGATC 12720
ProGlnValLysGluSerLysArgGlnPheIlePheAspValValAsnGluGlyGlyGluAlaGluLysMetGluLeuPheValSerPheCysGluAspThrIlePheGluMetGlnIle  4240

GCCGCGCAGATCTCGGAGCCCGAGGGCGAGCCGGAGACCGACGAGGACGAGGGCGCGGGCGCGGCGGAGGCGGGCGCGGAAGGCGCGGAGGAGGGCGCGGCGGGGCTCGAGGGCACGGCG 12840
AlaAlaGlnIleSerGluProGluGlyGluProGluThrAspGluAspGluGlyAlaGlyAlaAlaGluAlaGlyAlaGluGlyAlaGluGluGlyAlaAlaGlyLeuGluGlyThrAla  4280

GCCACGGCGGCGGCGGGGGGCGACGGCGCGGGTTGTGGCGGCCGCAGGCCGGGCCCTGCGAGGCCTCAGCTACCGCAGCCTGCGGCGGCGCGTGCGGCGGCTGCGGCGGCTTACGGCCCGC 12960
AlaThrAlaAlaAlaGlyAlaThrAlaArgValValAlaAlaAlaGlyArgAlaLeuArgGlyLeuSerTyrArgSerLeuArgArgArgValArgArgLeuArgArgLeuThrAlaArg  4320

GAGGCGGCCACCGCAGTGGCGGCGCTGCTCTGGGCAGCAGTGACGCGCGCTGGGGCCGCTGGCGCGGGGGCGGCGGCGGGCGCGCTGGGCCTGCTCTGGGGCTCGCTGTTCGGCGGCGGC 13080
GluAlaAlaThrAlaValAlaAlaLeuLeuTrpAlaAlaValThrArgAlaGlyAlaAlaGlyAlaGlyAlaAlaAlaGlyAlaLeuGlyLeuLeuTrpGlySerLeuPheGlyGlyGly  4360

CTGGTGGAGGGCGCCAAGAAGGTGACGGTGACCGAGCTCCTGGCAGGCATGCCCGACCCCACCAGCGACGAGGTGCACGGCGAGCAGCCGGCCGGGCCGGGCGGAGACGCAGACGGCGAG 13200
LeuValGluGlyAlaLysLysValThrValThrGluLeuLeuAlaGlyMetProAspProThrSerAspGluValHisGlyGluGlnProAlaGlyProGlyGlyAspAlaAspGlyGlu  4400

GGTGCCAGCGAGGGCGCTGGAGACGCCGCGGAGGGCGCTGGAGACGAGGAGGAGGCGGTGCACGAGGCCGGGCCGGGCGGTGCCGACGGGGCGGTGGCCGTGACCGATGGGGGCCCCTTC 13320
GlyAlaSerGluGlyAlaGlyAspAlaAlaGluGlyAlaGlyAspGluGluGluAlaValHisGluAlaGlyProGlyGlyAlaAspGlyAlaValAlaValThrAspGlyGlyProPhe  4440

CGGCCCGAAGGGGCTGGCGGTCTCGGGGACATGGGGGACACGACGCCTGCGGAACCGCCCACACCCGAGGGCTCTCCCATCCTCAAGAGGAAATTGGGGGTGGATGGAGTGGAGGAGGAG 13440
ArgProGluGlyAlaGlyGlyLeuGlyAspMetGlyAspThrThrProAlaGluProProThrProGluGlySerProIleLeuLysArgLysLeuGlyValAspGlyValGluGluGlu  4480

CTCCCGCCAGAGCCAGAGCCCGAGCCGGAACCAGAGCTGGAGCCGGAGAAAGCCGATGCCGAGAATGGGGAGAAGGAAGAAGTTCCCGAGCCCACACCAGAGCCCCCCAAGAAGCAAGCA 13560
LeuProProGluProGluProGluProGluProGluLeuGluProGluLysAlaAspAlaGluAsnGlyGluLysGluGluValProGluProThrProGluProProLysLysGlnAla  4520

CCTCCCTCACCCCCTCCAAAGAAGGAGGAAGCTGGAGGCGAATTCTGGGGAGAACTGGAGGTGCAGAGGGTGAAGTTCCTGAACTACCTGTCCCGGAACTTTTACACCCTGCGGTTCCTT 13680
ProProSerProProProLysLysGluGluAlaGlyGlyGluPheTrpGlyGluLeuGluValGlnArgValLysPheLeuAsnTyrLeuSerArgAsnPheTyrThrLeuArgPheLeu  4560

GCCCTCTTCTTGGCATTTGCCATCAACTTCATCTTGCTGTTTTATAAGGTCTCAGACTCTCCACCAGGGGAGGACGACATGGAAGGCTCAGCTGCTGGGGATGTGTCAGGTGCAGGCTCT 13800
AlaLeuPheLeuAlaPheAlaIleAsnPheIleLeuLeuPheTyrLysValSerAspSerProProGlyGluAspAspMetGluGlySerAlaAlaGlyAspValSerGlyAlaGlySer  4600

GGTGGCAGCTCTGGCTGGGGCTTGGGGGCCGGAGAGGAGGCAGAGGGCGATGAGGATGAGAACATGGTGTACTACTTCCTGGAGGAAAGCACAGGCTACATGGAACCGCCCCTGCGGTGT 13920
GlyGlySerSerGlyTrpGlyLeuGlyAlaGlyGluGluAlaGluGlyAspGluAspGluAsnMetValTyrTyrPheLeuGluGluSerThrGlyTyrMetGluProAlaLeuArgCys  4640

CTGAGCCTCCTGCATACACTGGTGGCCTTTCTCTGCATCATTGGCTATAATTGTCTCAAGGTGCCCCTGGTAATCTTTAAGCGGGAGAAGGAGCTGGCCCGGAAGCTGGAGTTTGATGGC 14040
LeuSerLeuLeuHisThrLeuValAlaPheLeuCysIleIleGlyTyrAsnCysLeuLysValProLeuValIlePheLysArgGluLysGluLeuAlaArgLysLeuGluPheAspGly  4680
```

FIG. 1—*continued*

B

```
CTGTACATCACGGAGCAGCCTGAGGACGATGACGTGAAGGGGCAGTGGGACCGACTGGTGCTCAACACGCCGTCTTTCCCTAGCAACTACTGGGACAAGTTTGTCAAGCGCAAGGTCCTG  14160
LeuTyrIleThrGluGlnProGluAspAspAspValLysGlyGlnTrpAspArgLeuValLeuAsnThrProSerPheProSerAsnTyrTrpAspLysPheValLysArgLysValLeu  4720

GACAAACATGGGGACATCTACGGGCGGGAGCGGATTGCTGAGCTACTGGGCATGGACCTGGCCACACTAGAGATCACAGCCCACAATGAGCGCAAGCCCAACCCGCCGCCAGGGCTGCTG  14280
AspLysHisGlyAspIleTyrGlyArgGluArgIleAlaGluLeuLeuGlyMetAspLeuAlaThrLeuGluIleThrAlaHisAsnGluArgLysProAsnProProProGlyLeuLeu  4760

ACCTGGCTCATGTCCATCGATGTCAAGTACCAGATCTGGAAGTTCGGGGTCATCTTCACAGACAACTCCTTCCTGTACCTGGGCTGGTATATGGTGATGTCCCTCTTCGGACACTACAAC  14400
ThrTrpLeuMetSerIleAspValLysTyrGlnIleTrpLysPheGlyValIlePheThrAspAsnSerPheLeuTyrLeuGlyTrpTyrMetValMetSerLeuLeuGlyHisTyrAsn  4800

AACTTCTTCTTTGCTGCCCATCTCCTGGACATCGCCATGGGGGTCAAGACGCTGCGCACCATCCTGTCCTCTGTCACCCACAATGGGAAACAGCTGGTGATGACCGTGGGCCTTCTGGCG  14520
AsnPhePhePheAlaAlaHisLeuLeuAspIleAlaMetGlyValLysThrLeuArgThrIleLeuSerSerValThrHisAsnGlyLysGlnLeuValMetThrValGlyLeuLeuAla  4840

GTGGTCGTCTACCTGTACACCGTGGTGGCCTTCAACTTCTTCCGCAAGTTCTACAACAAGAGCGAGGATGAGGATGAACCTGACATCAAGTGTGATGACATGATGACGTGTTACCTGTTT  14640
ValValValTyrLeuTyrThrValValAlaPheAsnPhePheArgLysPheTyrAsnLysSerGluAspGluAspGluProAspMetLysCysAspAspMetMetThrCysTyrLeuPhe  4880

CACATGTACGTGGGTGTCCGGGCTGGCGGAGGCATTGGGGACGAGATCGAGGACCCCGCGGGTGACGAATACGAGCTCTACAGGGTGGTCTTCGACATCACCTTCTTCTTCTTCGTCATC  14760
HisMetTyrValGlyValArgAlaGlyGlyGlyIleGlyAspGluIleGluAspProAlaGlyAspGluTyrGluLeuTyrArgValValPheAspIleThrPhePhePhePheValIle  4920

GTCATCCTGTTGGCCATCATCCAGGGTCTGATCATCGACGCTTTTGGTGAGCTCCGAGACCAACAAGAGCAAGTGAAGGAGGATATGGAGACCAAGTGCTTCATCTGTGGAATCGGCAGT  14880
ValIleLeuLeuAlaIleIleGlnGlyLeuIleIleAspAlaPheGlyGluLeuArgAspGlnGlnGluGlnValLysGluAspMetGluThrLysCysPheIleCysGlyIleGlySer  4960

GACTACTTTGATACGACACCGCATGGCTTCGAGACTCACACGCTGGAGGAGCACAACCTGGCCAATTACATGTTTTTCCTGATGTATTTGATAAACAAGGATGAGACAGAACACACGGGT  15000
AspTyrPheAspThrThrProHisGlyPheGluThrHisThrLeuGluGluHisAsnLeuAlaAsnTyrMetPhePheLeuMetTyrLeuIleAsnLysAspGluThrGluHisThrGly  5000

CAGGAGTCTTATGTCTGGAAGATGTACCAAGAGAGATGTTGGGATTTCTTCCCAGCTGGTGATTGTTTCCGTAAGCAGTATGAGGACCAGCTTAGCTGACACACCCCCAGCTGGCCCTCC  15120
GlnGluSerTyrValTrpLysMetTyrGlnGluArgCysTrpAspPhePheProAlaGlyAspCysPheArgLysGlnTyrGluAspGlnLeuSer***  5032

ACCCCCACCTCAAGTGCCTTATTCTCACAGCAAGCCCCTTAGTCCCCAAGCCCCTCCCCCTAAGGCAGCTGGGGGAGAGGTGACCTAGTACTGGAAAATAAATCTGTCGTACGCCCCCCA  15240

G
```

FIG. 1—*continued*

binding and $^{45}Ca^{2+}$ release activity by solubilization in CHAPS, followed by density gradient centrifugation through 5–20% sucrose in a Beckman SW 28 rotor as described previously (Lai *et al.*, 1988). The [^{3}H]ryanodine receptor peak, which sedimented with an apparent sedimentation coefficient of 30 S, was collected, concentrated in a Centriprep C-30 concentrator (Amicon), and recentrifuged as described above. Sodium dodecyl sulfate polyacrylamide gel analysis of the pooled [^{3}H]ryanodine receptor peak on a linear 5–12% polyacrylamide gradient gel revealed a single major high molecular weight band with an apparent relative molecular mass of a 400,000 (Lai *et al.*, 1988a).

Hydrophilic tryptic peptides from the purified ryanodine receptor (2 mg) were obtained by the procedure of Rao *et al.*, (1988), separated by reverse-phase-HPLC on a Vydac C_{18} column, and subjected to automated NH_2-terminal analysis by Edman degradation in an Applied Biosystems 470 gas-phase sequenator with an on-line HPLC system for phenylthiohydantoin derivative analysis. Peptides 1742–1748 and 3196–3210 in Fig. 1 were obtained in this way. Two attempts to obtain the NH_2-terminal sequence of the native (unproteolysed) ryanodine receptor following dialysis against 0.05% sodium dodecyl sulfate were unsuccessful. In an alternative approach, the ryanodine receptor was isolated from trypsin-treated heavy sarcoplasmic reticulum membranes (Meissner *et al.*, 1989) using the sedimentation procedure described above. The concentrated ryanodine receptor fractions were run on preparative 5–15% linear polyacrylamide gradient gels (60 μg/lane), then transferred electrophoretically with 80% efficiency to PVDF membranes (Immobilon, Millipore) at 8 °C and 500 mA for 12 h (Moos *et al.*, 1988; Otter *et al.*, 1987). Membranes were treated with 0.1% Coomassie Brilliant Blue R-250, and discrete, well-stained protein bands were excised and sequenced in a gas-phase sequenator as described above. Peptides 1629–1632 and 3119–3130 in Fig. 1 were obtained in this way.

Structural Analysis—Secondary structure was predicted by the methods of Garnier *et al.* (1978) and Chou and Fasman (1974). A search for Ca^{2+}-binding sites of the EF hand class was made using the template method of Taylor (1986). The scoring of the critical sites was adjusted so that when the program was run against the whole data base, it gave scores of about 100,000 to all known authentic members of the family. Any score less than about 3,000 was considered to be insignificant. Potential calmodulin-binding sites were identified as predicted α-helices containing clusters of 2–4 positive charges, separated by a predominantly hydrophobic region, which are the usual requirements for a calmodulin-binding site (Buschmeier *et al.*, 1987; Harris *et al.*, 1988; Lear *et al.*, 1988). There is no sequence generally diagnostic of a nucleotide-binding site, although many contain a glycine-rich loop G*X*G*XX*G(KT) following the first β-strand of a β-α-β alternation (Wierenga and Hol, 1983). Glycine-rich loops are found in many other situations and, since 90% of known nucleotide sites belong to a family of parallel β sheets, many irrelevant candidates were eliminated by restricting the search to predicted β-α-β regions. Potential transmembrane segments were identified by the methods of Kyte and Doolittle (1982) and Engelman *et al.* (1986) as implemented in the University of Wisconsin Genetics Computer Group program package for DNA and protein sequence analysis.

RESULTS

cDNA Isolation—In our initial screening of the λgt11 library, we isolated clones whose expressed product reacted with an antibody specific for the Ca^{2+} release channel protein (Zorzato *et al.*, 1989b). The fusion protein expressed by these isolated clones also reacted with a second antibody raised against the purified 30 S ryanodine receptor (Meissner *et al.*, 1989). As supporting evidence that we had isolated the correct clones, both rabbit and human probes from the coding region of the DNA hybridized to a message of about 15 kb in rabbit muscle mRNA (Fig. 2, *A* and *B*). These observations provided evidence that we had cloned cDNA encoding the ryanodine receptor. As we extended these cDNAs and analyzed their sequences, we found four deduced amino acid sequences that corresponded to the sequences of peptides isolated from the purified ryanodine receptor. These sequences are underlined in Fig. 1*B*. As further supporting evidence that the clones encoded the ryanodine receptor, we noted that the deduced amino acid sequence would give rise to a protein with several transmembrane passages at the carboxyl-terminal end and that the bulk of the protein was hydrophilic. Such a protein would match very well with the structure of the ryanodine receptor in which the bulk of the protein is cytoplasmic and only a small segment is transmembrane (Wagenknecht *et al.*, 1989). The simultaneous sequencing of both rabbit and human cDNAs gave us confidence that cloning artifacts did not arise in these very long sequences.

cDNA Sequence—In Fig. 1*A* we present the restriction map and sequencing strategy for the human ryanodine receptor cDNA. In Fig. 1*B*, we present the nucleotide and deduced amino acid sequences. The sequence was determined from linear clones 1, 3, 4, 5, 6, and 7 in Fig. 1*A*, which abutted each other at *Eco*RI sites between clones 1 and 3, 3 and 4, and 5 and 6. The junction between clones 1 and 3 was sequenced in clone 2, which contained an intact *Eco*RI restriction site, and

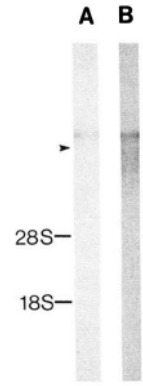


FIG. 2. **Northern blot analysis of RNA from neonatal rabbit skeletal muscle.** Poly(A)$^+$ RNA from neonatal rabbit tissues skeletal muscle was separated in formaldehyde-agarose gels and transferred to Hybond nylon membranes. Ribosomal RNA bands corresponding to 4.7 and 1.9 kb are indicated. *A*, hybridization of neonatal rabbit skeletal muscle mRNA to a human ryanodine receptor genomic DNA fragment covering the cDNA clone 6-clone 7 boundary, as indicated in Fig. 1. The *arrowhead* indicates the size of the dystrophin transcript (14 kb, Koenig *et al.*, 1987) which was analyzed in the same blot. *B*, hybridization of neonatal rabbit skeletal muscle mRNA to a rabbit ryanodine receptor cDNA probe (residues 8612–9215).

junctions between clones 3 and 4, 4 and 5 (a gap was introduced in this segment of cDNA by a second oligo(dT) primer initiation site), and 5 and 6 were obtained through sequencing of genomic DNA clones isolated from the human chromosome 19 library. The region around the *Eco*RI site in clones 6 and 7 was also sequenced in genomic DNA.

The 3′-untranslated region, beginning after the TGA termination codon, was 142 bp long. A canonical AAAATAAA polyadenylation signal (Proudfoot and Brownlee, 1976) was found 19 bases upstream of the polyadenylation site and this was followed closely by the TG-rich sequence TCTGTCGTACG, characteristic of sequences between the polyadenylation signal and the polyadenylation site (McLauchlan *et al.*, 1985).

The initiator methionine was found 15,096 bp upstream of the termination codon. The initiator methionine codon was present in the longer sequence ACATCATGG which closely resembles the consensus initiation sequence, CCA(G)CCATGG (Kozak, 1984). Although the 105 bp upstream of the initiator methionine were in frame, the sequence contained about 75% G+C residues, characteristic of 5′-untranslated sequences in other sarcoplasmic reticulum protein cDNAs (MacLennan *et al.*, 1985; Brandl *et al.*, 1986). We were unable to obtain an NH_2-terminal amino acid sequence from the ryanodine receptor protein, which would have been helpful in defining the position of the initiator methionine in the cDNA sequence, and we conclude that the NH_2-terminal methionine is blocked in the mature protein. The study of Takeshima *et al.* (1989), in which the cap site for the rabbit mRNA was found to lie 138 residues upstream of the initiator methionine codon, is our final guide to the placement of the initiator methionine in Fig. 1.

Amino Acid Sequence Analysis—Our human cDNA sequence encoded a protein of 5,032 amino acids with a molecular weight of 563,584. This deduced molecular mass is considerably larger than that previously predicted for the ryanodine receptor, on the basis of its mobility in sodium dodecyl sulfate gels. It is, however, consistent with the large mass of the foot protein (Saito *et al.*, 1988) and with measurements of ryanodine binding to the purified tetrameric protein (Lai *et al.*, 1988).

In Fig. 3, *third lane*, we present the deduced amino acid sequence of the human ryanodine receptor in a single-letter code. In the *second line* (above the linear sequence), we have indicated positions where we found differences with the rabbit sequence. In the *first line* (two above the linear sequence) we have noted discrepancies between our rabbit cDNA sequence and that presented by Takeshima *et al.* (1989). Two discrepancies are recorded. We did not find the sequence -Ala-Gly-Asp-Ala-Gln-, recorded by Takeshima *et al.* (1989) as residues 3481–3485, in either our rabbit or human cDNA sequences. Residue 2015 was recorded as Glu by Takeshima *et al.* (1989), but we find Asp at this position in rabbit and Gly at this position in the human sequence.

Amino acid differences between the rabbit and human sequences are scattered throughout the molecule and involve several deletions and/or additions. This is pronounced in the Glu-rich region lying between residues 1872 and 1923. In this region of the rabbit sequence, a single stretch of 18 Glu is found and the whole region has 39 Glu and 4 Asp residues. In the comparable human sequence there are 35 Glu and 4 Asp residues. A deletion of 3 Glu in a row and then 1 Glu in the human sequence are later compensated for by the inclusion of the sequence Thr-Ala-Gln and later still by the inclusion of a Gly so that, over the course of about 40 residues, the sequences realign. Overall, we noted 163-amino acid substitutions between the two proteins, while deletions or insertions in eight different positions accounted for many more differences between the two proteins.

Transmembrane Sequence Predictions—The hydropathy of the deduced amino acid sequence of the human ryanodine receptor is presented in Fig. 4. We identified 11 sequences which were long enough to span the membrane, gave mean hydropathy indices for a window of 17 amino acids ranging from 1.2 to 2.9, and are candidates for transmembrane sequences located in the baseplate of the protein. A 12th potential transmembrane sequence (sequence 9) is also included in Figs. 3 and 5. Although its hydropathy index is only 0.8, largely because of a cluster of 4 glycine residues which do not score highly in hydropathy plots but are compatible with a hydrophobic environment, this sequence resembles the other putative transmembrane sequences in other respects. M′ and M″ are isolated in the central region of the molecule in residues 3123–3134 and 3187–3205, while sequences M1 to M10 are distributed in the COOH-terminal fifth of the molecule between residues 3978 and 4932. In the amino acid sequences of these potential transmembrane sequences listed in Fig. 5, the charged and amide-containing residues are *boxed*. These sequences could form six pairs of transmembrane passages with loops in the lumen of the sarcoplasmic reticulum, 44, 18, 40, 67, 16, and 19 amino acids in length, progressing from NH_2 to COOH termini.

Homology Searches—A search of the EMBL/GenBank data base has revealed relatively little sequence identity between the ryanodine receptor and any other protein. The most conspicuous identities were with sequences from the predicted transmembrane regions of the ryanodine receptor and the Na^+ (Noda *et al.*, 1984) and Ca^{2+} (Tanabe *et al.*, 1987) channels and the acetylcholine receptor (Noda *et al.*, 1983) (Fig. 3).

Repeated Segments—We observed a repeating sequence motif of 114–120 amino acids, averaging 28% identity, and occurring four times in two doublets. The first and second repeats are 114 residues long and include residues 841–954 and 955–1068. The third and fourth repeats are 120 residues long and include residues 2725–2844 and 2845–2958. In order to make the appropriate alignment, a 6-residue gap was introduced into repeat sequence 4 between residues 2899 and 2900. A 16-residue segment of the longer motif is repeated twice more in residues 1344–1359 and 1371–1386.

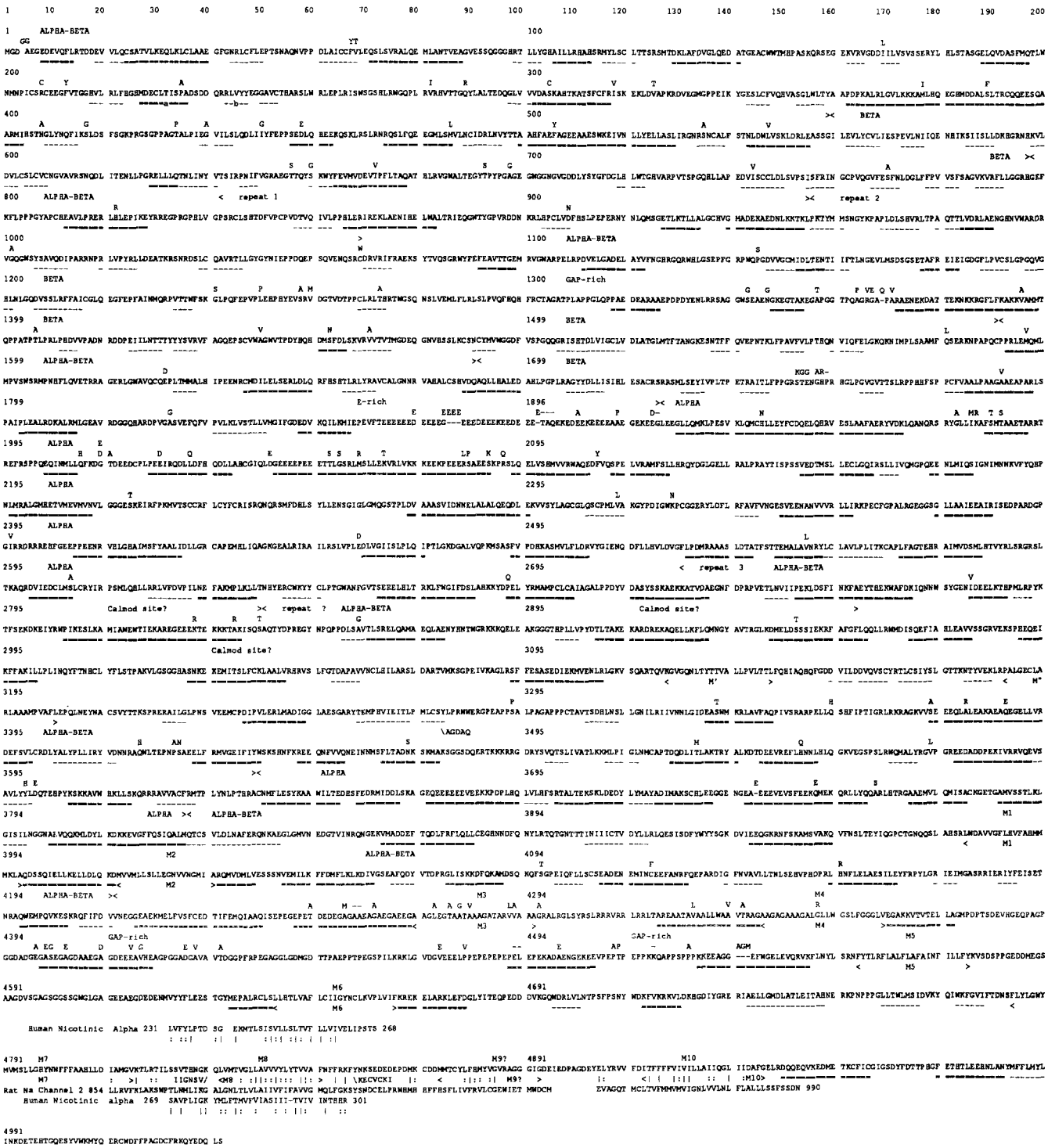


FIG. 3. **Secondary structure and domains of the human and rabbit ryanodine receptor amino acid sequences.** The *first line* provides information on sequences below; (i), the numbering for the human ryanodine receptors sequence at intervals of about 100 amino acids; (ii), a commentary on predicted domain structure over long sequences; (iii), locations where our rabbit cDNA sequence differed from the cDNA sequence determined by Takeshima *et al.* (1989); (iv) location of repeated sequences; (v), location of potential calmodulin binding sites. The *second line* indicates the rabbit amino acid sequence only where it differs from the human sequence. The *third line* gives the human amino acid sequence in full. The *fourth line* provides the predicted secondary structure (α helix = = =, or β strand – – –) and hydrophobic segments M′, M″, and M_1–M_{10} for the full sequence in the third line. In the last three grouping of lines, matches to the Na^+ channel (second repeat) and to the nicotinic acetylcholine receptor (M_2 and M_3) are shown. The matches to the former are shown above the sequence on the secondary structure line; those to the latter are below the sequence.

The repeated segments are aligned in Fig. 6. The sequence identity is not high, but it is sufficient to imply a common tertiary structure (Chothia and Lesk, 1986). Although the predicted secondary structure (Fig. 3) for the four repeats differ in some segments, the consensus (Fig. 6) shows a β-α-β-α pattern, with the possibility of a third β-α unit if the final helix is extended and interrupted by a short β strand. Two units in tandem would be predicted to give a viable parallel β sheet domain.

The "profile" method of Gribskov *et al.* (1987) was used to

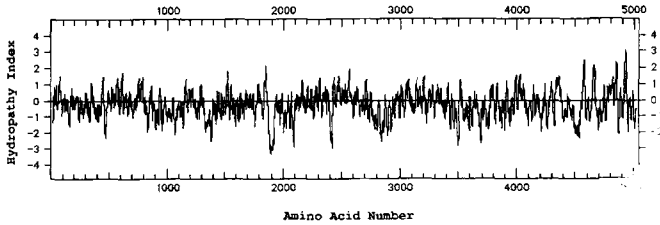


FIG. 4. **Hydropathy profile of the human ryanodine receptor.** The hydropathy plot (Kyte and Doolittle, 1982) was plotted using a window of 17 amino acids. The *vertical lines* indicate the hydropathy index; the *horizonatal line* indicates the amino acid residue numbers.

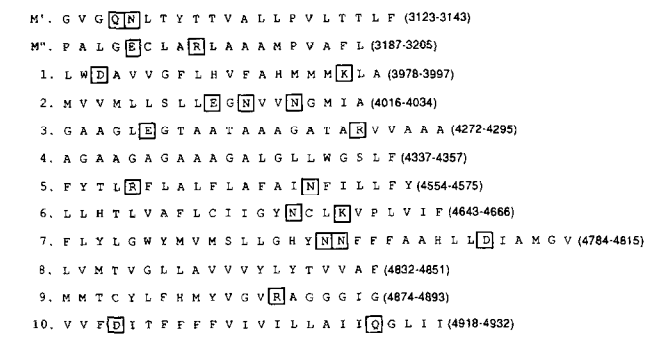


FIG. 5. **Potential transmembrane sequences in the human ryanodine receptor.** The sequences of 12 hydrophobic sequences near the COOH terminus of the human ryanodine receptor are compared. Charged and amide-containing residues are *circled.* Mean hydropathy indices with a window of 17 residues for sequences M′, M″, and M_1-M_{10} are 1.3, 1.5, 1.6, 1.6, 1.2, 1.4, 2.4, 2.0, 1.2, 2.3, 0.8, and 2.9, respectively.

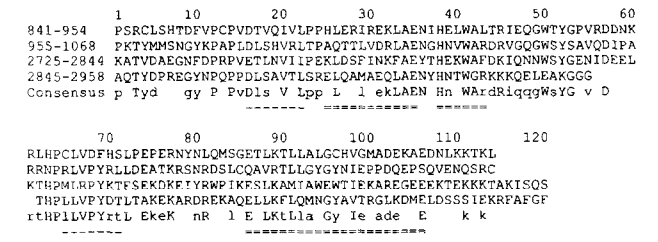


FIG. 6. **Alignment of four repeated segments.** The consensus sequence in the fifth line is shown in *uppercase* when three or more identities are present; *lowercase* indicates two identities. The predicted secondary structure of individual repeats is shown in Fig. 3. The consensus prediction in *line 6* was obtained by reevaluation of the original profiles (α helix = = =; β strand – – –).

scan the NBRF data base to see if any similar segments occurred elsewhere. The highest score of 37 (0.34 of the maximum score) was given by a segment of vinculin, but the resemblance was not significant.

Ligand Binding Sites—The Ca^{2+} release channel is modulated by four physiologically relevant molecules; Ca^{2+}, Mg^{2+}, ATP, and calmodulin (Meissner, 1986; Meissner *et al.*, 1986; Morii and Tonomura, 1983). Therefore, it is of interest to determine where in the sequence these ligands bind, especially since consensus high affinity binding sequences are available for three of them.

Ca^{2+} is bound with high affinity to EF hand structures in many proteins (Kretsinger, 1987). No sequence with the precise characteristics of an EF hand is present in either the human or rabbit ryanodine receptors, although several sequences meet the requirements in part. The maximum score obtained for all potential sequences was 750, well below the score that we consider to be significant. The glutamate-rich region between residues 1872 and 1923 is of interest as a potential low affinity Ca^{2+}-binding domain, in light of the capacity of such sequences to bind Ca^{2+} with low affinity (Fliegel *et al.*, 1987; Leberer *et al.*, 1989b). Magnesium-binding sites have not yet been defined and cannot be predicted in the primary structures of proteins.

We were also unable to detect a convincing consensus sequence for adenine nucleotide binding in the primary sequences of the rabbit and human receptors. G*X*G*XX*G motifs were found at positions 1194–1199 and 4447–4452 in Fig. 1. The first lies in a region that is predicted to contain several β strands. The second occurs in a glycine-proline-rich region where proline bends might give a rather rigid structure, unlike that of a typical nucleotide-binding site.

Potential calmodulin-binding sites made up from an amphipathic helix, with two clusters of positive charges separated by a hydrophobic region, were found between residues 2807 and 2840, 2909 and 2930, and 3031 and 3049. These sites are indicated in Fig. 3. One of these (2909–2930) showed some sequence homology to the calmodulin-binding site of β spectrin (Harris *et al.*, 1988).

The sites of cAMP and calmodulin-dependent protein kinase phosphorylation have been well characterized (Feramisco *et al.*, 1980). Potential phosphorylation sites were found in the protein at residues 3940–3945 and 4314–4317. Glycosylation sites are composed of the sequence N-*X*-T(S). We found N-*X*-T(S) sequences which would be glycosylated at positions 1064, 2773, 3127, 3943, 4142, and 4859 in the sequence, provided they were luminally located.

DISCUSSION

In this paper we have described the cloning and sequence analysis of cDNAs encoding one of the largest proteins analyzed to date, the rabbit and human forms of the ryanodine receptor. The rabbit and human sequences were found to be very similar. Major differences were found in an extremely acidic region of the protein (residues 1872–1923 in Fig. 1) in which several deletions and insertions were noted between the two sequences. We found several long amino acid sequences (residues 2948–3293, 3764–4096, 4534–5032) which exhibited complete identity with the corresponding rabbit sequence. The first conserved region contains putative calmodulin binding sequences, whereas the last two sequences contain putative transmembrane sequences M_1 and M_2 and M_5 to M_{10}. In other regions, the differences were minor and fully consistent with species differences in the same gene, as opposed to differences between different genes. We noted only one significant difference between our rabbit cDNA sequence and that published by Takeshima *et al.* (1989). A stretch of five amino acids reported in their sequence was absent from ours. We cannot readily explain this discrepancy as an allelic variation. It may represent an alternative splicing of a small exon or the retention of an unexcised intron in the Takeshima sequence. This sequence would not appear to be essential to function, since the rabbits used in both studies were considered normal. The region is predicted to form a turn (Fig. 3), so its loss or inclusion would not be likely to disrupt a helix or strand domain. The Asp for Glu replacement that we found at position 2015 is conservative and could represent an allelic variant.

A major goal in obtaining the primary sequence of a membrane protein is to deduce features relating to the structure and function of the protein. Hydropathy plots (Fig. 4) illustrate that, with the exception of the sequences labeled M′ and M″ in Fig. 3 (residues 3123–3143 and 3187–3205), the first 4000 amino acids are hydrophilic and are likely to constitute

the cytoplasmic domain of the ryanodine receptor. The clearest boundaries in this portion of the molecule are provided by the four 114 or 120 residue repeats (Fig. 6), occurring in two tandem pairs, and three regions rich in runs of glutamic acid residues (1870–1930, 2025–2090, and 3675–3750). In between these fairly well-defined segments are regions, typical of globular proteins, in which predicted α-turn-α, β-turn-β, or β-turn-α-turn-β supersecondary motifs predominate. Some of these are indicated in Fig. 3.

We have identified two potential transmembrane sequences near the middle of the molecule and 10 in the COOH-terminal fifth of the molecule which are candidate sequences for the transmembrane channel of the baseplate. The size of the baseplate, made up from transmembrane sequences of four subunits (Wagenknecht *et al.* (1989), is about 140 × 140 Å. A structure of this size could accommodate up to 150 transmembrane helices of 11 Å diameter (Engelman *et al.*, 1980), provided it were free of lipid or of polar segments of the protein embedded in the hydrophobic helices, while each monomeric subunit could accommodate about 36 transmembrane sequences. If sequences M′, M″, and M_1–M_{10} were all transmembrane, then six transmembrane hairpin loops, one near the center of the molecule and five in the COOH-terminal fifth of the molecule would anchor each monomer to the membrane and the total number of transmembrane sequences in the tetramer would be 48.

Of the 12 sequences proposed to be transmembrane, four, labeled M_5, M_6, M_8, and M_{10} in Fig. 3, have mean hydropathy indices over 17 residues ranging from 2.0 to 2.9. The remainder have mean hydropathy indices ranging from 0.8 to 1.6 and are less clear candidates for transmembrane sequences. In our earlier analysis of transmembrane segments of the Ca^{2+} ATPase (MacLennan *et al.*, 1985), we predicted that 10 transmembrane sequences with mean hydropathy indices ranging from 1.3 to 2.7 would exist in this protein, and these included sequences which were relatively rich in polar and charged amino acids. We have obtained evidence recently (Clarke *et al.*, 1989) that it is, indeed, these charged polar residues that are involved in forming the Ca^{2+}-binding sites and the Ca^{2+} channel in the transmembrane domain of the Ca^{2+} ATPase. By analogy, we believe that it is unlikely that the Ca^{2+} release channel of the ryanodine receptor would be made up only of very hydrophobic sequences. Lodish (1988) has presented a similar view of the structure of membrane transport proteins.

The COOH-terminal fifth of the ryanodine receptor molecule contains highly charged sequences in addition to the major hydrophobic stretches. In the folding model that would result from the assignment of sequences M′, M″, and M_1 to M_{10} (Fig. 3) to the transmembrane sector, rather highly charged sequences would lie in the lumen of the terminal cisternae. The sequences RRRVRRLRR (residues 4307–4314) and EEAEGDEDE (residues 4612–4620) represent especially concentrated regions of positive and negative charges. A high density of charge surrounding the luminal mouth of the Ca^{2+} release channel might influence the gating properties of the channel or act as an ion selective screen at the channel entrance. It might also influence the interaction of luminal proteins such as calsequestrin (MacLennan and Wong, 1970) or calsequestrin-binding proteins (Mitchell *et al.*, 1988) with the ryanodine receptor.

Sequences labeled M_3, M_4 and M_9 are rich in glycine and alanine residues and M_9 has a low hydropathy index, largely due to a cluster of glycine residues in the sequence. Glycine and alanine residues are compatible with transmembrane sequences, however, and one of the transmembrane sequences in subunit C of the bacterial F_1F_0 ATPase complex (Walker *et al.*, 1984; Senior, 1988) and transmembrane sequence D in the P-glycoprotein (Gros *et al.*, 1987) are glycine and alanine rich.

In a search for homology of the ryanodine receptor sequence with that of other known proteins, we noted amino acid identities between our proposed transmembrane segments M_6 and M_8 and segments M_2 and M_3 of the nicotinic acetylcholine receptor which, according to the model of Noda *et al.* (1983) has only four hydrophobic transmembrane segments. These identities are confined to two hydrophobic regions in the two molecules. While the regions of identity are contiguous in the acetylcholine receptor, they are separated by 160 residues encompassing putative transmembrane sequence M_7 in the ryanodine receptor sequence. Another match in the region containing putative transmembrane sequences M_8–M_{10} can be made to the S4, S5 region of the Na^+ channel (Noda *et al.*, 1984) and to the corresponding segment of the dihydropyridine-sensitive Ca^{2+} channel (Tanabe *et al.*, 1987). One of these matches is shown in Fig. 3. Since the matches are mainly hydrophobic in a region rich in such residues, they are unlikely to be significant. The lack of significant homology of the ryanodine receptor with other channel proteins suggests that the ryanodine receptor may be the first member of a novel family of channel proteins that might contain other intracellular Ca^{2+} release channels such as the cardiac form of the ryanodine receptor (Lai *et al.*, 1988b; Inui *et al.*, 1987b) or the inositol trisphosphate receptor (Supattapone *et al.*, 1988), which have been identified, but not yet cloned.

Our attempts to identify nucleotide-binding sites in the primary sequence were not successful. Known nucleotide-binding sites are almost invariably in parallel β sheet domains and perhaps half of them include a glycine-rich bend in the first α-β unit of the sheet. The glycine-rich sequence GLGDMG, (residues 4447–4452) noted earlier (Takeshima *et al.*, 1989), is present in a glycine-proline-rich region which would not be predicted to form a typical nucleotide-binding domain. Our present information on the structural features of the nucleotide-binding site derives largely from proteins such as muscle adenylate kinase (Fry *et al.*, 1986) which has a high affinity for ATP of the order of 30 μM (Noda, 1973). Half-maximal activation of the Ca^{2+} release channel, either in planar bilayers (Smith *et al.*, 1986; Smith *et al.*, 1988) or in intact heavy sarcoplasmic reticulum vesicles (Morii and Tonomura, 1983; Meissner *et al.*, 1986) occurs at about 2 mM ATP, indicating that the activation of Ca^{2+} release by ATP is due to the interaction of the ligand with low affinity binding sites which may not be closely related to those with high affinity.

The search for high affinity Ca^{2+}-binding sites of the EF hand type was also unsuccessful. Since high affinity Ca^{2+} binding occurs in many proteins of known structure which do not contain EF hand structures, this is not surprising. For example, in a recent study, Fliegel *et al.* (1989) have defined the primary sequence of the high affinity Ca^{2+}-binding protein (calreticulin) of the sarcoplasmic reticulum (Ostwald and MacLennan, 1974). Although this protein of 400 amino acids binds 1 mol of Ca^{2+}/mol with high affinity and 25 mol of Ca^{2+}/mol with low affinity, no clear EF hand sequences were present in the molecule. In analogy with the long acidic sequence in the ryanodine receptor (residues 1872–1923, Fig. 1), 32 out of 40 amino acids near the COOH terminus of calreticulin were acidic. Thus, in both calreticulin and the ryanodine receptor, high affinity Ca^{2+} binding occurs in the absence of a clear EF hand sequence, but in the presence of a long acidic sequence. The determination of the Ca^{2+} binding

properties of both of these long acidic sequences will be of great interest.

Our search for calmodulin-binding sites revealed a number of candidate sequences lying near the center of the molecule. If these sites are functional, they would indicate that modulation of the channel could occur in domains that are distant in the primary sequence from the sequences most likely to make up the release channel itself. Potential phosphorylation sites were found in the sequence just upstream from the probable channel forming sequences in regions that we predict to be cytoplasmic. Although phosphorylation of the channel has been reported (Seiler *et al.*, 1984), regulation of the channel by phosphorylation has not been reported.

Several consensus glycosylation sites were found in the sequence but all were in regions that we predict to be cytoplasmic. Since glycosylation reactions occurred in the lumen of the endoplasmic reticulum (Lennarz, 1987), which has the same orientation as the sarcoplasmic reticulum, none of these would be predicted to be glycosylated. An alternative folding pattern (Takeshima *et al.*, 1989) could lead to the luminal location and glycosylation of residue 4859, however. To date, no detailed study of glycosylation of the ryanodine receptor has been published.

The primary sequence of the ryanodine receptor provides us with the first important clues to the understanding of the structure/function relationships in the molecule. Further information is likely to arise from detailed investigation of the biochemical properties of altered forms of the molecule such as those which can be obtained through site-specific or naturally occurring mutations.

Acknowledgments—We thank Vijay Khanna and Stella de Leon for expert and invaluable technical assistance; Dr. David Klapper and Alan Harris for amino acid sequence determination; Drs. Michael Harpold and Steven B. Ellis (The Salk Institute Biotechnology Industrial Assocates, San Diego, CA) and Dr. Arnold Schwartz (University of Cincinnati) for the gift of their rabbit muscle cDNA expression library; Drs. Michel Koenig and Louis Kunkel (Harvard University) for the gift of their human muscle cDNA library; Drs. Henry Klamut, Peter N. Ray, and Ronald G. Worton (University of Toronto) for the gift of human muscle mRNA and for a dystrophin cDNA probe; Dr. Willie R. Taylor for invaluable assistance in structural predictions; and the American Type Culture Collection for providing the human chromosome 19-specific library LL19NL01 prepared by Dr. Pieter de Jong, Lawrence Livermore National Laboratory, Livermore, CA.

REFERENCES

Brandl, C. J., Green, N. M., Korczak, B., and MacLennan, D. H. (1986) *Cell* **44,** 597–607

Buschmeier, B., Meyer, H. E., and Mayr, G. W. (1987) *J. Biol. Chem.* **262,** 9454–9462

Campbell, K. P., Knudson, C. M., Imagawa, T., Leung, A. T., Sutko, J. L., Kahl, S. D., Raab, C. R., Madson, L. (1987) *J. Biol. Chem.* **262,** 6460–6463

Chothia, C., and Lesk, A. M. (1986) *EMBO J.* **5,** 823–826

Chou, P. Y., and Fasman, G. D. (1974) *Adv. Enzymol.* **47,** 45–140

Clarke, D. M., Loo, T. W., Inesi, G., and MacLennan, D. H. (1989) *Nature* **339,** 476–478

Ellis, S. B., Williams, M. E., Ways, N. R., Brenner, R., Sharp, A. H., Leung, A. T., Campbell, K. P., McKenna, E., Koch, W. J., Hui, A., Schwartz, A., and Harpold, M. (1988) *Science* **241,** 1661–1664

Engelman, D. H., Henderson, R., McLachlan, D. A., and Wallace, B. A. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2023–2027

Engelman, D. M., Steitz, T. A., and Goldman, A. (1986) *Annu. Rev. Biophys. Chem.* **15,** 321–353

Feramisco, J. R., Glass, D. B., and Krebs, E. G. (1980) *J. Biol. Chem.* **255,** 4240–4245

Ferguson, D. G., Schwartz, H. W., and Franzini-Armstrong, C. (1984) *J. Cell Biol.* **99,** 1735–1742

Fleischer, S., Ogunbunmi, E. M., Dixon, M. C., and Fleer, E. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 7256–7259

Fliegel, L., Ohnishi, M., Carpenter, M. R., Khanna, V. K., Reithmeier, R. A. F., and MacLennan, D. H. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 1167–1171

Fliegel, L., Burns, K., MacLennan, D. H., Reithmeier, R. A. F., and Michalak, M. (1990) *J. Biol. Chem.*, in press

Franzini-Armstrong, C. (1970) *J. Cell Biol.* **47,** 488–499

Fry, D. C., Kuby, S. A., and Mildvan, A. S. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 907–911

Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) *J. Mol. Biol.* **120,** 97–120

Gershoni, J. M., Davis, F. E., and Palade, G. E. (1985) *Anal. Biochem.* **144,** 32–40

Gribskov, M., McLachlan, A. D., and Eisenberg, D. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 4355–4358

Harris, A. S., Croall, E. E., and Morrow, J. S. (1988) *J. Biol. Chem.* **263,** 15754–15761

Hymel, L., Inui, M., Fleischer, S., and Schindler, H. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 441–445

Imagawa, T., Smith, J. S., Coronado, R., and Campbell, K. P. (1987) *J. Biol. Chem.* **262,** 16636–16643

Inui, M., Saito, A., and Fleischer, S. (1987a) *J. Biol. Chem.* **262,** 1740–1747

Inui, M., Saito, A., and Fleischer, S. (1987b) *J. Biol. Chem.* **262,** 15637–15642

Jenden, D. J., and Fairhurst, A. S. (1969) *Pharmacol. Rev.* **21,** 1–25

Koenig, M., Hoffman, E. P., Bertelson, C. J., Monaco, A. P., Feener, C., and Kunkel, L. M. (1987) *Cell* **50,** 509–517

Kozak, M. (1984) *Nature* **308,** 241–246

Kretsinger, R. H. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52,** 449–510

Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132

Lai, F. A., Erickson, H. P., Block, B. A., and Meissner, G. (1987) *Biochem. Biophys. Res. Commun.* **143,** 704–709

Lai, F. A., Erickson, H. P., Rousseau, E., Liu, Q.-Y., and Meissner, G. (1988a) *Nature* **331,** 315–319

Lai, F. A., Anderson, K., Rousseau, E., Liu, Q. Y., and Meissner, G. (1988b) *Biochem. Biophys. Res. Commun.* **151,** 441–449

Lai, F. A., Misra, M., Xu, L., Smith, M. A., and Meissner, G. (1989) *J. Biol. Chem.* **264,** 16776–16785

Lear, J. E., Wasserman, L. R., and De Grado, W. F. (1988) *Science* **240,** 1177–1181

Leberer, E., Charuk, J. H. M., Clarke, D. M., Green, N. M., Zubrzycka-Gaarn, E., and MacLennan, D. H. (1989a) *J. Biol. Chem.* **264,** 3484–3493

Leberer, E., Charuk, J. H. M., Green, N. M., and MacLennan, D. H. (1989b) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 6047–6051

Lennarz, W. (1987) *Biochemistry* **26,** 7205–7210

Lodish, H. F. (1988) *Trends Biochem. Sci.* **13,** 332–334

MacKenzie, A. E., Korneluk, R. G., Duff, C., Worton, R. G., and MacLennan, D. H. (1989) *Am. J. Human Genet* **45,** A149

MacLennan, D. H., and Wong, P. T. S. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 1231–1235

MacLennan, D. H., and de Leon, S. (1983) *Methods Enzymol.* **96,** 570–579

MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985) *Nature* **316,** 696–700

MacLennan, D. H., Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Lai, F. A., Meissner, G., Green, N. M., Willard, H. F., Britt, B. A., Worton, R. G., and Korneluk, R. G. (1989) *Am. J. Human Genet.* **45,** A205

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Marks, A. R., Tempst, P., Hwang, K., Inui, M., Chadwick, C. C., Fleischer, S., and Nadal-Ginard, B. (1989) *J. Cell. Biochem.* **13** (Suppl. E) p. 208

McLauchlan, J., Gaffney, D., Whitton, J. L., and Clements, J. B. (1985) *Nucleic Acids Res.* **13,** 1347–1368

Meissner, G. (1984) *J. Biol. Chem.* **259,** 2365–2374

Meissner, G. (1986) *Biochemistry* **25,** 244–251

Meissner, G., Darling, E., and Eveleth, J. (1986) *Biochemistry* **25,** 236–244

Meissner, G., Rousseau, E., and Lai, F. A. (1989) *J. Biol. Chem.* **264,** 1715–1722

Mitchell, R.-D., Simmerman, H. K. B., and Jones, L. R. (1988) *J. Biol. Chem.* **263,** 1376–1381

Miyamoto, H., and Racker, E. (1982) *J. Membr. Biol.* **66,** 193–201

Moos, M., Jr., Nguyen, N. Y., and Liu, T. Y. (1988) *J. Biol. Chem.* **263,** 6005–6008

Morii, H., and Tonomura, Y. (1983) *J. Biochem. (Tokyo)* **93,** 1271–1285

Noda, I. (1973) in *Enzymes* (Boyer, P. D., ed) Vol. III, Part A, pp. 298–299, Academic Press, New York

Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T., Shimizu, S., Kikyotani, S., Kayano, T., Hirose, T., Inayama, S., and Numa, S. (1983) *Nature* **305,** 818–823

Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., and Numa, S. (1984) *Nature* **312,** 121–127

Ostwald, T. J., and MacLennan, D. H. (1974) *J. Biol. Chem.* **249,** 974–979

Otter, T., King, S. M., and Witman, G. B. (1987) *Anal. Biochem.* **162,** 370–377

Pessah, I. N., Waterhouse, A. L., and Casida, J. E. (1985) *Biochem. Biophys. Res. Commun.* **128,** 449–456

Pessah, I. N., Francini, A. O., Scales, D. J., Waterhouse, A. L., and Casida, J. E. (1986) *J. Biol. Chem.* **261,** 8643–8648

Proudfoot, N. J., and Brownlee, G. G. (1976) *Nature* **263,** 241–246

Rao, U. S., Hennessey, J. P., Jr., and Scarborough, G. A. (1988) *Anal. Biochem.* **173,** 251–264

Ray, P. N., Belfall, B., Duff, C., Logan, C., Kean, V., Thompson, M. W., Sylvester, J. E., Gorski, J. L., Schmickel, R. D., and Worton, R. G. (1985) *Nature* **318,** 672–675

Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5463–5467

Saito, A., Inui, M., Radermacher, M., Frank, J., and Fleischer, S. (1988) *J. Cell Biol.* **107,** 211–219

Seiler, S., Wegener, A. D., Whang, D. D., Hathaway, D. R., and Jones, L. R. (1984) *J. Biol. Chem.* **259,** 8550–8557

Smith, J. S., Coronado, R., and Meissner, G. (1985) *Nature* **316,** 446–449

Smith, J. S., Coronado, R., and Meissner, G. (1986) *J. Gen. Physiol.* **88,** 573–588

Smith, J. S., Imagawa, T., Ma, J., Fill, M., Campbell, K. P., and Coronado, R. (1988) *J. Gen. Physiol.* **92,** 1–26

Supattapone, S., Worley, P. F., Baraban, J. M., and Snyder, S. H. (1988) *J. Biol. Chem.* **263,** 1530–1534

Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T., and Numa, S. (1989) *Nature* **339,** 439–445

Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., and Numa, S. (1987) *Nature* **328,** 313–318

Taylor, W. R. (1986) *J. Mol. Biol.* **188,** 233–258

Wagenknecht, T., Grassucci, R., Frank, J., Saito, A., Inui, M., and Fleischer, S. (1989) *Nature* **338,** 167–170

Wahl, G. M., Stern, M., and Stark, G. R. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 3683–3687

Walker, J. E., Saraste, M., and Gay, N. J. (1984) *Biochim. Biophys. Acta* **768,** 164–200

Wierenga, R. K., and Hol, W. G. (1983) *Nature* **302,** 842–844

Young, R. A., and Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 1194–1334

Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Lai, F. A., Meissner, G., Green, N. M., and MacLennan, D. H. (1989a) *7th International Congress on Cylic Nucleotides, Calcium and Protein Phosphorylation,* October 8–13, Kobe, Japan (abstr.)

Zorzato, F., Chu, A., and Volpe, P. (1989b) *Biochem. J.* **261,** 863–870