ment of the spacer with a stretch of GC pairs did not entirely diminish the joining (lane 4 in Fig. 4C). This observation is consistent with the notion that the length is more important than the sequence for the spacer (*8, 9*).

We analyzed V-(D)-J joining with synthetic RSS oligomers in a recombination-competent pre-B line. It was clearly demonstrated that 12-bp and 23-bp RSS's were sufficient substrates to cause DNA recombination in a manner analogous to the natural V-(D)-J joining. In the endogenous Ig and TCR genes, V-(D)-J joining takes place in a time-ordered fashion during lymphocyte development (*29, 30*) although all the V-(D)-J joining events follow the same 12- and 23-bp spacer rule (Table 1). It remains to be determined what regulates DNA rearrangement. Transcriptional activation of a gene is thought to be prerequisite for DNA rearrangement (*18*). A putative recombinational enhancer may also play an important role in making the chromatin structure open for DNA rearrangement (*30*).

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Deep-Sea Hydrocarbon Seep Communities: Evidence for Energy and Nutritional Carbon Sources

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Mussels, clams, and tube worms collected in the vicinity of hydrocarbon seeps on the Louisiana slope contain mostly "dead" carbon, indicating that dietary carbon is largely derived from seeping oil and gas. Enzyme assays, elemental sulfur analysis, and carbon dioxide fixation studies demonstrate that vestimentiferan tube worms and three clam species contain intracellular, autotrophic sulfur bacterial symbionts. Carbon isotopic ratios of 246 individual animal tissues were used to differentiate heterotrophic ($\delta^{13}C = -14$ to -20 per mil), sulfur-based ($\delta^{13}C = -30$ to -42 per mil), and methane-based ($\delta^{13}C = <-40$ per mil) energy sources. Mussels with symbiotic methanotrophic bacteria reflect the carbon isotopic composition of the methane source. Isotopically light nitrogen and sulfur confirm the chemoautotrophic nature of the seep animals. Sulfur-based chemosynthetic animals contain isotopically light sulfur, whereas methane-based symbiotic mussels more closely reflect the heavier oceanic sulfate pool. The nitrogen requirement of some seep animals may be supported by nitrogen-fixing bacteria. Some grazing neogastropods have isotopic values characteristic of chemosynthetic animals, suggesting the transfer of carbon into the background deep-sea fauna.

WE REPORT HERE A STUDY OF THE energy and nutritional carbon sources of mussels, clams, and tube worms from hydrocarbon seep communities on the Louisiana continental slope (*1*). The organisms were collected in trawls near hydrocarbon seep sites in water depths between 400 and 920 m on R.V. *Gyre* cruises 86-G-1/2. The northern Gulf of Mexico slope is extensively faulted and fractured by salt tectonics, thus providing conduits for the upward migration of oil and gas (*2*). The taxa at these sites are similar to those of the hydrothermal vent sites of the Pacific (*3*), the cold seep sites of the Florida Escarpment (*4*), and the Oregon Subduction Zone (*5*). The Louisiana sites are distinct in that the vent taxa are living in a high hydrocarbon environment derived from deeper reservoired petroleum. These vent-type taxa use organic matter produced in situ by chemoautotrophic, sulfide-oxidizing bacteria and endosymbiotic chemoautotrophs (*6, 7*). Methane use has been demonstrated for the mussels from the Louisiana site (*8*) and from the Florida Escarpment (*4, 9*) and has been suggested for the animals at the Oregon Subduction Zone (*5*).

A variety of tests were used to determine the nature (and presence) of endosymbionts in these seep fauna. The mussel is the only animal with confirmed methanotrophic symbionts (*8*) and is the only one of these seep species that possesses methanol dehydrogenase, an enzyme characteristic of methylotrophy (Table 1). The mussel is also the only animal tested whose bacterial symbionts contain stacked internal membranes (typical of type I methanotrophs). Mussel gills lack the enzymes characteristic of sulfur oxidation [adenosine triphosphate (ATP) sulfurylase and adenosine-5′-phosphosulfate reductase], lack elemental sulfur, and have only trace activities of ribulose-bisphosphate carboxylase (an enzyme characteristic of autotrophic carbon fixation); these factors indicate that symbionts of mussel gills are not sulfur-oxidizing chemoautotrophs.

The other three bivalves and the two

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vestimentiferans appear to harbor sulfur-oxidizing chemoautolithotrophic symbionts (Table 1). The enzyme activities, the presence of elemental sulfur in the symbiont-containing tissue, and electron microscopy provide evidence that both vestimentiferans and the lucinid clam, *Pseudomiltha* sp., contain chemoautotrophic, sulfur bacterial symbionts. The evidence for the vesicomyid clams is not as conclusive since no tissue from *Calyptogena ponderosa* was frozen in liquid nitrogen for enzymatic analysis and the one *Vesicomya cordata* collected died before dissection. The absence of specific enzyme activities is thus of questionable significance. Nonetheless, the high level of elemental sulfur in the gills of *C. ponderosa* and the high levels of ATP sulfurylase in *V. cordata* gills suggest that sulfur-oxidizing symbionts were present. The sulfide oxidase activities in all animals assayed are at the level expected for invertebrates exposed to a sulfide environment (*10*).

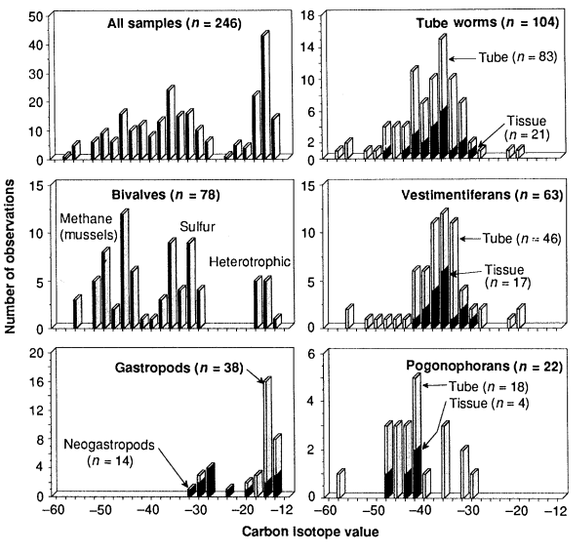

Fig. 1. Carbon isotopic values (per mil relative to Pee Dee belemnite standard) of 246 animals collected at seep sites on the Louisiana continental slope.

The seep fauna at the Louisiana site contain mostly "dead" carbon (Table 2). Several sources of dietary carbon are possible for the seep animals. First, carbon can be derived from particulate detritus fixed photosynthetically in the upper water column ($\Delta^{14}C = 100 \pm 20$ per mil; $\delta^{13}C = -18$ to -20 per mil; background fauna are in Table 3). Second, carbon can be derived from bacterial organic carbon synthesized chemoautotrophically from dissolved inorganic carbon (DIOC). The DIOC can be derived either from ambient bottom water ($\Delta^{14}C = -100$ per mil; $\delta^{13}C = -0$ per mil) (*11*) or from dead CO_2 ($\Delta^{14}C = -1000$ per mil). Dead DIOC can be derived from (i) seeping oil and gas, (ii) bacterial degradation of the seeping oil and gas, (iii) dissolu-

Table 1. Enzyme activities, elemental sulfur (S^0) content, stable carbon isotope ratio ($\delta^{13}C$), presence of symbiotic bacteria (S.B.), and methane consumption (CH_4) in individual Louisiana slope seep organisms. Assays were conducted on symbiont-containing tissues (bivalve gills and vestimentiferan trophosome). One unit of enzyme activity will convert 1 μmol of substrate to product. RuBP, ribulose-bisphosphate carboxylase; ATP, ATP sulfurylase; APS, adenosine-5′-phosphosulfate reductase; methanol, methanol dehydrogenase; sulfide, sulfide oxidase; ND, not detected; N, no; Y, yes; NT, not tested; WW, wet weight; and EM, electron microscopy.

Animal	Identification No.	Enzyme activity (unit/g WW/min)					S° (% WW)	$\delta^{13}C$	S.B. (EM)	CH_4*
		RuBP	ATP	APS	Methanol	Sulfide				
					Mollusca					
Lucinidae										
Pseudomiltha sp.									Y	N
	14-1	0.43	12.86	0.83	ND	2.1	0.02	−33.5		
	14-2	0.41	2.47	0.66	ND	1.94	0.06	−33.6		
	14-3	0.44	15.43	1.36	ND	2.04	ND	−32.5		
	14-14	NT	NT	NT		NT	0.5	−37.7		
Mytilidae										
Undescribed									Y	Y
	24-1	0.011	ND	ND	0.66	0.7	ND	−51.8		
	24-2	0.017	ND	ND	0.53	0.75	ND	−52.0		
	25-1	0.027	ND	ND	0.4	1.09	ND	−52.6		
Vesicomyidae										
V. cordata†									Y	NT
	18-1	0.003	29.58	ND	ND	NT	ND	−39.8		
C. ponderosa									Y	NT
	CAT-1	NT	NT	NT	NT	NT	0.4	−37.9		
	CAT-2	NT	NT	NT	NT	NT	8.3	−36.9		
	CAT-3	NT	NT	NT	NT	NT	ND	−39.1		
					Vestimentifera					
Lamellibrachiidae										
Lamellibrachia sp.									Y	N
	25-3	0.24	4.24	0.70	ND	1.77	4.5	−36.6		
	25-4	4.03	1.03	NT	ND	3.15	6.1	−36.8		
	25-5	4.97	0.51	0.78	ND	5.47	2.6	−37.4		
Undescribed family										
Undescribed									Y	N
	24-1	3.57	0.32	ND	ND	NT	0.1	−36.4		
	25-1	3.73	1.03	1.54	ND	5.32	NT	−39.9		
	25-2	5.40	1.90	ND	ND	2.98	1.9	−41.0		
	25-3	NT	NT	ND	NT	2.37	0.4	−37.0		

*Methane consumption from incubation of gill or trophosome tissue measured gas chromatographically (*8*). †The *V. cordata* died before dissection.

tion of ancient carbonate, or (iv) degradation of sedimentary organic matter. Dead carbon can also be derived from the direct use of methane by symbiotic bacteria and most likely results from direct use of methane by the mussels (*8*) and extensive biodegradation of the oil and gas by bacteria. Large amounts of isotopically light, authigenic carbonate and extensively biodegraded oil in sediments from the seep sites (*12*) indicate active CO_2 production. Although most reservoired gases in the Gulf of Mexico contain small amounts of CO_2 (*13*), this source of dead carbon is hypothesized to be minor relative to other CO_2 sources.

At the hydrothermal vent sites (Table 2), the principal source of dietary carbon for mussels and tube worms is DIOC (*6*). At the Florida Escarpment, where methane is apparently the major energy source for the mussels, the radiocarbon content of three tube worms and the mussels was older, although not predominantly dead (Table 2). In contrast to the hydrothermal vent and Florida Escarpment sites, many of the mussels, tube worms, and clams at the Louisiana site contain nearly dead carbon. The dead carbon in the mussels supports the metabolic and physiological studies that indicate there is a bacterial symbiosis between the mussel and methanotrophic bacteria. In contrast, the sulfur-based tube worms and clams must use dead DIOC derived from bacterial degradation of hydrocarbons. Thus much of their dietary carbon is derived ultimately from the sediments and not from the more recent DIOC of seawater. These observations are consistent with the hypothesis that oil and gas are the energy sources for these seep taxa.

The carbon isotopic content of these seep organisms reflects the isotopic fractionation that occurs during the synthesis of organic tissues (*6*) and the food source of the animals (*14*). Figure 1 presents the carbon isotopic compositions of 246 organisms from trawl samples collected on the Louisiana–Upper Texas slope (34 sites). The most striking feature of Fig. 1 is the three isotopically distinct groups of bivalves. The mussel tissues all have $\delta^{13}C$ values less than −40 per mil. The $\delta^{13}C$ values between −30 and −42 per mil represent clams with sulfur bacterial symbionts. These values are similar to those of the hydrothermal vent clams (*6, 15, 16*), which appear to derive their energy from hydrogen sulfide. We assume that clam $\delta^{13}C$ values typical of deep-sea fauna (−14 to −20 per mil) are heterotrophic.

The light carbon isotopic values of the mussels are characteristic of the methane symbiosis between the bacteria and the mussel (*8*). On Johnson *Sea-Link-I* dives 1877 and 1878, we collected mussels living in a bubbling gas stream at 630 m in Green Canyon (GC) Block 185. The mussels $\delta^{13}C$ (−40.6 per mil) closely reflected the composition of the methane (−41.2 per mil) used by the bacterial symbionts. Biogenic methane is characterized by $\delta^{13}C$ values less than −60 per mil with few, if any, longer chain hydrocarbons, whereas thermogenic gas contains higher hydrocarbon gases and $\delta^{13}C$ values heavier than −45 per mil (*17*). The mussels from GC-185 have thermogenic isotopic values. Most of the mussels collected from the trawls suggest an admixture of biogenic and thermogenic methane. The gill and mantle tissue from three mussels in our study have similar isotopic compositions, indicating a transfer of bacterial carbon from the symbionts in the gill to the mussel's other tissues.

Tube worm tissues and tubes from these sites show a range of $\delta^{13}C$ values from −20 to −58 per mil (Fig. 1). These values are atypical of the few previous reports from the hydrothermal vent (*6, 15, 16*) and other cold seep sites (*4, 5*). The vestimentiferans (*Riftia pachyptila*) from the hydrothermal vents all have $\delta^{13}C$ values near −10 per mil (*6, 15*; Table 2). One suggested explanation of the heavy values is that CO_2 limitation during growth precludes discrimination at the site of carbon fixation (*6, 15*). The other $\delta^{13}C$ values for tube worms from the Florida Escarpment and the Oregon Subduction Zone show lighter $\delta^{13}C$ values. Thus tube worm values heavier than −42 per mil are characteristic of sulfur-based endosymbionts. Values lighter than −42 per mil in the pogonophorans may indicate a contribution from methane endosymbionts. The three heavy values (−20 to −22 per mil) in the vestimentiferans from the Louisiana sites may reflect processes similar to those occurring at the hydrothermal vent sites. The wide range of values also reflect the multiple sampling sites, the patchiness of thermogenic hydrocarbon seepage, and perhaps a difference in the $\delta^{13}C$ of the DIOC utilized by the animals. All of the tissue and tube

Table 2. Stable carbon and radiocarbon measurements of seep and vent taxa from the deep sea. Numbers of individuals, without parentheses, refer to carbon isotopic measurements; numbers in parentheses refer to radiocarbon analyses. TR, values in this report.

Sample description	Number of individuals	$\Delta^{14}C$ (per mil)	$\delta^{13}C$ (per mil)	Reference
Louisiana hydrocarbon seep sites				
Clam tissue (*C. ponderosa*)	4		−31.2 to −35.3	(*1*)
Clam tissue (*C. ponderosa*)	3		−36.9 to −39.1	TR
Clam tissue (*Pseudomiltha* sp.)	17 (1)	−753	−30.9 to −37.7	TR
Mussel	38 (2)	−829, −840	−40.1 to −57.6	TR
Snail (neogastropod)	1		−31.5	(*1*)
Neogastropods	10 (2)	−210, −544	−14.6 to −32.8	TR
Tube worm (*Lamellibrachia*)				
Tissue	1		−27.0	(*1*)
Tube	1		−28.1	(*1*)
Tube worm (*Lamellibrachia*)*	37 (1)	−586	−29.8 to −57.2	TR
Tube worm (pogonophorans)*	22 (2)	−205, −749	−30.5 to −59.3	TR
Tube worm (Escarpia-like)*	24		−21.4 to −48.6	TR
Hydrothermal vent sites (Galápagos and 21°N)				
Clam tissue (*C. magnifica*)	2		−32.1, −32.7	(*15*)
Clam tissue (*C. magnifica*)†	4		−32.1 to −39.9	TR
Mussel tissue (*Bathymodiolus thermophilus*)	3 (3)	−270 to −228	−32.8 to −33.9	(*6*)
Mussel tissue (*B. thermophilus*)	1		−32.7 to −33.6	(*16*)
Mussel tissue (*B. thermophilus*)	24		−32.1 to −37.2	TR
Tube worm (vestimentiferan) tissue	1 (1)	−270	−10.9	(*6*)
Tube worm (vestimentiferan) tissue	1		−10.8 to −11.0	(*15*)
Tube worm tissue‡	4		−11.9 to −13.7	TR
Florida escarpment site				
Mussel tissue (mytilid)	10 (3)	−567 to −247§	−74.3 ± 2.0 (SD)	(*4*)
Gastropod tissue (trochid)	2		−59.9 ± 0.7 (SD)	(*4*)
Tube worm (vestimentiferan) tissue	3 (2)	−419, −424§	−42.7 ± 0.7 (SD)	(*4*)
Oregon subduction zone site				
Clam tissue (*Calyptogena* sp.)	1		−35.7	(*5*)
Clam gills (*Calyptogena* sp.)			−51.6	(*5*)
Clam tissue (*Solemya* sp.)	1		−31.0	(*5*)
Tube worm (*Lamellibrachia*)				
Tissue	1		−31.9	(*5*)
Segment			−26.7	(*5*)

*Includes both tubes and tissues of different individuals. †Values include isolated gills and remains. ‡Values include isolated trophosomes and vestimentum. §Values reported originally as percentage of modern. The conversion to $\Delta^{14}C$ assumed modern as 0 per mil.

Table 3. Stable carbon, nitrogen, and sulfur isotopic ratios and radiocarbon measurements of Louisiana slope seep organisms. Numbers in parentheses indicate the number of animals represented by the range. Locations are 9-square-nautical-mile Mineral Management Service lease areas and blocks (GC, Green Canyon; GB, Garden Banks; EB, Ewing Bank; and MC, Mississippi Canyon). All measurements are on animal soft tissue.

Animal	Location	$\delta^{13}C$ (per mil)	$\delta^{15}N$ (per mil)	$\delta^{34}S$ (per mil)	$\Delta^{14}C$ (per mil)
		Symbiont-containing animals (chemosynthetic)			
Bivalves					
Mussel (Mytilidae undescribed)	GC-272	−50.1 to −45.5 (9)	−12.9 to +3.0 (10)	+13.4, +7.5 (2)	−829 (1)
Clam (*C. ponderosa*)	GC-272, GC-234	−34.8, −35.0 (2)	+1.1 to +7.1 (3)	−0.1 to +2.1 (3)	
Clam (*V. cordata*)	GC-116	−36.3 (1)	−0.9 (1)		+254 (1)
Clam (*Pseudomiltha* sp.)	GC-79	−36.0 to −31.8 (3)	−3.5 to +6.1 (3)	−11.5 to +1.3 (4)	−753 (1)
Vestimentiferans					
Tube worm (Escarpia-like)	GC-272, GB-458	−40.9 to −30.4 (3)	+2.9, +5.4 (2)	−3.5 (1)	−205, −949(2)
Tube worm (*Lamellibrachia* sp.)	GC-33	−43.2 (1)	+2.7 (1)	−2.7 (1)	−586 (1)
		Heterotrophic deep-sea animals			
Neogastropods	MC-839, EB-1010, GC-33	−32.8 to −14.8 (5)	+2.8 to +13.0 (4)	0.0 to +18.7 (4)	−210, −544 (2)
Shrimp	GB-300	−19.5 to −18.6 (2)	+13.3 (1)	+13.3 (1)	+123 (1)
Clam (*Acesta*)	GC-272	−18.7 (1)	+8.9 (1)	+16.1 (1)	+96 (1)

pairs (more than 12 pairs) analyzed show similar carbon isotopic compositions (Fig. 1).

The sulfur isotopic content (Table 3) of the seep fauna also differentiates sulfur and methane energy sources. Most animals from food webs based on phytoplankton have sulfur isotopic compositions between 13 to 20 per mil, similar to the seawater sulfate pool (+20 per mil) (*18, 19*). Fry *et al.* (*20*) found that the fauna at hydrothermal vent sites had values between −5 to +5 per mil, similar to the sulfur-bearing minerals of the vents. Although the H_2S isotopic content of the Louisiana sites is unknown, the sulfide-based tube worms and clams have values between −12 to +2 per mil. Some neogastropods have values in this range, reflecting chemosynthetic dietary carbon and sulfur, whereas others have values characteristic of deep-sea heterotrophs. These values are consistent with Fig. 1, which shows that most of the gastropods contain heterotrophic carbon. However, some of the neogastropods show transfer of chemosynthetic carbon into the background slope fauna, which most likely results from the food source of these snails that often prey on bivalves. The methane-based mussels have sulfur values more characteristic of the heavier seawater sulfate.

In a manner similar to carbon and sulfur isotopes, nitrogen isotopes may indicate sources of nitrogen and food web relations. Hydrothermal vent communities (*21*) and seep communities of the Florida Escarpment (*4*) have unusually depleted nitrogen isotopic compositions compared to typical marine values (5 to 15 per mil) (*22*). Such ^{15}N-depleted values have been attributed to fractionations of source nitrogen either through assimilation of depleted nitrate (*21*) or ammonium (*4*). At the Louisiana sites, ^{15}N values range from similar to those previous studies (*4, 21*) to even more depleted values (−12 per mil). Such depleted ^{15}N values have been reported for laboratory algal cultures with high (millimolar) concentrations of ammonium or nitrate (*23*), ammonium-rich hot springs (*24*), and nitrate-rich lakes in Antarctica (*25*). In all of these cases, elevated inorganic nitrogen concentrations allow for marked discrimination in the uptake of ^{14}N rather than ^{15}N, with resulting cells being much depleted in ^{15}N. However, such unusual environments are not expected at the seep sites. Normal (micromolar) levels of nitrate have not been associated with large ^{15}N depletions or anomalous values (*22, 26, 27*).

An alternative source of nitrogen is fixation of nitrogen gas (N_2) associated with methane from the seeps. The N_2 of the vent gas, once fixed, may be the sole source of nitrogen for some of the organisms as indicated by their ^{15}N depletion. The N_2 gas isolated from a nearby oil well has a ^{15}N value of −2.9 per mil. Microorganisms fixing this gas would then have isotopic values near −6 per mil. Natural gases from other oil reservoirs have been reported to be as depleted as −14.6 per mil (*28*), so the N_2 gas associated with the seep area could be even more depleted.

The dietary carbon, nitrogen, and sulfur; energy sources; and trophic relations in the Louisiana seep ecosystems are complex. The wide isotopic ranges of organisms from these sites as opposed to other vent and cold seep sites suggest that either (i) the Louisiana seep communities are more complex and diverse or (ii) the fewer isotopic measurements at the other sites do not adequately reflect their diversity and complexity.

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A Synaptic Vesicle Protein with a Novel Cytoplasmic Domain and Four Transmembrane Regions

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Complementary DNA and genomic clones were isolated and sequenced corresponding to rat and human synaptophysin (p38), a major integral membrane protein of synaptic vesicles. The deduced amino acid sequences indicate an evolutionarily highly conserved protein that spans the membrane four times. Both amino and carboxyl termini face the cytoplasm, with the latter containing ten copies of a tyrosine-rich pentapeptide repeat. The structure of synaptophysin suggests that the protein may function as a channel in the synaptic vesicle membrane, with the carboxyl terminus serving as a binding site for cellular factors.

SYNAPTIC VESICLES PLAY A CRUCIAL role in neurotransmission. At the synapse, neurotransmitters are stored in synaptic vesicles and released by synaptic vesicle exocytosis. Although these functions must, to a large extent, be mediated by proteins in the synaptic vesicle membrane, the primary structure of none of these proteins has been characterized. Monoclonal antibodies have been used to identify an integral synaptic vesicle membrane protein named synaptophysin or p38 which constitutes 6 to 8 percent of the synaptic vesicle membrane protein but less than 0.1 percent of total brain protein (*1*). In addition to being a constituent of synaptic vesicles, synaptophysin is also found in a subset of brain coated vesicles, presumably as a result of vesicle recycling after exocytosis (*1, 2*). As one approach to the study of the molecular basis of neurotransmitter release, we undertook the molecular characterization of this protein using recombinant DNA and biochemical techniques and present here the primary structure of this integral synaptic vesicle membrane protein.

Synaptophysin was purified from rat brain cortex by monoclonal antibody affinity chromatography (*1*). Purified protein was proteolytically cleaved by trypsin and chymotrypsin, and peptides isolated by high-performance liquid chromatography were subjected to amino acid sequence analysis (*3*). A nonredundant oligonucleotide probe was designed from one of the three peptide sequences obtained [MATDPENIIKEMP (*4*), oligonucleotide sequence: ATGGCCACCGACCCCGAGAACATCATCAAGGAGATGCC] and was used to screen rat brain and human retina complementary DNA (cDNA) libraries (*5*). Of the 16 positive rat and 12 positive human clones isolated from the libraries, the majority proved to encode synaptophysin based on sequence analysis. RNA blots hybridized with probes derived from these clones revealed a single 3.4-kb species in rat brain (Fig. 1) (*6*).

Two overlapping rat clones and the largest human clone were selected for complete DNA sequence analysis on both strands (*7*). In all of these cDNAs an open reading frame extended to the very 5′ end, indicating that they did not contain the beginning of the coding sequence. Multiple attempts at isolating cDNA clones that extended more 5′ were unsuccessful. We therefore isolated rat genomic clones for synaptophysin from a rat genomic DNA library and used a novel approach to identify the 5′ end of the synaptophysin gene (*8*). The 5′ end of the synaptophysin messenger RNA (mRNA) from total rat brain RNA was sequenced directly by primer-extension in the presence of dideoxy nucleotides. An oligonucleotide designed from the sequence obtained was used to isolate the 5′ exon from the genomic clone. From our determination of the complete nucleotide sequence (*7*) we have deduced the protein sequence shown in Fig. 2.

A plot of the mean side-chain hydrophobicity of the protein sequence of synaptophysin shows four 23 or 24 amino acid stretches of high average hydrophobicity, suggesting the presence of four transmembrane regions that are separated by short hydrophilic sequences (Fig. 2). The four transmembrane regions exhibit a limited sequence homology to each other and may have arisen by gene duplication events. Synaptophysin is known to be *N*-glycosylated and disulfide linked (*1*). The only possible *N*-glycosylation site (Asn-X-Ser or Asn-X-Thr) in the amino acid sequence is found at residues 53 to 55, between the first and second transmembrane regions. Similarly, cysteines are only found between the first and second and between the third and fourth membrane-spanning regions. Therefore, the hydrophilic protein loops between the first and second and between the third and fourth transmembrane regions must extend into the intravesicular space, while the NH_2-terminal and COOH-terminal sequences of synaptophysin face the cytoplasm (Fig. 3). Since the NH_2-terminus is cytoplasmic, no signal sequence is observed. The nucleotide and amino acid sequences of synaptophysin are not significantly homologous to any reported sequence in the available data banks (*9*).

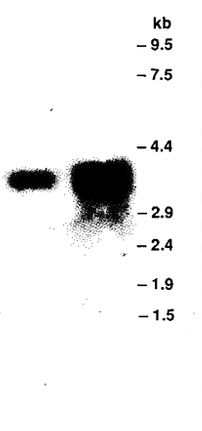

Fig. 1. RNA blot analysis of 5 μg and 20 μg of total RNA from rat brain hybridized with a single-stranded ^{32}P-labled DNA probe complementary to nucleotides 337 to 513 of the rat synaptophysin cDNA (*7*). Numbers on the right give the positions of RNA molecular size standards. The blot was exposed to film for 3 days at −70°C with an intensifying screen.

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