The Three Human Syntrophin Genes Are Expressed in Diverse Tissues, Have Distinct Chromosomal Locations, and Each Bind to Dystrophin and Its Relatives*

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(Received for publication, April 21, 1995)

The syntrophins are a biochemically heterogeneous group of 58-kDa intracellular membrane-associated dystrophin-binding proteins. We have cloned and characterized human acidic (α1-) syntrophin and a second isoform of human basic (β2-) syntrophin. Comparison of the deduced amino acid sequence of these two isoforms of syntrophin (together with the previously reported human β1-syntrophin) demonstrates their overall similarity. The deduced amino acid sequences of human α1- and β2-syntrophin are nearly identical to their homologues in mouse, suggesting a strong functional conservation among the individual isoforms. Much like β1-syntrophin, human β2-syntrophin has multiple transcript classes and is expressed widely, although in a distinct pattern of relative abundance. In contrast, human α1-syntrophin is most abundant in heart and skeletal muscle, and less so in other tissues. Somatic cell hybrids and fluorescent in situ hybridization were both used to determine their chromosomal locations: β2-syntrophin to chromosome 16q22-23 and α1-syntrophin to chromosome 20q11.2. Finally, we used in vitro translated proteins in an immunoprecipitation assay to show that, like β1-syntrophin, both β2- and α1-syntrophin interact with peptides encoding the syntrophin-binding region of dystrophin, utrophin/dystrophin related protein, and the Torpedo 87K protein.

Dystrophin, the protein product of the Duchenne muscular dystrophy locus, is a large membrane-associated cytoskeletal protein (1). In order to understand the function of this protein in skeletal muscle, it is important to establish the molecular organization of dystrophin in the context of the membrane cytoskeleton. Dystrophin copurifies with a group of integral membrane glycoproteins and membrane-associated proteins called the dystrophin glycoprotein complex (2–4). A number of these proteins have been further defined by their primary sequence and their biochemical properties.

A 58-kDa cytoplasmic peripheral membrane protein was independently identified in the Torpedo electric organ, and shown to localize to the postsynaptic neuromuscular junction in mammals (5). This 58-kDa synaptic protein also copurifies with dystrophin and is now known as syntrophin (6–9). Dystrophin-associated syntrophin isolated from rabbit skeletal muscle is heterogeneous; it appears as a triplet by one-dimensional SDS-electrophoresis, and when separated by two-dimensional gel electrophoresis, appears as two clusters of 58-kDa proteins with different isoelectric points (pl), one which is slightly acidic (α, pl = 6.4) and the other which is quite basic (β, pl = 9) (10). Phosphatase pretreatment of the isolated microsomes results in some signal consolidation (10), and phosphoamino acid analysis of syntrophin isolated from Torpedo electric organ shows that serine and tyrosine residues are phosphorylated (11).

The isolation of two distinct isoforms of syntrophin in mouse (9), and antibodies to a single cloned isoform of rabbit syntrophin (12), confirmed the biochemical evidence that there are at least two distinct genes. Based upon partial peptide sequences from purified rabbit muscle syntrophin, we independently isolated human β1-syntrophin cDNA which was also used to identify a distinct but related human muscle expressed sequence tag (EST)1, EST25263 (13). The deduced amino acid sequence of this human EST fragment was nearly identical to a portion of mouse β2-syntrophin (9). From all the available sequences, we proposed that there are at least three syntrophin genes in the mammalian genome. From their predicted amino acid sequences and their calculated pl values, the acidic isoform was named α1-syntrophin, and the two basic isoforms β1-syntrophin and β2-syntrophin (see Table I).

The widely expressed C-terminal product of the DMD gene, dystrophin protein of 71 kDa (Dp71), the dystrophin related protein (DRP or utrophin), and the 87K relative of dystrophin also copurify with syntrophin when isolated by immunoaffinity techniques (6, 8, 14, 15). The suggestion that dystrophin interacts with syntrophin via its C terminus was independently determined by blot overlay of dystrophin onto isolated syntrophin (16, 17). Recombinantly produced β1-syntrophin interacts with a small region within the C terminus of dystrophin, revealing a strong binding site within exon 74 of dystrophin (18). This result was independently determined by using bacterially expressed or in vitro translated portions of dystrophin to overlay onto purified syntrophin bound to a solid support and to show that syntrophin may have yet another binding site in a more distal region on dystrophin (19, 20). In addition, β1-syntrophin was shown to also interact with the homologous

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regions of utrophin/DRP and the Torpedo 87K protein (18).

Despite their mRNA expression in a wide variety of tissues (9, 12, 13), the syntrophin isoforms appear to have a remarkable specificity in their submembranous localization in muscle. Isoform-specific antibodies discriminate the localization of α1-syntrophin, which is expressed throughout the sarclemma, from β2-syntrophin, which localizes specifically to the neuro-muscular junction (NMJ) (21). The determinants of the isoform-specific localization are presumably due to structural differences between these isoforms, but these are yet to be determined.

Gibson and colleagues have noted that the syntrophins contain two pleckstrin homology (PH) domains (22), a small ~100-residue domain originally found as an internally duplicated motif in pleckstrin, the major substrate of protein kinase C in erythrocytes (23, 24). This domain has captured wider attention because it is also found in a number of other intracellular signaling and cytoskeletal proteins, such as β-spectrin, phospholipase Cγ, the β-adrenergic receptor kinase, a number of GTPases, and GTPase-activating proteins, many of which are membrane-associated (22). The deduced three-dimensional structure of the N-terminal pleckstrin domain and the PH region of β-spectrin has been determined (25, 26). A hydrophobic lip of the pleckstrin β-barrel has been shown to bind to phosphatidylinositol 4,5-bisphosphate, which may explain how many PH-containing proteins are associated with the membrane without containing classical membrane-anchoring groups (27).

Adams and colleagues (28) have noted the homology between a conserved region in the middle of the syntrophin genes and a number of other membrane-associated proteins, the PDZ domain, named for the Putative synaptic density protein-95 (P5D-95, 29)), the Drosophila discs large tumor suppressor protein (30), and the Zonula occludens-1 protein (ZO-1 (31)). Since this motif is shared among these intracellular peripheral membrane proteins, it may also be the basis by which the syntrophins interact with another component of the membrane or membrane cytoskeleton.

We report here the cloning and characterization of human β2-syntrophin and α1-syntrophin. By comparing the amino acid sequences of human β1-, β2-, and α1-syntrophin, we have identified the C-terminal 57 amino acids of syntrophin as a conserved syntrophin-unique domain. The mRNA of β2-syntrophin is expressed in a wide variety of tissues, whereas α1-syntrophin is predominantly expressed in striated muscle. The human chromosomal sublocalization of β2-syntrophin is 16q23–24, and that of α1-syntrophin is 20q11.2. We have also verified the functional conservation of β1-, β2-, and α1-syntrophin in their interaction with dystrophin and its relatives in an in vitro binding assay.

MATERIALS AND METHODS

PCR Cloning—Oligonucleotides were synthesized by phosphoramidite chemistry (ABI, Foster City, CA). PCR primers to amplify β2-syntrophin, designed from the sequence of EST25263, were reported previously (33): 5'-TGG TAC AGG TCA AGT TCA TC-3' and 5'-TGG TGT TCT GGT GTT TT-3'. For α1-syntrophin, the oligonucleotide pair 5'-TGG GAT CCA GGA GAA GA-3', amplified a reverse-transcribed cDNA template from human adult muscle, using 100 ng of template DNA and 100 pmol Taq enzyme (U. S. Biochemical Corp.) or an ABI automated sequencer with Taq DNA polymerase (Perkin-Elmer). Sequences were analyzed and aligned using the GCG software suite (Wisconsin University) in their default settings. Gaps determined pairwise identity scores, PileUp produced multiple alignments (Figs. 1–3), and ProteinStructure made local secondary structure predictions.

Isolation of Genomic DNA Clones—Genomic clones were isolated from an EMBL3 human genomic DNA library (34). The radiolabeled β2-syntrophin cDNA insert 19-1 hybridized to the 4 phages E3.2, E16.1, E19.3, and E4.1. Genomic clones of α1-syntrophin were obtained similarly with the PCR probe described above, yielding the 8 clones E3.1, E15.1, E18.1, E19.2, E20.1, E9.1, E11.1, and E19.1. The DNA from these phages was isolated and mapped by restriction digest and electrophoresis to show that the clones within each group were related to each other (data not shown).

The mRNA Expression of the Syntrophins—A panel of poly(A)+ RNA from human tissues blotted onto positively charged membrane (Clontech) was hybridized to PCR probes using standard conditions of high stringency (32). For β2-syntrophin, the primer pair 5'-GGA AAA CAG GCT ACA TTC-3' and 5'-AGA GGT GCT GTG GAA CC-3' amplified a 401-bp fragment from the open reading frame of clone 19-1. In addition, the gel-purified insert of clone 19-1 was radiolabeled (OLB, Boehringer) and used in a separate hybridization of an identical blot. For α1-syntrophin, the primer pair described above in “PCR cloning” was used to amplify the 250-bp open reading frame region of α1-syntrophin from the subcloned PCR fragment.

Somatic Cell Hybrid Mapping of α1-Syntrophin—Based upon the sequence of the PCR product for α1-syntrophin, another pair of internal primers was designed to amplify the human sequence specifically, so that only the human sequence would be amplified from human-rodent somatic cell hybrids. The DNAs from the NIGMS monochromosomal hybrid collection (35, 36) were amplified in pools as described by Backes and colleagues (35). PCR using the primer pair 5'-GGA AAA CAG GCT ACA TTC-3' and 5'-GAG GGC CGT GTG GAA CC-3' was performed in a 50-μl reaction volume with 200 ng of genomic DNA, 50 pmol of each primer, and 2.5 units of Taq DNA polymerase. The annealing, elongation, and denaturation cycles were the same as for the PCR cloning of α1-syntrophin. The 78-bp reaction product was separated by 7% polyacrylamide gel electrophoresis and detected with ethidium bromide.

FISH Localization of Syntrophins—FISH analyses of human β2-syntrophin and α1-syntrophin were performed essentially as by Lichter and colleagues (37, 38). The β2-syntrophin clones E4.1 and E16.1 and the α1-syntrophin clones E3.1 and E19.2 were labeled with biotin-dUTP and hybridized to phytohemagglutinin-treated lymphocytes dropped onto glass slides and 4,6-diamidino-2-phenylindole (DAPI) counterstained. The avidin fluoroscein signal and their DAPI counterstain were imaged by a Zeiss axiopt microscope, acquired digitally by CCD camera, and the images merged using IPLabs software.

Immunoprecipitation of In Vitro Translated Syntrophin, Dystrophin, DRP, and Torpedo 87K Protein—The translation of the syntrophin binding site of dystrophin, DRP, and 87K protein, and their use in the co-precipitation of β1-syntrophin was described in detail previously (18). The dystrophin peptides C2979 and Dp71D110, the utrophin/DRP peptide TDR3, and the full-length 87K peptide T87 were translated in E. coli using their respective expression vectors without the presence of [35S]methionine; we also translated the exon 74 containing region of dystrophin or its homologous region of DRP or the Torpedo 87K protein as fusion proteins with the FLAG octapeptide (IBI/Kodak, New Haven,
Cloning of Human α-1-Syntrophin—Based upon the published sequence of α1-syntrophin in mouse and rabbit, a pair of PCR primers (see "Materials and Methods") were designed to amplify a conserved region of the cDNA from a human muscle cDNA template. This PCR product was subcloned into plasmid, sequenced to confirm the specificity of the reaction, and then used as a template to screen a human left ventricle cDNA library. The six resulting clones yielded five partial cDNAs and a single full-length cDNA. The full-length cDNA, LV31-1, is 2136 bp long and encodes a single large open reading frame. The cDNA clone LV6-2 spans a 3' portion of the open reading frame (Fig. 2A; GenBank accession no. U40571).

The deduced peptide is 505 amino acids in length, predicted to be a molecular mass of 54 kDa, and have a pl of 6.4 (Fig. 2B). The open reading frame begins with the first ATG start codon in the cDNA, which is in a favorable context for the initiation of translation, and is flanked at the 3' end with a polyadenylation signal at the appropriate distance from a poly(A) tail. At the amino acid level, this human isoform is 94% identical to the published mouse sequence and 93% identical to the published rabbit sequence. All three sequences contain homologous start codons. In comparison to rabbit and human, the mouse cDNA bears an internal deletion of 6 amino acids near its N terminus (GAPREQ). The 4-amino acid internal insertion in mouse (SSAIE) is considered to represent a rare splicing event to a nearby splice acceptor (28).

Comparison of the Three Human Syntrophins—The deduced amino acid sequence of human β2-syntrophin is 57% identical to human β1-syntrophin. The human α1-syntrophin peptide sequence is 54 and 50% identical to human β1- and β2-syntrophin, respectively. Our alignment of three human syntrophin isoforms indicates the two tandem pleckstrin homology domains as aligned in Ref. 22 and represents them schematically as well (Fig. 3, A and B). The first PH domain is split into two...
regions (PH1a and PH1b) by a large region of variable sequences flanking a core of close homology among the three syntrophins. This core of high homology, the PDZ domain, is aligned as by Adams and colleagues (28) (Fig. 3 A, black box), and is predicted to have mainly \( \alpha \)-helical secondary structure by Chou-Fasman and Garnier-Osguthorpe-Robson analyses (see "Materials and Methods").

The C-terminal 57 amino acids (Fig. 3 in gray) also forms a region of strong homology among the three human syntrophins, but does not have homology to other characterized proteins. This 57-amino acid sequence has been labeled the syntrophin-unique domain, and is predicted by Chou-Fasman and Garnier-Osguthorpe-Robson analysis to consist of from three to five strands of \( \beta \)-sheet separated by as many turns (see "Materials and Methods").

Tissue Expression of the Syntrophin mRNA—Hybridization to Northern blots of a series of human tissues reveal that each of the syntrophins are expressed distinctly (Fig. 4). We have previously reported the expression of \( \beta \)-1-syntrophin (13), but show it here for comparison (Fig. 4 A). The Northern analysis of \( \beta \)-2-syntrophin shows that it also has a wide ranging pattern of expression, but that it is expressed in relatively low but evenly levels throughout all tissues (Fig. 4 B). It also has at least three distinct transcript classes, of 10, 5, and 2 kilobase pairs. The
diverse distribution of distinct transcript classes is also observed in the mRNA expression of mouse β2-syntrophin as well (9). Neither our PCR amplifications nor our cDNA clones reflect any differences in the open reading frame that would account for this size heterogeneity.

A similar hybridization with α1-syntrophin probe reveals a distinct pattern of expression from that of the β-syntrophins (Fig. 4C). A single 2.5-kilobase pair transcript is expressed in relatively high levels in both skeletal muscle and heart, with some low level expression in brain, pancreas, liver, kidney, and lung, and none detected in placenta.

Chromosomal Localization of Syntrophins—Using the same NIGMS somatic cell hybrids that were used to map β1- and β2-syntrophin (13), we determined the chromosomal location of α1-syntrophin. Using a specific pair of oligonucleotides (see “Materials and Methods”), a 780-bp PCR product was amplified in pools of human-rodent somatic cell hybrids containing chromosome 20 (not shown). DNA from the cell lines was then used individually to further confirm that this PCR product uniquely amplifies from chromosome 20-derived cell lines, and not from cell lines containing other members of the pool or either of the other two syntrophin genes, chromosomes 8 or 16 (Fig. 5). The 78-bp product is amplified in human genomic DNA, but not in DNA isolated from rat or hamster cell lines. Furthermore, two independent somatic cell lines, each containing human chromosome 20, amplify the specific PCR product.

Human genomic clones of both β2-syntrophin and α1-syntrophin isolated from an EMBL3 human genomic library were used for FISH analysis to independently confirm the mapping panel results (see “Materials and Methods”). The β2-syntrophin signal localized to the region between 16q23 and 16q24 (Fig. 6A), and α1-syntrophin uniquely localized a signal to 20q11.2 (Fig. 6B). No secondary hybridization signals were consistently seen to suggest other closely related loci elsewhere in the genome.

Translated Syntrophins Bind to Translated Dystrophin, DRP, and 87K Proteins—The C-terminal two-thirds of β1-syntrophin can be translated in vitro and can bind to translated...
portions of dystrophin and the homologous regions of utrophin/DRP and the entire Torpedo 87K protein (18). We translated similar portions of β2-syntrophin and α1-syntrophin in the presence of [3H]leucine and combined these peptides with translated portions of dystrophin, utrophin/DRP, and the 87K protein (see "Materials and Methods"). When the dystrophin, DRP, and 87K peptides are combined with translated β2-syntrophin or α1-syntrophin peptides, anti-dystrophin, anti-utrophin, and anti-87K antibody can precipitate their respective complexes (Fig. 7A). As was shown previously with β1-syntrophin (18), dystrophin lacking the alternatively spliced region encoded on exons 71–74 fails to precipitate β2- or α1-syntrophin (Fig. 7A, lanes 4 and 9) over levels in which no dystrophin was added (Fig. 7A, lanes 5 and 10).

The syntrophin proteins used in this assay consistently showed a low level rate of aggregation that is also seen in the control lanes (Fig. 7, A, lanes 4, 5, 9, and 10, B, lanes 4 and 8). To address this issue, the exon 74 homologous regions of dystrophin, utrophin, and 87K protein were produced as FLAG fusion protein.

FIG. 7. β2- and α1-syntrophin interact with dystrophin and its relatives. A, translated peptides of dystrophin (dys), utrophin/DRP (drp), and the Torpedo 87K protein (87K) are used to coprecipitate β2-syntrophin (lanes 1–5) and α1-syntrophin (lanes 6–10) peptides. The syntrophin peptides were also combined with Dp71A110 (18), which lacks the syntrophin binding region (lanes 4 and 9), or with d11 antibody alone (lanes 5 and 10). B, FLAG fusion proteins of the syntrophin binding domains of dystrophin (1 and 5), utrophin/DRP (2 and 6), and the Torpedo 87K protein (3 and 7) are used to coprecipitate translated partial cDNAs of β2-syntrophin and α1-syntrophin. In the control lane (4 and 8) an identical precipitation was performed in the absence of FLAG fusion protein.

Table 1

|                  | α1-Syntrophin (mouse syntrophin 1 Torpedo syntrophin rabbit 59-DAP 1) | β1-Syntrophin (human β-syntrophin-2) | β2-Syntrophin (mouse syntrophin 2 human EST25263) |
|------------------|------------------------------------------------------------------------|-------------------------------------|--------------------------------------------------|
| **M r**          | 54,000                                                                 | 58,000                              | 58,000                                           |
| Isoelectric point | 6.4                                                                    | 9.0                                 | 9.4                                              |
| Binds dystrophin  | Yes                                                                    | Yes                                 | Yes                                              |
| Distribution in muscle | Sarcolemma                                      | Unknown                             | NMJ                                               |
| mRNA distribution | Striated muscle                                             | Ubiquitous                           | Ubiquitous                                        |
| mRNA classes     | One                                                                    | Five                                | Three                                            |
| Phosphorylated    | Yes                                                                    | Probably                             | Probably                                          |
| Chromosomal location | 20q11.2                               | 8q23-24                             | 16q23-24                                         |
| Assigned human gene name | SNT A1                              | SNT B1                              | SNT B2                                           |

In this report we conclusively confirm our previous hypothesis that there are three distinct but homologous human syntrophin genes. Their biochemical and genetic characteristics are summarized in Table I. We have also shown that these homologous proteins are functionally conserved with respect to their in vitro binding properties to dystrophin, utrophin, and the 87K protein.

Comparisons of the three human syntrophins to each other, as well as those sequences available in mouse and rabbit, demonstrate that for a particular isoform of syntrophin there is a high degree of interspecies conservation, with 96% identity for β2-syntrophin and at least 93% for the three mammalian α1-syntrophins. In contrast, the three human syntrophins are less strongly conserved with respect to each other. The α1-syntrophin is 54% and 50% identical to its β1- and β2-syntrophin counterparts, respectively, and the β-syntrophins are only 57% identical to each other.

The syntrophins contain two tandem PH domains (Fig. 3A, in plain box) (22). The first PH domain is interrupted by a 162- to 182-amino acid region in which 80 amino acids are highly conserved among the three syntrophins (Fig. 3A, in black box). Adams and colleagues (28) have noted the homology between this conserved region and a number of other membrane-associated proteins, the PDZ domain. The PH and PDZ domains, either together or individually, may determine the specific membrane localization of syntrophin, either directly to a lipophilic membrane component (27) or via an integral membrane protein (41).
these two isoforms of syntrophin had unique binding properties to the respective dystrophin and utrophin/DRP proteins. The finding that all three syntrophins can each bind to dystrophin and its relatives in vitro falls short in providing some clue as to how either the syntrophins or dystrophins can localize to different specializations of the sarcolemma. The coprecipitation procedure used here (Fig. 7) does not quantitatively address this question. However, the differences among the three syntrophins, which are especially marked in the connecting loops to the PDZ domain (Fig. 3A), may reflect the specialization of these individual genes to a particular function, such as to interact with another protein, or as determinants of their distinct subcellular localization.

At the level of the mRNA, the β-syntrophins share a common characteristic in that they give rise to a set of transcript classes (Fig. 4). In contrast to β1-syntrophin, whose five transcript classes are most abundant in liver, β2-syntrophin transcripts are more homogeneously expressed, most abundant in lung, and have three transcript classes (Fig. 4B). These results are similar to those found in mouse β2-syntrophin, but the relative abundance of β2-syntrophin in human brain is much lower than that observed in mouse (9). In the cDNA clones that we have isolated so far, we have not noticed any large differences in the sequences among the clones that can account for these alternative forms, and the other reported cDNAs from rabbit and mouse (9, 12, 28).

The α1-syntrophin transcript is expressed as a single-sized transcript of 2.5 kilobase pairs (Fig. 4C), and is strongly dominant in cardiac and skeletal muscle. This representation of α1-syntrophin mRNA expression shows somewhat more expression in extrasomal tissues than that reported in mouse and rabbit tissues previously (9, 12), but may be attributable only to a higher sensitivity in detection.

The tissue distribution of the respective syntrophin mRNA also allow us to hypothesize what kinds of inherited disorders may be caused by defects of these genes. The sublocalization of β2-syntrophin to 16q23-24 (Fig. 6A), and the widespread tissue distribution suggests that a defect of this gene would have consequences in multiple organs. Because α1-syntrophin is so abundantly expressed in striated muscle, we would predict that a defect of this gene would be more inclined to result in a myopathic phenotype. The question of whether this 20q11.2-encoded gene (Fig. 6A) is linked to any autosomal neuromuscular diseases is currently under investigation.

Acknowledgements—We are indebted to S. C. Froehner and M. E. Adams for sharing their PDZ alignment and unpublished data on mouse β1- and β2-syntrophin, to J. B. Cohen for anti-87K antibody, to H. S. Selig for advice and assistance with FISH, to E. M. McVally for use of the human heart cDNA library and careful reading of the manuscript, and to J. Knoll for assistance in verifying chromosomal localizations.

REFERENCES

1. Emery, A. E. H. (1993) in Duchenne Muscular Dystrophy: Oxford Monographs on Medical Genetics (Matulsky, A. G., Harper, P. S., Bobrow, M., and Scriver, C., eds) 2nd Ed., Oxford University Press, New York
2. Campbell, K. P., and Kahl, S. D. (1989) Nature 343, 259–62
3. Yoshida, M., and Ozawa, E. (1990) J. Biochem. (Tokyo) 108, 748–752
4. Froehner, S. C., Murano, A. A., Tobler, M., Peng, H. B., and Sealock, R. (1987) J. Cell Biol. 104, 1633–1646
5. Butler, M. H., Douville, K., Murano, A. A., Kramer, N. R., Cohen, J. B., Sealock, R., and Froehner, S. C. (1992) J. Biol. Chem. 267, 6213–6218
6. Cartaud, A., Stetkowsky-Marden, F., and Cartaud, J. (1993) J. Biol. Chem. 268, 13019–13022
7. Kramer, N. R., Vidai, A., Froehner, S. C., and Sealock, R. (1994) J. Biol. Chem. 269, 2670–2676
8. Adams, M. E., Butler, M. H., Dwyer, T. M., Peters, M. F., Murano, A. A., and Froehner, S. C. (1993) Neuron 11, 531–540
9. Yamamoto, H., Hagiwara, Y., Mizuno, Y., Yoshida, M., and Ozawa, E. (1993) J. Biochem. (Tokyo) 114, 132–139
10. Wagner, K. R., and Huginar, R. L. (1994) J. Neurochem. 62, 1947–1952
11. Yang, B., Ibraghimov-Beskrovnaya, O., Moonaw, C. R., Slaughter, C. A., and Campbell, K. P. (1994) J. Biol. Chem. 269, 6040–6044
12. Ahn, A. H., Yoshida, M., Anderson, M. D. S., Feener, C. A., Selig, S., Hagiwara, Y., Ozawa, E., and Kunkel, L. M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 4446–4450
13. Carr, C., Fischbach, G. D., and Cohen, J. B. (1989) J. Cell Biol. 109, 1753–1764
14. Wagner, K. R., Cohen, J. B., and Huginar, R. L. (1993) Neuron 10, 511–522
15. Suzuki, A., Yoshida, M., Yamamoto, H., and Ozawa, E. (1992) FEBS Lett. 308, 154–160
16. Suzuki, A., Yoshida, M., Hayashi, K., Mizuno, Y., Hagiwara, Y., and Ozawa, E. (1994) J. Biol. Chem. 269, 283–292
17. Ahn, A. H., and Kunkel, L. M. (1995) J. Cell Biol. 128, 363–371
18. Pfeffer, S. R., Raft, D. J., and Bruce, P. (1995) J. Biol. Chem. 270, 4975–4978
19. Peters, M. F., Kramer, N. R., Sealock, R., and Froehner, S. C. (1994) Neuron 5, 1577–1580
20. Gilson, T. J., Hyvonen, M., Musacchio, A., Saraste, M., and Birney, E. (1994) Trends Biochem. Sci. 19, 349–353
21. Haslam, R. J., Koide, H. B., and Hemmings, B. A. (1993) Nature 363, 309–310
22. Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993) Cell 73, 629–638
23. Yoon, H. S., Hajduk, P. J., Petros, A. M., Oleniczak, E. T., Meadows, R. P., and Fesik, S. W. (1994) Nature 369, 672–675
24. Mancias, J. M., Musacchio, A., Pérotin, H., Nilges, M., Saraste, M., and Oshikiri, H. (1994) Nature 369, 675–677
25. Harlan, J. E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) Nature 371, 168–170
26. Adams, M. E., Dwyer, T. M., Dowler, L. L., White, R. A., and Froehner, S. C. (1995) J. Biol. Chem. 270, 25859–25865
27. Cho, K. O., Hunt, C. A., and Kennedy, M. B. (1992) Neuron 9, 929–942
28. Woods, D. F., and Bryant, P. J. (1991) Cell 66, 451–464
29. Itoh, M., Nagafuchi, A., Yonemura, S., Kitani, Y. T., Tsukita, S., and Tsukita, S. (1993) Cell 71, 491–502
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
31. McVally, A. P., Neve, R. L., Colletti, F. C., Bertelson, C. J., Kurnit, D. M., and Kunkel, L. M. (1986) Nature 323, 646–650
32. Dubois, B. L., and Naylor, S. L. (1993) Genomics 16, 315–319
33. Becker, J. S., Powers, L. S., Sheffield, V. C., and Murray, J. C. (1993) Am. J. Hum. Genet. 53, (suppl.) 974
34. Lichter, P., Cremer, T., Borden, J., Manuelidis, L., and Ward, D. C. (1988) Hum. Genet. 80, 224–234
35. Lichter, P., Tang, C. C., Call, K., Hermanson, G., Evans, G. A., Hausman, D., and Ward, D. C. (1990) Science 247, 64–69
36. Masutani, C., Sugawara, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takai, K., Tanaka, K., Spek, P. V. D., Bootma, D., Hoeijmakers, J. H. J., and Hanaoka, F. (1994) EMBO J. 13, 1813–1843
37. Kozak, M. (1986) Cell 44, 283–292
38. Itoh, M., Hiraoka, K., Yonemura, A., Yonemura, S., Tsukita, S., and Tsukita, S. (1994) J. Cell Biol. 127, 1617–1626
39. Ohlendieck, K., Ervasti, J. M., Matsunura, K., Kahl, S. D., Leveille, C. J., and Campbell, K. P. (1991) Neuron 7, 499–508