Molecular diversity of Ca^{2+} channel α_1 subunits from the marine ray *Discopyge ommata*

W. A. Horne^{*†}, P. T. Ellinor[‡], I. Inman[‡], M. Zhou^{*†}, R. W. Tsien[‡], and T. L. Schwarz^{‡§}

[‡]Department of Molecular and Cellular Physiology, Stanford University Medical Center, Stanford, CA 94305; and ^{*}Department of Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850

Communicated by James A. Spudich, January 7, 1993

ABSTRACT In many neurons, transmitter release from presynaptic terminals is triggered by Ca²⁺ entry via dihydropyridine-insensitive Ca²⁺ channels. We have looked for cDNAs for such channels in the nervous system of the marine ray Discopyge ommata. One cDNA (doe-2) is similar to dihydropyridine-sensitive L-type channels, and two cDNAs (doe-1 and doe-4) are more similar to the subfamily of dihydropyridineinsensitive non-L-type channels. doe-4, which encodes a protein of 2326 aa, most closely resembles a previously cloned N-type channel, doe-1, which encodes a protein of 2223 aa, is a member of a separate branch of the non-L-type channels. Northern blot analysis reveals that doe-1 is abundant in the forebrain. doe-4 is more plentiful in the electric lobe and, therefore, may control neurotransmitter release in motor nerve terminals. These results show that the familial pattern of Ca²⁺-channel genes has been preserved from a stage in evolution before the divergence of higher and lower vertebrates >400 million years ago. The cloning of these channels may be a useful starting point for elucidating the role of the Ca²⁺ channels in excitation-secretion coupling in nerve terminals.

Delineation of the molecular diversity of voltage-gated Ca²⁺ channels is essential for understanding how these channels control a wide spectrum of cellular functions ranging from secretion and contraction to metabolism and gene expression. Several types of voltage-gated Ca²⁺ channels (L, T, N, and P types) have been distinguished by functional criteria and show different tissue distributions and differential sensitivity to modulators and drugs (1, 2). Much of the known diversity among Ca²⁺ channels may arise from the existence of multiple forms of the α_1 subunit (3–10), which are large (200–260 kDa) transmembrane proteins and are responsible for Ca²⁺-channel voltage dependence, selectivity for Ca²⁺, and sensitivity to pharmacological modulation.

Two structural subfamilies of α_1 subunits have emerged from molecular cloning of mammalian cDNAs (11, 12). The first subfamily includes α_1 cDNAs originally derived from skeletal muscle (3, 13), heart (4), aorta (14), lung (15), and brain (7, 8). Expression of these channels demonstrated that they are responsive to 1,4-dihydropyridines (DHPs) and may thus be classified as L-type channels. The second α_1 subfamily consists so far of cDNAs derived from mammalian brain. The individuals within this subfamily show upward of 60% identity with each other but only ~45% with members of the first subfamily (5, 6, 9, 10), and, when expressed, lack the characteristic DHP response of L-type channels (5, 10).

We chose the marine ray *Discopyge ommata* as a source for additional neuronal Ca^{2+} -channel cDNAs. The electric organ of marine rays has served as a valuable model system for studying the biochemical properties of synaptic proteins. The nerve terminals within the electric organ of *D. ommata* are the richest known source of receptors for the cone snail

doe-1 rbB	$\label{eq:light_constraint} \begin{split} \textbf{IGMQLFGNIGLDDHTPINRENNFHTFINALMLLFRSATGESWQ} \\ \textbf{IGMQVFGNIALDDGTSINRENNFRTFLQALMLLFRSATGEAWH} \end{split}$
L ₁ (skel)	IGMQMFGKIALVDGTQINRNNNFQTFPQAVLLLFRCATGEAWQ
L ₂ (card)	IGMQWFGKIALNDTTEINRNNNFQTFPQAVLLLFRCATGEAWQ
L ₃ (neur)	IGMQMFGKVAMRDNNQINRNNNFQTFPQAVLLLFRCATGEAWQ
doe-2	IGMQ#FGKIAMVDGTQINRNNNFQTF#QAVLLLFRCATGEAWQ

FIG. 1. PCR-derived Ca²⁺-channel fragments from *D. ommata* fall into both subfamilies of high-voltage-activated channels. The fragments are from the region after IVS5. The sequences encoded by the oligonucleotides for PCR are marked with arrows; the position of the detection oligonucleotide is marked with a dashed line. Residues that differ from the sequence of the skeletal muscle L_1 channel are shaded. doe-1 is grouped with the DHP-insensitive rbB channel from rat brain (9, 11); doe-2 is grouped with the DHP-sensitive L-type channels (3–8).

toxin ω -conotoxin GVIA (16), a selective probe for N-type Ca²⁺ channels. Here we report the molecular cloning of two full-length cDNAs that encode putative Ca²⁺-channel α_1 subunits[¶] that are differentially expressed in the forebrain and in the electric lobe that innervates the electric organ.

METHODS

PCR and Molecular Cloning. Phage template DNA was isolated from plate lysates (17) of an oligo(dT)-primed λ gt10 cDNA library (kindly provided by F. Rupp and R. Scheller, Stanford University). Forty cycles of PCR (95°C for 1 min, 52°C for 2 min, and 72°C for 1 min) were performed using standard conditions (17). PCR products were separated through a 1.2% agarose gel, transferred to Hybond membranes (Pharmacia), and hybridized with a ³²P-end-labeled degenerate oligonucleotide probe (see Results). Positive DNA fragments were gel-purified, blunt-ended with T4 DNA polymerase, phosphorylated with T4 polynucleotide kinase, and ligated into the Sma I site of pBluescript SK+ (Stratagene). Plasmid DNA was purified and sequenced by the dideoxynucleotide termination method (18). Ca²⁺-channel cDNAs were isolated from the oligo(dT)-primed λ gt10 library from electric lobe and additional randomly and specifically primed forebrain and electric lobe cDNA libraries in λ ZAP II (Stratagene). Libraries were probed with the PCR-derived clones or successive cDNA isolates. DNA sequencing was performed by creating nested deletions on both strands (Promega Erase-A-Base) and with specifically synthesized primers. Sequence analysis was carried out using GCG software (19).

Isolation of RNA and Northern Blot Analysis. Poly(A)⁺ RNA was isolated from either forebrain or electric lobe regions of *D. ommata* by using the guanidinium isothiocya-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: DHP, dihydropyridine.

[†]Present address: Neurex Corp., 3760 Haven Avenue, Menlo Park, CA 94025.

[§]To whom reprint requests should be addressed.

The sequences reported in this paper have been deposited in the GenBank data base [accession nos. L12531 (doe-1) and L12532 (doe-4)].

	· · · · · _ ·	
doe-1	MARFGEAVGSLSADASSEQGRSRHQVPVTGETAVAAAAAAVVAGAAQGSAGFKQTRAQRARTMALYNPIPVRHNCLTANRSLFLFGEDNIVRRSARRVIE	100
BI-2	MARFGDEMPAL. RYGGGGAGAAAGVVVGAAGGRGAGGSROGGOPGAORMY. KOSMAORARTMALYNPIPVRONCLTVNRSLFLFSEDNVVRKYAKEITE	96
rbB	MURECORTIGG RYGOTOGOERA BOGOGOGOERACOROCORVIV KOSTAORARTMALYNEI DVRONOFTUNESI DVR SEDWUVEKVARETTE	03
don-4		35
006-4	INARUGADVIA. QIGGSFAG	86
doe-1	wppfeymilatiianCv/vlaleQhlpNgDktpM <u>akSleQ</u> tepyfigifCfeagikiv/algfvfhkgsylrngwnvMdf12vvlSGLLataAThfNLRtlra	200
BI-2	WPPFEYMILATIIANCIVLALEQHLPDDDKTPMSERLDDTEPYFIGIFCFEAGIKIIALGFAFHKGSYLRNGWNVMDFVVVLTGILATVGTEFDLRTLRA	196
rbB	WPPFEYMILATIIANCIVLALEOHLPDGDKTPMSERLDDTEPYFIGIFCFEAGIKIIALGFWFHKGSYLRNGWNMDFVVVLTELLATAGTDFDLRTLRA	193
doe-4	WDDFFYMTIATTIANCTVIAIFOUIDDCDKTDMSEDIDDTEDYFICTFCFFACTKTTAICFAFUKCSVIDNCWNMDFVVVITCTTÄTTCTDFDDTDA	196
406-4	ISI TO THE ADDRESS AND THE FEAST CERTIFICATION AND AND AND AND AND AND AND AND AND AN	100
doe-1	VRVLRPLKLVSGIPSLQHVLKSIMKAMVPLLQIGLLLFFAILMFAIIGLEFYYGKUHRTCYTDDAAAEELDLQFPCGTQEPTRLCPNGTVQ.SYWIGPND	299
BI-2	VRVLRPLKLVSGIPSLQVVLKSIMKAMLPLLQIGLLLFFAILLIFAIIGLEFYMGKFHLTCFELGTDDIQGESPAPCGTEEPARLCPNGTRQPYWEGPNN	296
rbB	VRVLRPLKLVSGIPSLQVVLKSIMKAMVPLLQIGLLLFFAILMFAIIGLEFYMGKFHKACFPNSTDAEPVGD.FPCGKEAPARLCDSDTECREYWPGPNF	292
doe-4	VRVLRPLKLVSGIPSLOVVLKSIMKAMVPLLOIGLLLFFAILMFAIIGLEFYMGKFHKTCFSEETN, EPVEE, FPCGTKYPSRLCPNGTVCKGYMMGPNF	284
4 1		200
00e-1	GIIGEDNILEALLIVEGCIIMEGWILIIINIDDALGAMWWWIIEIPLIIIGSEEVLALUGVLSGEEAARERVENRASPLALARGVGGIERELMGIRAMI	399
BI-2	GITQFDNILFAVLTVFQCITMEGWTDLLYNSMDASGNTWNWLYFIPLIIIGSFFMLNLVLGVLSGEFAKERERVENRRAFLKLRRQQQIERELNGYMEWI	396
rbB	GITNFDNILFAULTVFQCITMEGWTDILYNTNDAAGNTWNWLYFIPLIIIGSFFMLNLVLGVLSGEFAKERERVENRRAFLKLRRQQQIERELNGYLEWI	392
doe-4	<u>GITNFDNILFAVLTVFQCITMEGWTDMLYTANDALGNTWNWLYFIPLIVIGSFFMLNLVLGVLSGEFAKERERVENRRAFLKLRRQQQVEQEFNRYLRWI</u>	384
	IH5	
doe-1	DEAREVMILEEMENAGESSIH, VIRRATIKKGRMEMTOTESSEDOYTEISSVGSPLARAŠIKŠTKULEGSSYFRREEMIRISTRHMVKSHAFYWIVIG	498
BT_2	CERA DE ANDER DE	405
D1-2		401
TDB	FICAEEVMLAEEDKNAEEKSPLDAVLKRAATKKSKNDLIHAEEGEDRIVDLCAAGSPFARASLKSGR. TESSSIFRKEEMMFRELIKKMVRAQSFIMUVLC	491
doe-4	HIAEEVMLAEEDKNAEDKICALD.VLKRATTKKSKNDLINAEEGEDHFTDISSVGFNRPSLKSVKJ.NERSSYFRKEKRFRFFIRRMVKSOSFYWIVLC	480
	▲ 8+ 000 +	
doe-1	LVALNTVCVAVVHYDQPLWLSNFLYYAEFTFLGLFS SEMFLKMYGCGPRLYFHSSFNCFDCGVTIIGSIFDVVWTIIIRPETSFGISVLRALRLLRIFKIITK	598
BT-2	LVALNTLCVALVHYNOPEWISDELYYAEFIFIGLEMSEMFTKMYGLGTRPYFHSSENCEDOGVTIGSTFEVITWAVTROCTSEGTSVLBALDILBTEKVTK	595
 	WALNTLOVAMUUMODON TRALVER PUPICIPIC PLANCE CODEVERSENCE DECUTOR CENTRE UNAL TECHNER CENTRE INTERNAL	501
TDB	VVALATILCVANVATING VALATILALTERET VELGLE LIERSLAATGLEGERSTERSSERCEDE GVIVGSTE EV VMAATGUSET STANDALTERVIL	591
doe-4	LVGLNTLCVAIVHYDQPPILTDALYFAEFVFLGLFLTEMSLKMYGLGPRNYFHSSFNCFDFGVIVGSIFEVVWTAVKPDTSFGISVLRALRLLRIFKVTK	580
doe-1	YWAŚLRNLVVSIMSSMKSIISLLFLLFLFIVVFALLGMQLFGGQFNFEEGTPPTNFDTFPAAIITVFQILTGEDWNEVMYMGIKSQGGVNSGMWSSVYFI	698
BI-2	YHASLENI, VYSLINSMESTISLIFLIFLEFIVYFALLGMOLFGGOFNFDEGTPPTNFDTFPAAIMTVFOILTGEDWNEVMYDGIRSOGGVOGGMVFSTYFI	695
	WENCE DNE WESTENESTED TELETINE TOWART CONTROLOGY AND THE DATE DATE TO A TERMAN WHICE TESO CONCECUTES SERVET	691
TDB		C00
doe-4	YWNSLRNLVVSLLNSKKSIISLLFLFIVVFALLGMQLFGGQFNFEDGTPPTNFDTFPAAILTVFQILTGEDWNEVMIGIEAHGGVRKGMFSSVIFI	680
doe-1	VITIFGNYTIINVFIAIAVDNIANAQEITKEEQEEEAINQKHAIQKAKEVSPMSAPGFPSTEREFRRHKHMSIWEARTSQIRRRMOMSSREAIF	793
BI-2	VITIFGNYTLINVFLAIAVDNLANAOELTKDEOEEEEAVNOKLALOKAKEVAEVSPISAANMSIAMKEOOKNOKPAKSVWEORTSEMRKONLLASREALY	795
rhB	VITE FORVELL NUFLA TAVDNI, ANA OFI. THE FEMERALANOKIALOKAKE VAFUS FMSAANTS TAAR DOMSAK ARSVWEORASOL REDNIRASCEALV	789
IDB		770
d0e-4	ILITLEGNITLLNVELAIAVDNLANAQELTKDEEEREERIIQUNTIQUAMEVADVSPISAINLSIAAADVQUASEN. SASINLQKISQLAAQHIQISQLAADI	113
doe-1	TD	875
BI-2	SEMDPEERWKASYARHILRPDMKTHLDRPLVVDPQENRNNNTNKSRVAEPTVDQ.RIGQORAEDFLRKQARHHDRARDPSAHAAAGLDARPWAGS	889
rbB	SEMDPEERLEVASTRHVRPDMKTHMDRPLVVEPGRDGLRGPÄGNKSKPEGTEATEGADPPRRHHRHRDRDKTSÄSTPAGGEQDRTDCP.KAES	881
doe-4	NETIDE TO BE WYVS SHOTE POMETHID BPL VVEP BNSTRKSAD KVCPSDCOEGEOERIVOPESCEAPRSHRHRDK	869
doe-4	NEIDDEORMYVSSHOIRPDMKTHLDRPLVVEP.RNSTRKSADKVCPSDCOEGEORIVOPESCEAPRRSHRHRDKLGEODKGDGALDTGE	869
doe-4		869
doe-4 doe-1	NEIDDEORRMYVSSHOTRPDMKTHLDRPLVVEP. RNSTRRSADKVCPSDCOEGEORRIVOPESCEAPRRSHRHRDKLOOLCEEOEGODKGDGALDTGE	869 949
doe-4 doe-1 BI-2	NETDDE ORRMYVSSHOTRPDMKTHLDRPLVVEP. RNSTRIKSAD KVCPSDCQEGEQERTIVQPESCEAPRRSHRHRDKLQQLCEQDKGDGALDTGE VEAGASFRMARPIRARRYRSLYKEARMGLEESAETSLSRRPGKNKEGRLLQQLCEEQESQLTQTPEVMDAQG QEAETISREGPYGRESDHQAREGGLEPPGFWEGELERGKAGDPHRRHAHRQGVGGSGGSRSGSPRTGTADGEPRRHRVHRRPGEDGPDD	869 949 979
doe-4 doe-1 BI-2 rbB	NEIDDEORMYVSSHOIRPDMKTHIDRPLVVEP. RNSTRKSADKVCPSDCOEGEORETVOPESCEAPRRSHRHRDKLGEODKGDGALDTGE voo VEAGASFRMAREPIRARRYRSLYKEARMGLESSAEGSAEGEAERGKNKEGRLLQQLCEEGESGQLTQHPEVMDAQG OEAEISREGPYGRESDHQAREGGLEPFGFWEGEAERGKAOPHRRHAHRQGVGGSGGSRSGSPRTGTADGEPRRHRWERRPGEDGPDD	869 949 979 958
doe-4 doe-1 BI-2 rbB doe-4	NEIDDEGRAMIVSSEGIRPDMKTHLDRPLVVEP. RNSTRKSADKVCPSDCGEGEGERIVGPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMAEPIRARRYRSLYKEARMGLEESAETSUSRFGKNKEGRL	869 949 979 958 949
doe-4 doe-1 BI-2 rbB doe-4	NEIDDEGRAMIVSSEGIRPOMKTHLDRPLVVEP. RNSTRRSADKVCPSDCGEGEGERIVGPESCEAPRRSHRHRDKLGEODKGDGALDTGE VEAGASFRMAEPIRARRYRSLYNEARMGLE SAETSLSRPGKNKEGRLLQQLCEEGEGESGSLTQTPEVMDAQG OF AEISREGPYGRESDHOAREGGLEPFGFWEGEAERGKAGDPHRRHAHRQGVGGSGSGSRSGSPRTGTADGEPRRHRVHRRPGEDGPDD	869 949 979 958 949
doe-4 doe-1 BI-2 rbB doe-4	NEIDDEGRAMVVSBRUT <u>RPDMKTHIDRPLVVEP</u> .RNSTRKSADKVCPSDC <u>QEGEQERI</u> V <u>QPESCEAPRRSHRHRD</u> KL <u>GEQD</u> KGDGALDTGE VEAGASFRMAEDIRARRYRSLYKEAKMGLESSAEJSRFGKNKEGRLLQQLCEEQESGQLTQEPEJMDAQG QEAEISREGPYGRESDHQAREGGLEPPGFWEGEAERGKADPHRRAMEQQVGGSGGSRSGSPRTGTADGEPRRHRVERRPGEDGPDD	869 949 979 958 949 1041
doe-4 doe-1 BI-2 rbB doe-4 doe-1	NEIDDEGRAMVSSBUTRPDMKTHLDRPLVVEP. RNSTRKSADKVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMAZETRARRYRSLYKEARMGLEESAETGLGRRFGKNKEGRLLQQLCEEQESGQLTQTPEVMDAQG QEAETSREGPYGRESDHQAREGGLEPPGFWEGEAERGKAGDEHRRHAHRQGVGSGSGSRSGSPRTGTADGEPRRHRVHRRPGEDGPDD	869 949 979 958 949 1041
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2	NEIDDEORMYVSSHOIRPOMKTHLDRPLVVEP. RNSHRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKCDGALDTGE	869 949 979 958 949 1041 1068
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NEIDDEORMYVSSHOIR <u>PDMKTHIDRPLVVEP</u> .RNSTRKSADKVCPSDC <u>QEGEQERI</u> VQPE <u>S</u> CEA <u>PRRSHRHRD</u> KI <u>GEQD</u> KGDGALDTGE VEAGASFRMAEDIRARRYRSIYKDARMGLESSAEGSAEGAL VEAGASFRMAEDIRARRYRSIYKDARMGLESSAEGSAEGAL OC QEAEISREGPYGRESDHQAREGGLEPPGFWEGEAERGKAGDPHRRHAHRQGVGGSGGSRSGSPRTGTADGEPRHRVHRRPGEDGPDD	869 949 979 958 949 1041 1068 1048
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NEIDDEGRAMVVSSHOTRPDMKTHIDRPLVVEP.RNSTRKSADKVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMAREFIRARRYRSLYKEARMGLEESAETGLSRFGKNKEGRLLQQLCEEQESGSLYGESGOLTQEPEYMDAQG QEAEISREGPYGRESDHQAREGGLEPGFWEGEAERGKAGDEHRRHAHRQGVGGSGGSRSGSPRTGTADGEPRRHRVHRRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NEIDDEORMYVSSHOTRPDMKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLQQLCEEQESQUTQBPEYCAPRSHRHRDKLQQLCEEQESQUTQBPEYDDAQG VEAGASFRMADEPIRARRYRS YEAGASFRMADEPIRARRYRS OCAE QEAEISRECPYGRESDHQAREGGLEPPGFMEGEAERGKAGDPHRRHAHRQQVGGGGGGSRSGSPRTGTADGEPRHRVHRRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1	NEIDDEORMYVSSHOTRPDMKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMALETIRARRYRSIYKEARGIESSAETGEKSRFGKNKEGRLLQQLCEEQESGQLTQHPEWMDAQG OC QEAEISRBCPYGRESDHQAREGGLEPPGFWEGEAERGKAGDPHRRHAHRQQVGGSGGSRSGSPRTGTADGEPRRHRVHRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041 1055
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2	NEIDDEORMYVSSHOTRPDMKTHLDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LLGEQDKGDGALDTGE VEAGASFRMAZETRARRYKSHYKEARMGLESSESSESSESSESSESSESSESSESSESSESSESSESS	869 949 979 958 949 1041 1041 1048 1041 1055 1168
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2	NEIDDEORMYVSSHOTRPDMKTHLDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKCDGALDTGE VEAGASFRMAEPIRARRYRSHIXKEARMGLESSAFTGKNKEGRLLQQLCEEQESGQLTQBPEYMDAQG QEAEISREGPYGRESDHQAREGGLEPFGFMEGEAERGKAGDPHRRAHRQQVGGGGGGGSRSGSPRTGTADGEPRHRVHRRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NEIDDEORMYVSSHOTRPDMKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMALETIRARRYRSIYKEARGE SAFTSKRAGE SAFTSKRAGE SAFTSKRAGE SAFTSKRAGE STOLE SAFTSKRAGE STOLE SAFTSKRAGE STOLE SAFTSKRAGE SAFTSKRAGE STOLE	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NEIDDEGRAMIVSSHUTRPDMKTHLDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LGEQDKGDGALDTGE VEAGASFRMALETRARRYRSLYKEARMGLESSESAETGEAGKAGDEHRRHAFROGVGGGGGGSRSGSPRTGTADGEPRRHRDK LQQLCEEQESGQLTQEPEVMDAQG QEAEISREGPYGRESDHQAREGGLEPPGFWEGEAERGKAGDEHRRHAFRQGVGGGGGGSRSGSPRTGTADGEPRRHRVHRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084
doe-4 BI-2 rbB doe-4 doe-4 doe-4 doe-4 doe-4 doe-1 BI-2 rbB doe-4	NEIDDEORMYVSSHOTRPDMKTHLDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LGEQDKCDGALDTGE VEAGASFRMADEPIRARRYRSHSLYKEIARMGLESAFTSLESREGKNKEGRL. LQQLCEEQESGQLTQHPEYMDAQG QEAEISREGPYGRESDHQAREGGLEPPGFMEGEAERGKAGDPHRRAMRQVGGSGGSRSGSPRTGTADGEPRHRVMRRPGEDGPDD. KA TETGAREERARPRRSHSKEAPG. ADTQVR. GERS. RRHRAGYGVGSGGSRSGSPRTGTADGEPRHRVMRRPGEDGPDD. KA TETGAREERARPRRSHSKEAPG. ADTQVR. GERS. RRHRAGYGVGSGGSRSGSPRTGTADGEPRHRVMRRPGEDGPDD. KA TETGAREERARPRRSHSKEAPG. ADTQVR. GERS. RRHRAGYGVGSGGSRSGSPRTGTADGEPRHRVMRRPGEDGPDD. KA TETGAREERARPRRSKETEKERDEKGRK. GERSRSHEGGRRHHRAGVSSLDAPER. EPRRHRVHRAHRAQDSSKEGKEGTAPVJLVPKG PRANSKDDKRCSHRSHSKETEKERDEKGRK. GERSRSHEGGRRHHRAQSSLDAPER. ERRHRSHRHGTEQQUREANGT. KG GMKAFSWQGEPHSSGMTRTPDVDTDPSGGNLEK. ESGRTFENGKEESANTSEQVNEQSNWLMLQLNQQATPGDRELTGGTRDTKQDKTQEQTE ERRGRHREGSRPARSGEGEAEGPDGGGGGGGGERRRRHRHGPFAYDPDARRD. DRERRHRRRRDTGGSGVPVSGPMJSTTRPIQQDLSRQ ERRARHR.GERTG. FRETE. NSEEPTRHRRRDFFRAUHVPTTLEPFF.REVAEKESNVVEGORGETRNHGPKEERCDLEAIAVMGVGSLHMLFSTCLQK # IDVDCENTETPMDS. EPRINSKKDGSRSG. GREGEAVSRSHHAEGAERRRKHRQKVASTMESEEKREIGEKERETVL. RERRVHRVKETQPSQDSGTQGNVS. LHPFIGLQH # IDVDCENTETPMDS. EPRINSKNGSQ. PSDL. STTVHVPVTTTGPPGRAM UDEOPEDADNQRWVTRMGSQ. FSDLAERRSTDPAGPTPATAANPQNSTASRRTPNNPGNPSNFGPERTPENSLIVTNPVTTGPPGRA. EPSDL. TGDAQMENTYNIEVTVTTPAAEM. ISVICEPC	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4	MEIDDEQRAMYVSSHQIRPDMKTHIDRPLVVEP.RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDKLGEQDKGDGALDTGE VEAGASFRMALETIRARRYVSHARMGLESSAFTGKNKEGRLLQQLCEEQESGQLTQTPEVMDAQG QEAEISRAGPYGRESDHQAREGGLEPPGFMEGEAERGKAGDPHRRHAHRQVGSGGSGSRSGSPRTGTADGEPRRHRVHRRPGEDGPDD	869 949 979 958 949 1041 1068 1041 1055 1168 1089 1084 1120
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2	NEIDDEORMYVSSHOTRPDMKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LGEQDKGDGALDTGE VEAGASFRMALETIRARRYRSIYKDARMGLESSATGSLORVEGKNKEGRL LQQLCEEQESGQLTQTPEVMDAQG QEAEISREGPYGRESDHQAREGGLEPGFMEGEAERGXAODPHRRHAHRQGVGGSGGSRSGSPRTGTADGEPRHRVHRPGEDGPDD KK TETGAREERARPRRSHSKEAPG ADTQVR CERS RRSHSHEGGRRHHAQSSEEATER. EPRHRAHRHAQDSSKEGKEGTAPVLVPKG PRANSKDDKRCSHRSHSKETEKERDEKGRK GERSRSHEGGRRHHAQSSLDDAPER. EPRHRAHRHADDSSKEGKEGTAPVLVPKG PRANSKDDKRCSHRSHSKETEKERDEKGRK GERSRSHEGGRRHHAQSSLDDAPER. EFRRHRSHRHGTEQQHREANGT KG VEAGASFRARPTRSHSKETEKERDEKGRK GERSRSHEGGRRHHAQSSLDDAPER. EPRHRAHRHADDSSLDDAPER. EFRCHRSHRHGTEQQHREANGT KG VEAGASFRARCSRPARSGEGEAEGPDGGGGGGERRRRHRGPFPAYDPDARRD DESKTESANTSEQVNEQSNWLMLQLNQQATFGDRELTGSRDTKQDKTQEQTE ERRGRHREGSRPARSGEGEAEGPDGGGGGGGERRRRHRGPFPAYDPDARRD DESKESNTSEQVNEQSNWLMLQLNQQATFGDRELTGSRDTKQDKTQEQTE ERRARHR GERTG PRETE. NSEEPTRHRAKHKVPFTLEPFE REVAEKESNVVEGDKETRNHOPKEPRCDLEAIAVEGVGSLHMLFSTCLQK ERRARHR GERTG. GREGEAGYDSGLGGGGERRRRHRGKVASTNESEEKREIGEKERETVL RERVHRVKETQPSQDSGTQGKVS. LHPFIGQDLSR ERRARHR GERTG PRETE. NSEEPTRHRAKHKVPFTLEPFE REVAEKESNVVEGDKETRNHOPKEPRCDLEAIAVEGVGSLHMLFSTCLQK ERRARHR GERTG PRETE. NSEEPTRHRAKKKVPFTLEPFE REVAEKESNVVEGDKETRNHOPKEPRCDLEAIAVEGVGSLHMLFSTCLQK ERRARHR GERGEAVSRSHAEGAERRRKHRQKVASTNESEEKREIGEKERETVL RERVHRVKETQPSQDSGTQGKVS. LHPFIGLQH T	869 949 979 958 949 1041 1068 1041 1055 1168 1089 1084 1120 1268
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	MEIDDEQRAMYVSISHQIRPDMKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LGEQDKCDGALDTGE VEAGASFRMAEPIRARRYKSHUTEDRKTHIDRPLVVEP. RNSTRKSAD KVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LGQLCEEQESGQLTQTPEVMDAQG VEAGASFRMAEPIRARRYKSHQIESSTETSKERGEGEREGERAGDEHRRAHROQVGGSGGSRSGSPRTGTADGEPRRHRDK LQQLCEEQESGQLTQTPEVMDAQG QEAEISREGPYGRESDHQAREGGLEPPGFMEGEAERGKAGDEHRRAHRQQVGSGGGSRSGSPRTGTADGEPRRHRVHRPGEDGPDD	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084 1120 1268 1163
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	MEIDDE ORMANVSSHOT RPDMKTHIDRPLVVEP. RNSTRRSAD KVCPSDCQEGE QERIVQPESCEAPRRSHRHRDKLGEQDK GDGALDTGE VEAGASFRMALE IRARRYKSHIDRE SAFTSKER GKNKEGRLLQQLCEE QESGQLTQTPEVMDAQG VEAGASFRMALE IRARRYKSHIDRE SAFTSKER GKNKEGRLLQQLCEE QESGQLTQTPEVMDAQG QEAE ISRBCPYGRESDHQAREGGIDE PGFMEGE AERGKACDPHRRHAMRQVGSGGSGSRSGSPRTGTADGEPRRHRVERPGED GPDD	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1084 1120 1268 1163
doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NETDOEORAMYVSSHQIRPOMKTHLDRPLVVEP ENSTREKSADKVCPSDCQEGEQERIVQPESCEAPRRSHRHRDK LQELCHEQESGQLTQTPEQMADAGE VEAGASFRMADEPIRARRYRSLYKEARMGLEESAETSLORFGKNKEGEL LQQLCHEQESGQLTQTPEQMADAGE QEAELISKEGPYGREGDHQAREGCLEPPGFWEGEAERGKACDPHRRHAMERQCVGGSGGSRSGSPRTGTADGEPRHRVHRREGEDGPDD KA TETGAREERARFRKENTEKENDEGTAREGKACDPHRRHAMERQCVGGSGGSRSGSPRTGTADGEPRHRVHRREGEDGPDD KA TETGAREERARFRKENTEKENDEGTAREGKACDPHRRHAMERQCVGGSGGSRSGSPRTGTADGEPRHRVHRREGEDGPDD KA TETGAREERARFRENERSKENTEKENDEGGRAGGEARREGKACDPHRRHAMERQCVGGSGGVGGSGSRSGSPRTGTADGEPRRHRVHRREGEDGPDD KA TETGAREERARFRENERSKENTEKENDEGGRAGGEARREGKRALLANDEGGREHERALDAPER EPRRHRHAMEGTEQQHREANGT PRANSKDDKRCSTARSHTENDEGGGGGGGGGERRERHERGEFFRAUPPDARED ERGRHAMEGSRDANGEPKSENTENDEGGGGGGGGGERRERHERGEFFRAUPPDARED OMKAFSWQGEPHSSSMTRTPDDDDTPSGCNLEK ESGRTFENGKEESANTSEQUNEQSNULMEQLUNQQATFGDRELTTGTRDTKQDKTQEQTE ERRGRHREGSRDARSGEGEAEGPDGGGGGGGGERRERHERGEFFRAUPPDARED DEGRTG CHRMARSKENTGSSG NSEEPTREMERARKWYPTLEPPERNENED ERRARHE, OBRTG PRETER NSEEPTREMERARKWYKNTETPDDEDREDER ERRARHE, OBSTGSG, GERGEAEGPDGGGGGGGGERRERERHERGKVASTMESEEKREIGEKERETVL. RERENTREREGESGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSG	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084 1120 1268 1163 1162
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NETODE ORAMY SHOIR PDMKTHIDRPLVVEP. RNSTRK SAD KVCP SDCOZ GEOREN OPESCEAP RRSHRHRDK	869 949 979 958 949 1041 1068 1041 1055 1168 1084 1120 1268 1163 1162
doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1	NEIDDEORNUVSSED IRPOMETHLDRPLVVEP. RNSTRESSADEVCPSDCOEGEORIVOPESCEAPERSHRHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084 1120 1268 1162 1220
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2	NETDDEOR NUVSSED IRPOMETHLDRPLVVEP.RNSTREKSADKVCPSDCOEGEORIVOPESCEAPRESHRHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1089 1084 1120 1268 1163 1162 1220 1365
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NEIDDEDERMEVSSEDTRPDMKTHLDRPLVVED. IN STERKSADKVCP SDCQECEQERUVQPESCEAPRSHRHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NELDDEQRAMYVSSHQIRPDMKTHLDRPLVVEP. RNSTRKSADKVCPSDCQEGEGERIVQPESCEAPRSHRHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1084 1120 1268 1162 1220 1365 1263 1258
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NEIDDEQRAMYSKIGIRPDMKTHLDRPLVVEP. RNSTRIKSADKVCPSCQEGEGERIVQPESCEAPRSHRHRDK	869 949 979 958 949 1041 1068 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NETDDEQRALTVSSTRITED MKTHIDRPLVVEP RISTER SÅD KVCP SDCQEGE GEGER LVGPESCEAPRENHENDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258 1319
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1	NELDDEDERATVSSHOTRPDMKTHLDRPLVVEP P. NOT STAKSAD KVCPSDCQEGEGERIVQPESCEAPRSHRHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1041 1055 1168 1084 1120 1268 1162 1220 1365 1258 1319 1465
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NELDDEDERANTVESHOTRPDMKTHLDRPLVVEP RATERSALDKVCPSDCQEGEGEREIVQPESCEAPRSHRHRDKLGEODKGDGALDTGE VEAGASPRMARE_IRARRYRSLYKEARMGLEESAETGSLEAREGKAGDEHERHAMEGQVGEGESREGSPRGTADGEPRRERVHRPGEDGEDDKA GEAETISAEGPYGESDHOAKEGGLEDPGEWEGGALEKGAGDEHERHAMEGQVGEGSGSRGSPRGTADGEPRRERVHRPGEDGEDD	869 949 979 958 949 1041 1068 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258 1319 1465 1363
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NEIDDEORINATIVOS HUTELDRPLVVEP. EN STATAS KADKVCP SDCOZEGE MELVOPESCEAPERSHRHEDKLGEODKODCALDTGE VEAGAS RAMADEDIRARRYRLIYKAABMGLEE SARTSUGREG SARDSUGREG SCALTARD LVOLCEE OF SGULTON LEDDKODCALDTGE VEAGAS RAMADEDIRARRYRLIYKAABMGLEE SARTSUGREF GKACOPHERHAHEN OVGESGE SGREGS SPECTAD GEPREHRVERRY DE SGULTON LA VEAGAS READING READING AND TO WILL THE SARD STATE	869 949 979 958 949 1041 1068 1048 1048 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258 1319 1465 1363
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NETDOREGRENDUS HUT ILD RPLVVEP. EN STATUS SADE VOCP SD COE GEORET VOR SKREN RAMENDEN	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1084 1120 1268 1162 1220 1365 1263 1258 1319 1465 1363 1358
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NETDOREGNENVSERGIRPONKTHLDRPLVVEP. RNSTRESADOKVCPSDCQEGEGEREIVGPESCEAPRESHEHRDK	869 949 979 958 949 1041 1068 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1263 1162 1258 1319 1465 1363 1358
doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doo	NETDOREORIANYSERGIRADMAKTHLDRPLVVEP. RNSTRÄSKADAVCPSDCQEGEGURTVGPESCEAPRESHHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258 1319 1465 1363 1358 1419
doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB	NETDOBEORALVSSEDTREDMKTHLDRPLVVZE RINSTERSKADDRVCPSDCQEGEGEREVOPESCAPRESHHRDK	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1041 1055 1168 1084 1120 1268 1162 1220 1365 1263 1258 1319 1465 1363 1358 1419 1565
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NETDOBEOBRAMYS BUTEPONKTEIDRPLVVEP. RNSTRAKSADKVCPSDCQEGEQERIVQPESCEAPERSHRHDKLOEODKODGALDTGE VEAGAS RMALEDIGARRYRELY KEINEN KEINER SATUGLÖR KEKKE SALVCPSDCQEGEGERELYGPESCEAPERSHRHDKLOEOLCEEGESGUTQEPEMADAGG QEAELISHEFY GARESDEAL AND ALEGALER SATUGLÖR REKKEARAGUGGEGGERESSPRTGTADGEFREHRVERRUTGEGED	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1258 1319 1465 1363 1358 1419 1565 1463
doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB	NETDOBOORNALVIS HOTRPDMKTHLDRPLVVEP NUMERIKSADKVCPSDCQEGEQERIVQPESCEAPERSERREDK	869 949 979 958 949 1041 1068 1048 1048 1041 1055 1168 1049 1084 1120 1268 1162 1220 1365 1263 1258 1319 1465 1363 1358 1419 1565 1463
doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbB doe-4	NETDDEGRENTVESERUTEDENTHLDRPLVVEP. NIN STRESS ADVOCPSDCZGEGERIVOPESCEAPERSHRHRDKLGEODKADGALDTGE VEAGAS RANDEJIRARRYRSLYKENDEKONGLESSARTGEN OKALISHKEY GESCHUT GENERALE SARTGEN GRAELISHKOPY GESCHUT GENERALE SARTGEN SKALAPY GESCHUT GENERALE SARTGEN GRAELISHKOPY GESCHUT GENERALE SARTGEN GRAELISHKOPY GESCHUT GENERALE SARTGEN SKALAPY GENERALE SARTGEN GRAELISHKOPY GESCHUT GENERALE SARTGEN SKALAPY SKALAPY SKALAPY SKALAPY STALE SARTGEN SKALAPY SKALAPY SKALAPY SKALAPY STALE SARTGEN SKALAPY SKALAPY SKALAPY SKALAPY SKALAPY STALE SKALAPY SKALAP	869 949 979 958 949 1041 1068 1041 1055 1168 1089 1084 1120 1268 1163 1162 1220 1365 1258 1319 1465 1365 1358 1419 1565 1463 1458
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4	NETDOBEORENUTVSSEGTEPONKTHLDRPLVVEP. ENSTREMALAD.KVCPSDC2GEEGERUVOPESCEAP.RSHRHRDKLGEODKGDGALDTGE VEAGAS RAMDETIRARRYRSLYKERDEKGLYREGER GARTGSEGREGYNKEGELLOULGEGESGUTOD DE DODD (GAREJSROCPYGREGED GARIGELEP FORWEGELER GARAGENER GRUNNEGELLOULGEGESGUTOD DE DODD (GAREJSROCPYGREGED GARIGELEP FORWEGELER GARAGENER GRUNNEGELLOULGEGESGUTOD SKEEKEGER HUDDEN FROM FENDERSHER SHERTER FROM CONSTANT OF MOST AND THE AND	869 949 979 958 949 1041 1068 1048 1048 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1358 1319 1465 1358 1358 1458
doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-4 doe-4 doe-4 doe-4 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 doe-4 BI-2 rbB doe-4 doe	NETDOBEORIANTY SEGUEPOKTHLDRPLVVEP. EN STREKALADKVCP SDCQEGEGERENVOPESCEAPERSHRHRDKLGEODKGDGALDTGE VERGAS DRAWDEFIGARREY NELVKERADEKGALDTGE ORAELISHED YGREGOHOMALEGGEDPG WEGGALEGGALEGGALEGGALEGGALEGGEREGGEREGGER	869 949 979 958 949 1041 1068 1048 1048 1041 1055 1168 1049 1084 1120 1268 1162 1220 1365 1263 1258 1319 1465 1363 1358 1419 1565 1463 14519
doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB BI-2 rbB doe-4 BI-2 rbB doe-4 BI-2 rbBI R BI-2 rbBI R R R R R R R R R R R R R R R R R R R	NETDOBOBOM (MSGB) TAREA TRADUCTED ENTREDATED APPLAVED ENTRESON SADAK VC PSDCQEGEGERIVQDEZCEAPRASHERDAT	869 949 979 958 949 1041 1068 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1258 1319 1465 1358 1419 1565 1463 1458
doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB	NETDOBOBOM MYSEB TREPARTED DRY THE DARP LVVEP! RNSTRAKSAD KVCP SDCQEGE GERTVQPESCEAPRESHEHEDKLOGLCHE GESGCLTCHE BYDD SUDAGE VEAGS FAMDRES HEARER KREST VERBARGLEDE STEDSTER REAL AND STATE AND	869 949 979 958 949 1041 1068 1048 1041 1055 1168 1049 1084 1120 1268 1163 1162 1220 1365 1263 1358 1319 1465 1358 1419 1565 1465 1563
doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB	NETDOBOBOM WYSS BÖTKP DAKTHLDRPLVYES. RUSTRA SADAVCP SOCOLOGING REALWORKS APREHENDK	869 949 979 958 949 1041 1068 1048 1048 1041 1055 1168 1089 1084 1120 1265 1168 1162 1220 1365 1263 1158 1319 1465 1363 1358 1419 1565 1563 1558
doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-1 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB doe-4 doe-4 BI-2 rbB	NET_DOBOBANY MADE DARTAL DRPLAVED. RUSTER SADAVCP SOCOLOGICAL DESCRIPTION OF SOCAPERA RHENDER	869 949 979 958 949 1041 1068 1041 1055 1168 1041 1055 1168 1084 1120 1268 1163 1162 1220 1365 1263 1358 1319 1465 1365 1358 1419 1565 1563 1558

FIG. 2. (Figure continues on the opposite page.)

	0 0 0	
doe-1	LSFLKLFRAARLIKLLRQGITIRILLWTFVQSFKALPYVCLLIAMLFFIYAIIGMQLFGNIGLDDHTPINRHNNFHTFFNALMLLFRSATGE	1611
BI-2	LSFLRLFRAARLIKLLRQGYTIRILLWTFVQSFKALPYVCLLIAMLFFIYAIIGMQVFGNIGIDMEDEDSDEDEFQITEHNNFRTFEQALMLLFRSATGE	1765
rbB	LSFLRLFRAARLIKL@RQGYTIRILLWTFVQSFKALPYVCLLIAMLFFIYAIIGMQVFGNIÄLDD@TSINRHNNFRTFLQALMLLFRSATGE	1655
doe4	LSFLRLFRAARLIKLLRQGYTIRILLWTFVQSFKALPYVCLLIAMLFFIYAIIGMQVFGNIELDDDGAINRHNNFRTFLQAVMLLLRSATGE	1650
	IVS4 0 0 IVS5 + IVH5	
doe-1	SWQEIMLACLSGRECEGTREPSCGTDVAYFYFVSFIFLCSFLMLNLFVAVIMDNFEYLTRDSSILGPHHLDEFVRVWAEYDBAACGRIHYTDMYQML	1708
BI-2	ĨĂĦĔŊĨIMLSCLSGKPCDKNSGĪLTEECGNEFAYFYFVSFIFLCSFLMLNLFVAVIMDNFEYLTRDSSILGPHHLDETVRVWAEYDPAAMGRMLYRDMYAMI	1865
rbB	ANTEINISCLENRACOPHANASECGSDFAYFYFVSFIFLCSFLMLNLFVAVIMDNFEYLTRDSSILGPHHLDEFIRVWAEYDPAACGRISYNDMFEML	1753
doe-4	GWQEIMLACLWQSPCDARSGIDGDECGSWFAYFYFVSFIFFSSFLMLNLFVAVIMDNFEYLTRDSSILGPHHLDEFIRVWAEYDPGARGRITYNDMYEML	1750
	+ IVS6 +	
doe-1	TIMSPPLGLGKKCP <u>SK</u> VAYKRLVLMNMPVT.EDKTVHFTSTIMGLIRTALQIKULARGGADKQQLDAELRKEIMTIWPHLŠQKTLDLLVPMRTYSDLTVGK	1807
BI-2	RHMEPPLGLGKMCPARVAYKRLERMELPVA.DOMTVHFMSTLMALIRTALDIKIAKGGADKOQMDAELRKEMMAIWPNLSQKTLDLLVEPHKSTDLTVGK	1964
rbB	ĸĦMSPPLGLGKKCPARVAYKRLVRMNMPISMEDMTVHFTSTLMALIRTALEIKEAPAGTKOHOCDAELRKEISSVWANLEOKTLDLLVPP <u>HK</u> PDEMTVGK	1853
doe-4	RHMCPPLGLGKKCPARVAYKRLVRMNMPIA.EDGSVHFTSTLMALIRTALDVKISPGGAYQQQCDAELRKEITAVWPNLSQKFLDTLVPPQRASELTVGK	1849
	+	
doe-1	IYAAMMINDYYKQSKNKKYQKLQEEQSRTPNFQRMEASSLPPQII	1875
BI-2	IXAAMMIMEYYEQSKAKKUQAMEEGNRTPLMFQRMEEPPDEGGAGQNALPSTQDPAGGLMAHEDGLEDSPSWVTQRAQEMFQRTGT	2052
rbB	ŴŶĂĂĹMĨĔŊĔŸĸŶŇĸŦŦŔŊŶŦĦŊĂŀĠĊĿŚŊMĠŀŶŜĹĿ <u>ĦĔ</u> ĹŔŊŦĹĿŶŦŎŀŇVĿŔĠŔĸVŦĿŔŶĸŚŊŦŚĹŚŇĠĠĂĬŶŦ <u>ŎĔŚĠ</u> ĬĸĔŚĹŚŴĠ <u>ŦŶ</u> ŔŦŶŊŊĹĿĿĔ.ĂŔ	1952
doe-4	<u>VYAALMIYDYYKQNKSKKVQQQQDSGLSG.</u> TRKSFFQRVVGVLAATQEEPSSY <u>STS</u> HKNSVNPLYQGGRQKEFFSMLRSRDTCAE.GK	1936
	• • • • • • • • • • •	
doe-1	T <u>EI</u> VPLPPVMFQQ <u>GRT</u> SSQCEE <u>IHKQ</u> RPKELKKIKIETPHYGHYGPIENQGRAVSMPRIEIESAEDTSPIERSISTFAANHSNSTW	1961
BI-2	WSPERAPPADMADSOPKPOSVEMREMSOD. GYSDSEHCLPMEGOARAASMPRLPAEMORRRGEPRGSDLSTICDTSPMKRSASVTGP.KA.SER	2143
rbB	A <u>PLER</u> GHSAEIPVGQP <u>GAI</u> AV <u>DVQMQ</u> NMTURGPDGEPQPGLESQGRAASMPRLAAETQPAP <u>NA</u> SPMKRSISTLAP.RPHGTQ	2033
doe-4	KEVPESHPEREAGUTKSSSQAVEMREMGSDINHADQSSIENYGRAASMPRITAETO.KISRPSGRVRAPIADTSPMKRSVSTITP.Q.RSHV	2024
	· · · · · · · · · · · · · · · · ·	
doe-1	LNEYSLERAGPEDL	2010
BI-2	LDDYSLERVPPEERQRHHPRRRERAHRTSERSLGRYTDVDTGLGTDLSMTTQSGDLPSREREGERGR <u>FRD</u> RHHRPHHHHHHHHHHHHHH	2234
rbB	<u>LICNTVLORP</u> PISOVSHHHHHRCHRRDKKQRSLEKGPSLSVOTTEGAPSTAAGSGLPHGEGSTGCRRERKOERGRSQERRQPSSSSSE	2120
doe-4	MP <u>DYSLERV</u> IEVOMPH <u>HHHHHHRCHHRREKKORSLER</u> ATINRHADEEAGOLDAOLRD	2101
doe-1	ERSVCSTGQCAHESQHRGLDORLSRSPSPGYSHREPREQVNSSVSESEVPSSSGTS.EPKQGQRQLPQTPSKERPLUSYSPVAQRGDVSGHCSEMC	2104
BI-2	KERYGPOR PDHGHGRARAROORWSRSPSEGREHTTHRO	2273
rbB	KQREYSCDRFGSREPPQPKPSISSHPISPTAALEEPGPHPQGSGSVNGSPLMSTSGASTPGRGGRRQLPQTPLTPRPSUTYKTANSSPVHFAEGQSGLPAF	2220
doe-4	<u>KORYYSCDRYCSREPPOPR</u> STDHBREASPSTGTEOGFHROGSGSVNDSPLQBASGSSTPSR. <u>GRROLPRTPLTPRPSVTYKTANSSPAHFG</u> NLHDALPPS	2200
doe-1	ĸĔŦŖŶQSĹŔŶQPŚĸAĽWŚĿĘŚPĠŖŚŔĔſŚĿĔŚQHŚŦPĹŖŶĬĠĿĿŖŶĬĿĿĔŎĠĿĹĦŊĠP <u>ĠŚ</u> ĿĹŊŎĂĹĠ <u>ĔĔŦĹŦŦĔŎĂŶŎ</u> ŦŚĹĠŖſŚĦŦĬ <u>ŚŚ</u> ĂPPĹŔQĠŴĦĹŶŇĠŚ <u>Ŷ</u>	2198
BI-2		
rbB	SPGRLSRGLSEHNALLQREPLSGPLASGSRIGSDPYLGQRLDSEASAHNLPEDTLTFEEAVATNSGRSGRTSYVSSLTSQSHPLRRVPNGY	2311
doe-4	<u>ISPGRLSRGQSEHNHUL</u> SGESQNRPYAGGDSRQPMGTRLS <u>SDPYLG</u> FBSSCGSEDLELLEETLTFEVAVAATUA <u>TGRS</u> PRT <u>SS</u> FUTQPPQS <u>RRVPNGY</u>	2297
4		
a0e-1	KTRARUSSAFSTPUPUTHAREDDRU 2223	
B1-2		
TDB		
008-4	II CHILLING TO F STARS AND A TIKE THEN ING 2320	

FIG. 2. Alignment of the deduced amino acid sequences of doe-1 and doe-4 with representative mammalian clones of the non-L subfamily [rbB (9) and BI-2, the longer variant of the BI type (5)]. Dots represent gaps introduced by the BESTFIT program (GCG) to optimize alignment. When two or more residues at a position are identical, they are boxed. If two pairs of boxes are possible at a single position, the pair is boxed that includes the doe-4 sequence. The predicted positions of transmembrane (S1–S6) and pore-forming (H5) regions in each of the homologous repeats (I–IV) are indicated by brackets below. The position of an insert encountered in some doe-4 clones is indicated by a solid triangle. Predicted glycosylation sites in either doe-1 or doe-4 are indicated by open triangles. Potential sites for phosphorylation are marked by solid circles (cAMP-dependent protein kinase), open circles (protein kinase C), and plus marks (multifunctional $Ca^{2+}/calmodulin-dependent protein$ kinase).

nate method and selection on oligo(dT) resin (17). Poly(A)⁺ RNA (3 μ g) from each tissue was electrophoresed through a 1% agarose/0.8% formaldehyde gel and transferred to a nylon membrane for hybridization (17).

RESULTS AND DISCUSSION

Molecular Cloning. We used PCR to amplify candidate Ca²⁺-channel cDNA sequences from a cDNA library derived from the electric lobe of D. ommata. Degenerate primers were synthesized based on amino acid sequences, IGMQ-(V/M)FG and ATGEAW(Q/H), flanking 29 as in the region between transmembrane domains IVS5 and IVS6 (Fig. 1). To identify putative Ca²⁺-channel sequences, the PCR products were probed with an oligonucleotide based on a sequence, INR(N/H)NNF, within the predicted amplified region. DNA sequencing of positive clones yielded two distinct 128-bp fragments [D. ommata-1 (doe-1) and doe-2]. Sequence alignment indicates that doe-2 is as homologous to Ca²⁺ channels from skeletal muscle or heart as these L-type channels are to each other. Within this limited region of 29 aa, the doe-2 fragment differs from the skeletal muscle sequence at only 3 aa positions (Fig. 1).

In contrast, the doe-1 fragment is less closely related to L-type channels and much more similar to a Ca^{2+} -channel clone from rat brain (rbB; ref. 11). Starting with the doe-1

PCR fragment, we isolated overlapping cDNAs encoding the complete open reading frame (Fig. 2). The screening procedure also led to isolation of a distinct class of cDNA encoding doe-4, an additional putative Ca^{2+} channel. The doe-1 sequence consists of an open reading frame of 6669 bp, encoding a protein of 2223 aa with a calculated molecular mass of 251.8 kDa. doe-4 consists of an open reading frame of 6978 bp, corresponding to a protein of 2326 aa and a calculated molecular mass of 264.5 kDa. Like other Ca^{2+} -channel α_1 subunits (3), doe-1 and doe-4 consist of four largely hydrophobic repeats (I–IV), separated by largely hydrophilic linkers. Each repeat includes an H5 region, which is thought to line the conduction path, and six putative transmembrane segments (S1–S6).

In analyzing doe-4 cDNA, we repeatedly encountered a variant of the loop that joins the first two homology domains, a region that is otherwise well-conserved among this family of channels. This variation is likely to arise from alternative splicing and results in the insertion of a 20-aa sequence (DGLGIIYEPEQKPEDIQSVY) at a position marked in Fig. 2. Potential sites for phosphorylation by protein kinases are also marked. Because Ca^{2+} channels undergo physiological modulations by several second messenger systems (20, 21), these sites are potentially significant.

Distribution of mRNAs. Northern blot analysis of the tissue distribution of doe-1 and doe-4 mRNAs is illustrated in Fig.



FIG. 3. Differential expression of doe-1 and doe-4 transcripts in the central nervous system of *D. ommata.* Poly(A)⁺ RNA (3 μ g per lane) from the forebrain (lanes FB) or electric lobe (lanes EL) were probed with ³²P-labeled cDNA from regions likely to be specific for the individual gene (the 5' untranslated region of doe-1 or the cytoplasmic linker between repeats II and III of doe-4). Size standards from an RNA ladder (BRL) are indicated at the left.

3. doe-1, an 8-kb transcript, is much more abundant in forebrain than in the electric lobe; in contrast, doe-4, a 12- to 13-kb transcript, is more plentiful in electric lobe than in forebrain. Since the electric lobe consists largely of the electromotor nucleus which innervates the electric organ, this suggests that doe-4 may play an important role in regulating transmitter release from motor nerve terminals. Consistent with this hypothesis, neurotransmitter release in the electric organ is blocked by ω -conotoxin GVIA (16), which also blocks the human homolog of clone rbB (10).

Relationships to Previously Described Ca²⁺-Channel Structures. Fig. 4 provides an overview of structural homologies between doe-1 and doe-4 and mammalian Ca²⁺-channel cDNAs (see below). The tree diagram is based on sequence homology, but it also appears representative of an emerging pattern of functional differences. The upper branch consists of three α_1 genes designated as L₁, L₂, and L₃ (1, 12). To date, the Ca²⁺-channel activity expressed from members of this group has displayed responses to DHP antagonists and agonists and other functional characteristics expected for L-type Ca²⁺ channels. Individual members of this L subfamily display at least 60–70% amino acid identity with each other.

Members of the second subfamily show only $\approx 40\%$ amino acid identity with members of the L subfamily but >60%homology with each other. They are functionally distinct from the L subfamily in several respects (1). Most notably, they encode high-voltage-activated Ca²⁺ channels that are not inhibited by DHP antagonists or stimulated by DHP agonists (5, 10). By grouping the nearly identical genes from different species, three types of mammalian channels can be distinguished within this subfamily. They are represented by clones BI (rbA, CaCh4) (5, 6, 11, 12) and BII (22), which were isolated from rabbit brain, and rbB (CaCh5) (9-12), which was cloned from rat brain. The BI cDNA encodes an α_1 subunit with some key features expected for P-type Ca²⁺ channels, which are highly expressed in cerebellar Purkinje cells and certain nerve terminals (5). The rbB cDNA encodes an ω-conotoxin-sensitive, DHP-resistant activity character-



% identity

FIG. 4. Structural homologies of the mammalian and marine ray Ca^{2+} -channel α_1 subunits. Percent sequence identity, as determined by the BESTFIT program using the full amino acid sequence, is indicated. The subfamily of L-type channels is represented by L₁ from rabbit skeletal muscle, L₂ from rabbit cardiac muscle, and L₃ from human brain. doe-2 is not included because a comparable percent identity could not be determined from the limited sequence available. The subfamily of non-L channels includes doe-4, rbB (an N-type channel from rat brain; ref. 9), and BI (a rabbit cerebellar channel with some similarity to the P-type channel; ref. 5) and the more distantly related doe-1. In the nomenclature for mammalian brain channels of Snutch *et al.* (11), channels like L₂ are class C, those like L₃ are class D, those like BI are class A, and those like rbB are class B.

istic of an N-type Ca^{2+} channel (9, 10). The recently reported BII channel has not been physiologically characterized (22).

doe-1 and doe-4 belong to the non-L subfamily. The degree of homology to BI or rbB clones ranges between 60 and 72% identity; in contrast, the homology between doe-1 or doe-4 and various L-type Ca²⁺ channels is <45%. Some distinguishing structural features now emerge from a comparison of the L and non-L subfamilies. In contrast to all known L-type channels, for example, doe-1, doe-4, and BI or rbB cDNAs encode proteins containing an extra positive charge in repeat III (Fig. 5A) and a different pattern of spacing of charged residues in repeat IV (Fig. 5B). Similar considerations hold for the cytoplasmic loop between repeats III and IV (Fig. 5C), a segment thought to be important for inactivation (23, 24). doe-1, doe-4, BI, and rbB are highly homologous along this stretch but display significant differences with respect to L-type channel cDNAs.

From sequence analysis, we suggest that doe-1 and BII, which are 68% identical, represent a distinct branch of the non-L subfamily. The deduced amino acid sequence of doe-1 shows somewhat less identity to BI and rbB than they do to each other. The overall identities are 60% for doe-1 and rbB, 63% for doe-1 and BI, and 68% for BI and rbB. The most striking sequence divergence between doe-1 and the other branch of the non-L subfamily occurs in the cytosolic loop between repeats II and III.

doe-4 can more easily be grouped with BI and rbB than with doe-1 and BII (68, 72, 61, and 61% identities, respectively). In particular, where the subfamily shows the greatest divergence, some regions of doe-4 appear especially similar to rbB. This is most striking in the C termini of the channels: rbB and doe-4 extend, respectively, 174 and 185 aa beyond the end of BI and are identical to each other at 110 of these residues. Similar comparison can be made in the cytoplasmic linker between domains II and III.

CONCLUSIONS

From a comparison of sequences, the doe-1 channel reported here and the mammalian BII clone (22) can be recognized as

A	doe-1	IKSLRVLRVLRPLKTIKRL	В	doe-1	LSFLELFRAARLIKLLRDG		
	doe-4	IKSLRVLRVLRPLKTIKRL	-	doe-4	LSFLRLFRAARLIKLLRQG		
	B1	IKSLRVLRVLRPLKTIKRL		B1	LSFLRLFRAARLIKLLRQG		
	rbB	IKSLRVLRVLRPLRTIKRL		rbB	LSFLRLFRAARLIKLCRQG		
	L ₂	VKILRVLRVLRPLRAINRA		L ₂	ITFFRLFRVMRLVKLLSRG		
	L ₁	VKILRVLRVLRPLRAINRA		L ₁	SAFFRLFRVMRLIKLLSRA		
C	d 1						
C	doe-1	I IF QEQGUAMLEESSLEANERA	CIDEAL	SARPLIF	TWEGNEGLEGIKAMÖLAASE		
	doe-4	ITFQEQGDKVMSDCSLEKNERACIDFAISAKPLTRYMPQNKQTFQYKMWKFVVSP					
	B1	ITFQEQGDKMMEEYSLEKNERA	CIDFAI	SAKPLTR	HMPQNKQSFQYRMWQFVVSP		
	rbB	ITFQEQGDKVMSECSLEKNERA	CIDFAI	SAKPLTE	YMPONKOSFOYKTWTFVVSP		

- ^L2 L1 VTFQEQGEQEYKNCELDKNQRQCVEYALKARPLRRYIP--KNQHQYKVWYVVNST
 - VTFQEQGETEYKNCELDKNQRQCVQYALKARPLRCYIP--KNPYQYQVWYVVTSS

FIG. 5. Comparison of motifs between representatives of the L and non-L subfamilies. (A) IIIS4. (B) IVS4. (C) III/IV linker. (A and B) The positively charged repeating residues have been shaded to highlight the additional charge in IIIS4 and the shifted spacing in IVS4 that appear to distinguish members of the non-L family from the L family. (C) The sequence of doe-1 and any matches to its sequence have been shaded to illustrate the structural similarities of the non-L family that diverge from the L-type channels.

a distinct branch of the non-L subfamily of Ca^{2+} channels. We have also cloned a Ca^{2+} channel (doe-4) that is likely to play a role in transmitter release from terminals in the electric organ and is likely to correspond to the ω -conotoxin binding protein that we (25) and others (16) have studied. The electric organ channel is of special interest because of the high density of synapses in this tissue. The sequence of a channel that may be a component of the presynaptic release site in this organ (16) will be a useful tool in the purification of presynaptic plasma membranes and for elucidating the role of the Ca^{2+} channels in vesicle docking and fusion (26).

Analysis of the marine ray Ca²⁺ channels has provided some evolutionary perspectives on Ca²⁺-channel diversity. Marine rays are cartilaginous fish and diverged from animals with bony skeletons $>4 \times 10^8$ years ago. doe-2 represents a Ca²⁺ channel homologous to members of the mammalian L-type subfamily, whereas doe-1 and doe-4 fit within the non-L subfamily. Evidently, L-type and non-L-type subfamilies separated before the divergence between cartilaginous and higher vertebrates, hundreds of millions of years ago. Interestingly, homology analysis of Na⁺ channels points toward the opposite relationship; the electric-eel Na⁺ channel appears to have branched away from the mammalian genes before the major division of mammalian Na⁺ channels into muscle types and neuronal types (27). Among K⁺ channels, divisions into subgroups appear to be very ancient; classes have been conserved between Drosophila and mammals (28). Physiologic studies indicate that the division of Ca^{2+} channels may also be that old; both DHP-sensitive and DHP-insensitive Ca^{2+} channels have been described in Aplysia and Drosophila (29, 30).

Sequence comparisons of doe-1, doe-4, and mammalian clones have provided information regarding the structural properties of voltage-gated Ca²⁺ channels. These comparisons highlight structural domains, both conserved and divergent, that could play key roles in providing functional diversity among Ca²⁺-channel subtypes. For example, the presence of an extra positive charge in the III S4 region of non-L type channels may be related to findings of a steeper voltage dependence of activation of expressed channels. By analogy to excitation-contraction coupling in skeletal muscle, which depends critically on the II-III loop (31), variability in the II-III loop and the C-terminal tail of neuronal structures might allow the various Ca²⁺ channels to play distinct roles in excitation-secretion coupling.

- 1. Tsien, R. W., Ellinor, P. T. & Horne, W. A. (1991) Trends Pharmacol. Sci. 12, 349-354.
- Snutch, T. P. & Reiner, P. B. (1992) Curr. Opin. Neurobiol. 2, 247-253. Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., 3.
- Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. & Numa, S. (1987) Nature (London) 328, 313-318. Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, 4.
- H., Narumiya, S. & Numa, S. (1989) Nature (London) 340, 230-233. 5.
- Mori, Y., Friedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuichi, T., Mikoshiba, K., Imoto, K., Tanabe, T. & Numa, S. (1991) Nature (London) 350, 398-402. Starr, T. V. B., Prystay, W. & Snutch, T. P. (1991) Proc. Natl. Acad.
- 6. Sci. USA 88, 5621-5625.
- Williams, M. E., Feldman, D. H., McCue, A. F., Brenner, R., Veli-celebi, G., Ellis, S. B. & Harpold, M. M. (1992) *Neuron* 8, 71–84. Hui, A., Ellinor, P. T., Krizanova, O., Wang, J.-J., Diebold, R. J. & 7.
- 8 Schwartz, A. (1991) Neuron 7, 35–44. Dubel, S. J., Starr, T. V. P., Hell, J., Ahlijanian, M. K., Enyeart, J. J.,
- Catterall, W. A. & Snutch, T. P. (1992) Proc. Natl. Acad. Sci. USA 89, 5058-5062
- Williams, M. E., Brust, P. F., Feldman, D. H., Patthi, S., Simerson, S., 10. Maroufi, A., McCue, A. F., Velicelebi, G. & Ellis, S. B. (1992) Science 257, 389-395.
- Snutch, T. P., Leonard, J. P., Gilbert, M. M., Lester, H. A. & Davidson, N. (1990) Proc. Natl. Acad. Sci. USA 87, 3391-3395.
- Perez-Reyes, E., Wei, X., Castellano, A. & Birnbaumer, L. (1990) J. 12. Biol. Chem. 265, 20430-20436.
- Grabner, M., Friedrich, K., Knaus, H.-G., Striessnig, J., Scheffauer, F., 13. Staudinger, R., Koch, W. J., Schwartz, A. & Glossmann, H. (1991) Proc. Natl. Acad. Sci. USA 88, 727-731.
- Koch, W. J., Ellinor, P. T. & Schwartz, A. (1990) J. Biol. Chem. 265, 14. 17786-17791
- 15. Biel, M., Ruth, P., Bosse, E., Hullin, R., Stuhmer, W., Flockerzi, V. & Hofmann, F. (1990) FEBS Lett. 269, 409-412.
- Ahmad, S. N. & Miljanich, G. (1988) Brain Res. 453, 247-256.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A 17
- Laboratory Manual (Cold Spring Harbor Lab., Plainview, NY). 18. Sanger, F., Nicklen, F. & Coulson, A. R. (1977) Proc. Natl. Acad. Sci.
- USA 74, 5463-5467. Devereaux, J., Haeberli, P. & Smithies, O. (1984) Nucleic Acids Res. 12, 19. 387-395.
- Bean, B. P. (1989) Annu. Rev. Physiol. 51, 367-384. 20.
- 21. Anwyl, R. (1991) Brain Res. Rev. 16, 265-281.
- 22 Niidome, T., Kim, M.-S., Friedrich, T. & Mori, Y. (1992) FEBS Lett. 308, 7-13.
- 23. Stuhmer, W., Conti, F., Suzuki, H., Wang, X., Noda, M., Yahagi, N., Kubo, H. & Numa, S. (1989) Nature (London) 339, 597-603
- Vassilev, P., Scheuer, T. & Catterall, W. A. (1988) Science 241, 1658-24. 1661.
- 25. Horne, W. A., Hawrot, E. & Tsien, R. W. (1991) J. Biol. Chem. 266, 13719-13725.
- 26. Bennett, M. K., Calakos, N. & Scheller, R. H. (1992) Science 257, 255-259
- 27. Strong, M., Chandy, K. G. & Gutman, G. A. (1993) Mol. Biol. Evol. 10, 221-242.
- Salkoff, L., Baker, K., Butler, A., Covarrubias, M., Pak, M. D. & Wei, 28. A. (1992) Trends Neurosci. 15, 161–166.
- 29. Edmonds, B., Klein, M., Dale, N. & Kandel, E. R. (1990) Science 250, 1142-1147
- Pelzer, S., Barhanin, J., Pauron, D., Trautwein, W., Lazdunski, M. & 30. Pelzer, D. (1989) EMBO J. 8, 2365-2371.
- 31. Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T. & Numa, S. (1990) Nature (London) 346, 567-569.

We thank Elvse Jung and Jackie Phillips for technical assistance. This work was supported by National Institutes of Health Grants GM42376 (T.L.S.) and NS24067 (R.W.T.) and gifts from Miles, Parke-Davis, Wyeth-Ayerst, and Eli Lilly (R.W.T.).