Dynamin is a GTP, microtubule-, and phospholipid-binding protein that is expressed primarily in brain. In Drosophila, the shibire gene encodes a homologue of dynamin; mutations in this gene result in a defect in endocytosis, suggesting a function for dynamin in endocytic membrane traffic. In the present study we show that there are at least two distinct dynamin genes in mammals whose products are referred to as dynamins I and II. The two dynamins are similar to each other (79% identity) and are both equally homologous to the Drosophila shibire gene product (66% identity). The highest degree of identity between dynamins is observed in their N-terminal halves, whereas their C termini exhibit little homology. Transcripts of both dynamin genes are subject to two alternative splicing events, the first of which is identically found in both dynamins, whereas the second site of alternative splicing is different between the two types of dynamins. The first alternatively spliced sequence of the dynamins consists of an interior region that is present in two distinct homologous forms in both dynamins, suggesting alternative use of exons in both genes at identical positions. The second site of alternative splicing results in the generation of different C termini in dynamin I and II in the inclusion or exclusion of an interior four-amino-acid sequence in dynamin II.

The two dynamins exhibit remarkable differences in their tissue distribution and regulation. Dynamin I is almost exclusively expressed in the central nervous system. Conversely, dynamin II is expressed ubiquitously in all tissues tested. Previous studies revealed that the GTPase activity of dynamin I is regulated by phosphorylation by protein kinase C in nerve terminals. Expression of dynamins I and II by transfection in COS cells demonstrates that only dynamin I but not dynamin II is a substrate for protein kinase C. Our data suggest a specialization in the endocytic functions and the regulation of dynamins between neural and non-neural tissues in mammals.

Dynamin is a microtubule-binding protein that was originally identified as a possible microtubule-based motor protein (Shpeter and Vallee, 1989). The structure of dynamin as deduced by cDNA cloning revealed the presence of a GTP binding consensus sequence (Obar et al., 1990), and purified dynamin was shown to have a potent microtubule-activated GTPase activity (Shpeter and Vallee, 1992; Maeda et al., 1992). Dynamin is expressed primarily in the nervous system, with a developmental time course paralleling synaptogenesis and almost no detectable mRNA in peripheral tissues (Nakata et al., 1991; Faire et al., 1992; van der Bieken et al., 1993). In view of the microtubule-binding properties of dynamin, it came as a surprise when the product of the shibire gene in Drosophila was identified as a closely related homologue of dynamin (van der Bieken and Meyerowitz, 1991; Chen et al., 1991). Temperature-sensitive mutants of the shibire gene show a paralytic phenotype at the restrictive temperature, caused by the inability of synaptic vesicles to recycle after endocytosis. In addition, defects in endocytosis in oocytes and garland cells have also been observed, suggesting a general function of dynamin in endocytosis (Poodry and Edgar, 1979; Kosaka and Ikeda, 1983a, 1983b; Kessell et al., 1989; Massar et al., 1990). Recent transfection experiments of mutants of mammalian dynamin confirm a role for dynamin in endocytosis (Herskovits et al., 1993; van der Bieken et al., 1993). Endocytosis is not known to depend on microtubule function, and the relation of the microtubule binding activity of dynamin to its function in endocytosis is currently unclear.

The N-terminal 300 amino acids of dynamin are homologous to proteins identified in a wide range of functional contexts: VPS1/SPO1, a yeast protein with a function in vacuolar protein sorting and meiotic spindle pole separation (Rothman et al., 1990; Yeh et al., 1991); MGM1, a yeast protein required for mitochondrial DNA maintenance (Jones and Fangman, 1992); and vertebrate Mx proteins, interferon-inducible nuclear proteins implicated in resistance to influenza virus infections (Staeheli et al., 1986). The fact that the homologies between these proteins extend beyond their GTP binding domains suggests that their functional similarities are not restricted to GTP binding. Conversely, the C-terminal half of VPS1 that is not homologous to dynamin has been shown to be necessary for its function (Vater et al., 1992). In dynamin, the functional importance of the C terminus was also revealed in recent transfection experiments using dynamin mutants (Herskovits et al., 1993). These data suggest that the function of a common N-terminal domain may be differentially specified by distinct C-terminal domains in different proteins of this family of GTP-binding proteins.

Studies on 32P-labeled synaptosomes, pinched-off nerve endings from brain, have demonstrated the presence of a major M, 96,000 component that is quantitatively dephosphorylated upon nerve terminal depolarization and neurotransmitter release (Robinson et al., 1987). This protein, originally named dephosphin, was recently identified as dynamin (Robinson et al., 1993). Thus, dynamin is highly enriched in nerve terminals where it is phosphorylated by protein kinase C and quantitatively dephosphorylated in parallel with synaptic vesicle exocytosis and endocytosis. This finding suggests that dynamin may be
regulated in parallel with synaptic vesicle recycling. In vitro, phosphorylation of dynamin was shown to be at its C terminus and to enhance the GTPase activity of dynamin more than 10-fold (Robinson et al., 1993). Together with the phenotypic effects of the shibire mutations, these data strongly implicate dynamin in a regulatory function in synaptic vesicle recycling in the nerve terminal.

Analysis of shibire mutant flies suggested an endocytic function of the shibire gene product outside of the nervous system in addition to its synaptic function (Poodry and Edgar, 1979; Ko- saka and Ikeda, 1983a, 1983b; Kessel et al., 1989; Masur et al., 1990). However, little expression of dynamin was found outside of the nervous system in mammals (Nakata et al., 1991; van der Bieke et al., 1993). This raises the question if the currently characterized form of dynamin is simply expressed at much higher levels in neurons than in other cells but is actually present in all cells, as has recently been suggested (van der Bieke et al., 1993). An alternative explanation could be that dynamin is part of a gene family that comprises homologous but distinct proteins with differential functions in general, constitutive pathways and specialized regulated pathways. In an attempt to address this question, we have investigated the protein expression of the subfamilies and their properties. We now report the structure of a new dynamin, referred to as dynamin II, that is expressed in all tissues, whereas dynamin I shows an almost exclusive neuronal expression. Dynamin II is highly homologous to dynamin I in the N terminus but diverges significantly in the C terminus and is not regulated by protein kinase C phosphorylation, suggesting distinct functional specializations between neuron-specific membrane trafficking pathways and general trafficking pathways.

**EXPERIMENTAL PROCEDURES**

**cDNA Cloning and Sequencing**—A rat brain library in AZAP was screened as described (Sudhof et al., 1989) with two oligonucleotides complementary to the 5' end of the coding region of dynamin (Obar et al., 1990) (oligonucleotide sequences (redundant bases are shown in parentheses): GCCGGTACCGCGCAACATGCGGCAACCG and CTG- GAGCCTGCACTACATGTTGAGGATCTCTGCTGATATCTC. Positive clones were plaque purified and analyzed by restriction enzyme mapping and sequencing, revealing the presence of cDNA clones encoding the previously described form of dynamin (here referred to as dynamin I) (Obar et al., 1990) as well as additional clones encoding a novel form of dynamin, referred to as dynamin II. Since the initial clones isolated for dynamin II were not full-length, clones extending more 3' were isolated after further screening using additional specific oligonucleotides (sequences: CTGCTGAGTCGTTGTCTTG and GCCTCTAGATCTAAG- GTTGAACTGGGT). All cDNA clones were at least partially sequenced after subcloning into single-stranded M13 vectors using the dyeoxy nucleotide-chain termination method with fluorescently labeled sequencing primers, Taq DNA polymerase, and an ABI 370A automated sequencer. The dynamin I cDNA clones were described earlier (Robinson et al., 1993) and were identical with the published dynamin I sequence except for the discovery of two sites of alternative splicing. All DNA sequences were deposited with GenBank (accession number L25605).

**RNA Purification and Blotting**—Total RNA was purified from rat tissues after homogenization in guanidinium thiocyanate by centrifugation through a 5.7 M CsCl cushion as described elsewhere (Sambrook et al., 1989). RNA blots were obtained after electrophoresis of glyoxal-denatured samples and hybridized with uniformly 32P-labeled single-stranded DNA probes as previously described (Ushkaryov et al., 1992). RNA blot analysis of poly(A)-rich RNA from rat brain, liver, spleen, and heart was performed using the following oligonucleotides: 5'-CTGCTGAGTCGTTGTCTTG-3' (sense) and 5'-GCCTCTAGATCTAAGGTGGAACTGGGT-3' (antisense).

**RESULTS**

Identification of a Novel Dynamin—Upon screening a rat brain cDNA library with oligonucleotides complementary to sequences from dynamin, sequence analysis of the isolated clones revealed the presence of a novel type of dynamin in addition to the previously described form (Obar et al., 1990; Robinson et al., 1993). Full-length cDNA clones encoding the new forms of dynamin were isolated using specific oligonucleotide probes, and a complete sequence of the coding region was obtained. Since the new form of dynamin described here is a true homologue of dynamin but the product of a distinct gene (see below), we will refer to it as dynamin II and to the previously characterized form of dynamin as dynamin I.

Fig. 1 shows the characteristics of the dynamin II cDNA clones analyzed, and their nucleotide and translated amino acid sequence is shown in Fig. 2. cDNA clones contained 5' untranslated regions of up to 111 nucleotides without an in-frame stop codon. The first ATG in the sequence is likely to represent the initiation codon as it conforms well with the consensus sequence of initiation codons (Kozak, 1991) and is followed by an open reading frame highly homologous to that of dynamin I. At the 3' end, two closely spaced polyadenylation signals are observed (underlined in Fig. 2). Multiple polyadenylation signals appear to be used in dynamin II since the five analyzed cDNA clones that contain the 3' end of the message had three different closely spaced poly(A)-tail attachment sites 10–20 bp 3' to the polyadenylation signals (Fig. 2).

Sequencing of multiple cDNA clones encoding dynamin II revealed that they differ at two positions from each other (Figs. 1 and 2). Each variant was observed in independent cDNA clones, suggesting that they are caused by alternative splicing and not by a cDNA cloning artifact. The two alternatively...
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#### Figure 1: Structure of the mRNAs encoding rat dynamin II isoforms and localization of cDNA clones analyzed.

The structure of the dynamin II message is shown diagrammatically on top, with the open bar denoting the coding region. The stippled region represents the first alternatively spliced sequence. Two different variants were observed here in different cDNA clones, presumably due to alternative usage of exons labeled a and b in the diagrams of the cDNA clones below the mRNA diagram. The asterisk denotes the position of the second site of alternative splicing, consisting of a 12-nucleotide sequence that was present in some cDNA clones and absent from others (represented by + and − signs above the cDNA clone diagrams at the appropriate position). Note that all four combinations of splice variants were observed in the cDNA clones. The names of the cDNA clones are shown on the left, and the scale of the drawing is given on the bottom.

Spliced sequences will be referred to as the first and second site of differential splicing of dynamin II. The first differential splice site of dynamin II consists of a middle region that was present in two different sequences in different cDNA clones. The second alternatively spliced region of dynamin II consists of a four amino acid sequence that was either present in or absent from different cDNA clones. In addition to these two alternatively spliced sequences observed in multiple cDNA clones, one of six cDNA clones of dynamin II had a single amino acid deletion at position 848. It is unclear if this represents another alternatively spliced sequence or a polymorphism or cloning artifact.

Previous studies had demonstrated that dynamin I transcripts are subject to at least two differential splicing events (Robinson et al., 1993; van der Bliek et al., 1993). The first of these is similar to the first alternative splice site of dynamin II, suggesting that the first alternative splice site is conserved between dynamins I and II. The second site of differential splicing is very different in dynamin I and dynamin II, consisting of a small insertion or deletion of an interior sequence in dynamin II and the generation of different C termini in dynamin I. Independent cDNA clones containing all four combinations of the two events of alternative splicing were isolated for both dynamin I (Robinson et al., 1993) and dynamin II (Fig. 1), demonstrating the presence of at least four different dynamin I and II proteins. To avoid confusion in referring to these proteins, we suggest that the different gene products be identified by different Roman numbers (e.g., dynamins I and II) and the different splice forms by letters in an N- to C-terminal order, with the largest form or first described form being referred to as a, the next b, and so on. Accordingly, the form of dynamin I containing the middle insert originally described (Obar et al., 1990) would be referred to as dynamin Iab since it also contains the shorter C-terminal version, and the form of dynamin II containing the homologous insert in the middle and an insert in the second splice site would be dynamin Iaa (Fig. 2).

Dynamin II is highly homologous to dynamin I, with an overall sequence identity of 79% (Fig. 3). Both sequences are equally homologous to the Drosophila shibire gene product (66% sequence identity), suggesting that shibire is functionally no closer to one dynamin than to the other. The homologies between dynamins are unevenly distributed in the structure of the dynamins. The highest degree of identity is observed in the N-terminal 400 amino acids that are homologous to VPS1/SPO1, MGM1, and Mx proteins, and the lowest degree of identity is observed in their C-terminal domains. Alignment of the nucleotide and amino acid sequences of the two sequence variants observed for the first alternative splice site of dynamins I and II demonstrates that they are highly homologous to each other not only between dynamins but also within a type of dynamin (Fig. 4). The presence of alternative sequences suggests that they are caused by alternative exon usage. The two alternatively used inserts at these positions show much greater divergence at the nucleotide level than at the amino acid level, suggesting an evolutionary selection for retaining homologous amino acid sequences in the alternatively used exons.

The pattern of the sequence characteristics and the alternative splicing of dynamins allows formulation of a domain model for dynamins dividing the molecule into five parts (Fig. 5). The N-terminal half of the protein is composed of its highly conserved GTP binding domain which is followed by the alternatively spliced middle region and the conserved C-terminal region. At the C terminus, there is a sequence of almost a hundred amino acids that is not very well conserved between dynamins and contains 30% proline residues, followed by the highly divergent C terminus of the dynamins.

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#### Table 1: Alternative Splicing of Dynamin mRNAs

| mRNA | Alternative Sites |
|------|------------------|
| p914-4 | a/b | A_1 |
| p914-5 | a/b | A_2 |
| p758-12 | a/b | A_3 |
| p843-7 | a/b | A_4 |

#### Figure 6: Differential Distribution of Dynamins—Total RNAs from different rat tissues were analyzed to determine the expression levels of dynamins I and II. Upon hybridizing RNA blots with a probe specific for dynamin I, two message sizes were observed (Fig. 6A). As mentioned above, dynamin I is subject to alternative splicing at at least two positions, of which only the C-terminal alternative splicing results in mRNAs of different sizes. Sequences of our cDNA clones predict that the longer mRNA contains a 611-base pair insert as compared to the shorter mRNA (data not shown), with the longer mRNA encoding the shorter form of the protein (i.e., dynamins Iab and Ibb). The size difference between the longer and shorter mRNA corresponds exactly to the size differences between the two dynamin I transcripts observed on the RNA blots (Fig. 6A). The shorter mRNA is expressed at much higher levels than the longer mRNA. The two different dynamin I mRNAs appear to be expressed differentially since PC12 cells contained only the longer but not the otherwise more abundant shorter mRNA.

Short exposures of the RNA blots hybridized with the dynamin I-specific probe suggest a brain-specific expression that even excludes adrenal gland which expresses many otherwise neuron specific genes (Fig. 6A). However, long exposures of the same blot demonstrate that the longer mRNA is also expressed at very low levels in adrenal glands and liver but not in testis, lung, kidney and intestine (Fig. 6B). The long exposure shown in Fig. 6B also reveals non-specific hybridization of the probe with abundant 18 and 28 S ribosomal RNAs and cross-hybridization with dynamin I mRNAs. The latter can be recognized by a comparison to the dynamin II RNA blots (Fig. 6, C and D) and serve as an internal control for RNA integrity, clearly demonstrating the high degree of tissue specificity of the dynamin I expression.

RNA blots hybridized with dynamin II-specific probes demonstrate similar levels of expression of this mRNA in all tissues tested (Fig. 6, C and D). The expression of dynamin II was rather low as judged by the exposure times required for a signal, with highest levels being observed in testis. With the longer and higher specific activity probe used in Fig. 6C, cross-hybridization with dynamin I is observed in tissues with high levels of dynamin I (cerebellum, brain, and spinal cord). Two
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**Fig. 2. Primary structure of rat dynamin II.** The translated amino acid sequence is shown in single-letter code below the nucleotide sequence, and both sequences are numbered on the right. The region sequences subject to alternative splicing are shaded. For the first of these regions (nucleotides 1306-1443), two types of sequences were found that are homologous to each other and are encoded by alternative splicing (see Fig. 3 for the second variant). In the second region (residues 1657-1668), the shaded sequence was found to be present in three and absent from two different cDNA clones. Finally, the AGC-trinucleotide encoding serine 848 was present in all clones except for one (p914-11, Fig. 1). This shows the sequence of dynamin IIaa since it contains the "a" version of the first splice site and an insert in the second splice site. The two underlined AATAAA-sequences at the 3' end denote potential polyadenylation signal sequences; two cDNA clones exhibit poly(A)-tails following nucleotide 3437 (~914-10 and p914-11), one cDNA clone following nucleotide 3455 (p914-15), and two cDNA clones following residue 3463. The mRNA sizes are also observed for dynamin I (3.5 and 4.0 kilobase). The ratio between the two mRNAs for dynamin I does not change between different tissues, and the large mRNA is uniformly more abundant than the shorter mRNA. It is currently unclear if the dynamin I mRNAs reflect an as yet unidentified differential splicing of dynamin I at its C terminus, as shown for dynamin II, or if they are caused by differential polyadenylation sites. The co-expression of dynamin I and II in PC12 cells demonstrates that a single cell line can express both dynamins, suggesting different functions for the two forms.
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Fig. 3. Alignment of the amino acid sequences of different rat dynamins as compared with the Drosophila shibire gene product. Sequences in single-letter code are numbered on the right and identified on the left. Dynamins Iaa and Ibb correspond to the different splice variants of dynamin I (Obar et al., 1990; Robinson et al., 1993; van der Bliek et al., 1995), and dynamins Iaa and Ibb to those of dynamin II (Figs. 2 and 3). shi. A and shi. B represent the two splice variants of the Drosophila dynamin homologue that is the product of the Altire gene (Chen et al., 1991; van der Bliek and Meyerowitz, 1991). Sequences subject to alternative splicing are shaded. The positions of the GTP binding consensus sequences are indicated by bars above the respective amino acid residues.

Fig. 4. Nucleotide and translated amino acid sequences of the alternatively spliced middle region of the dynamins. The names of the dynamins are given in the sequences above the line and the residue numbers on the right. Periods denote identities with the top sequence, and only a variant of dynamin I in this region corresponding to the originally published sequence (Obar et al., 1990). We propose to identify the products of different genes by different Roman numbers and the variants of different splice sites by different letters in a 5' to 3' order. Note that most of the nucleotide changes in the two variants at each site are conservative, suggesting evolutionary pressure to retain homologous amino acid sequences in the respective dynamins.

Therefore tested whether transfected dynamin I and dynamin II were substrates for protein kinase C to determine if dynamin II could also potentially be regulated by the same mechanism. Cytosolic extracts from COS cells transfected with dynamins I or II or control cells were phosphorylated by purified rat brain protein kinase C in the presence of [γ-32P]ATP and in the presence or absence of Ca2+ and phosphatidylserine. Phosphorylation of dynamins was then assessed by immunoprecipitation of the reactions followed by SDS-PAGE and autoradiography. Immunoprecipitations were performed relatively non-stringently to allow immunoprecipitation of dynamin II with dynamin I antibodies.

Only phosphorylation of dynamin I by protein kinase C was observed whereas dynamin II was not phosphorylated (Fig. 7A). This suggests that dynamin II is not a substrate for purified protein kinase C. To ensure that the immunoprecipitation
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were equally well immunoprecipitated as judged by the signal intensity; in addition the relatively low stringency of the immunoprecipitation protocol resulted in the immunoprecipitation of multiple radiolabeled proteins that were identically observed in transfected and control COS cells.

DISCUSSION

Dynamin is an abundant GTP-binding protein in brain with a high microtubule-activated GTPase activity (reviewed in Vallee (1992)). Studies on the Drosophila mutant shibire revealed that it is caused by mutations in a gene encoding a close homologue of dynamin (Chen et al., 1991; van der Bliek and Meyerowitz, 1991). shibire mutants show a general defect in endocytosis, suggesting a ubiquitous function for dynamin in endocytosis (Poodry and Edgar, 1979; Kosaka and Ikeda, 1983a, 1983b; Kessell et al., 1989; Masur et al., 1990). Such a general role for dynamin in endocytosis was confirmed in recent transfection experiments demonstrating that expression of mutant dynamins can inhibit endocytosis in fibroblasts (Herkovits et al., 1993; van der Bliek et al., 1993). shibire phenotype typically affects the nervous system first, impairing neurotransmission presumably because the active recycling of synaptic vesicles is inhibited. Very high levels of dynamin are observed in mammalian neurons whereas peripheral tissues were reported to express no dynamin or little dynamin (Nakata et al., 1991; van der Bliek et al., 1993). Although the observed high neuronal levels of dynamin agree well with the particularly active membrane traffic observed in presynaptic nerve terminals, the virtual absence of dynamin from peripheral tissues is difficult to reconcile with a general role for this protein in endocytosis in all cells.

The current study resolves this paradox by demonstrating that there are two distinct dynamin genes in rats, one of which is expressed almost exclusively in brain and the other is expressed in all tissues. It suggests that dynamins are evolutionarily specialized into two classes, neuronal dynamins with a primary role in the nerve terminal and ubiquitous dynamins with a general function. A similar relation between neuronal and ubiquitous forms was described for the synaptic vesicle protein synaptobrevin, of which a ubiquitous homologue is present in a general pathway in all cells (McMahon et al., 1993). The identification of dynamin I as a nerve terminal trafficking protein is supported by its enrichment in synaptosomes and its quantitative phosphorylation and dephosphorylation in parallel with synaptic vesicle exocytosis and recycling (Robinson et al., 1993). The presence of two forms of dynamin, one specialized for the nerve terminal and one active in all cells, lends further support to the emerging concept of the synaptic vesicle pathway as a specialization of a general exo- and endocytotic vesicular recycling pathway in all cells (Johnston et al., 1989b). The fact that the two dynamins are equally related to the

expression of the two dynamins (5 μg/lane) was analyzed by agarose gel electrophoresis and blotting using probes derived from coding regions of the following dynamin cDNAs: A and B, dynamin I. The photographs show the same blot exposed for 9 h (A) and 7 days (B) with a screen at −70 °C. The long exposure reveals nonspecifically hybridizing 28S and 18S ribosomal RNAs (at 4.9 and 1.8 kilobases, respectively) and cross-hybridization to dynamin II. Note that only the larger mRNA for dynamin I is co-expressed at very low levels with dynamin II in some peripheral tissues (e.g., liver), whereas the shorter mRNA of dynamin I is brain-specific. C and D, dynamin II. C was hybridized with an interior probe from dynamin II (exposure time: 14 h at −70 °C with screen) that also weakly hybridizes with dynamin I to illustrate the relative distributions and mobilities of dynamin I and II mRNAs. D was hybridized with a dynamin II-specific probe (exposure time: 10 days at −70 °C with screen). Panels A and D exclusively display the expression of dynamins I and II, respectively, whereas Panels B and C demonstrate by cross-hybridization between the two dynamins the relations between the expression of the two dynamin genes. Numbers on the right indicate positions of molecular weight markers.
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Drosophila shibire gene product is compatible with the notion that a single gene may have both functions in Drosophila, although this remains to be established.

Two previous studies have addressed the distribution of dynamin I mRNAs in mammalian tissues with different results (Nakata et al., 1991; van der Bliek et al., 1993). Nakata et al. observed a brain-specific expression, whereas van der Bliek et al. observed a ubiquitous expression with highest levels in brain. It is likely that the ubiquitously expressed mRNA observed by van der Bliek et al. actually corresponds to dynamin II because its size is different from the dynamin I mRNAs they observed in brain and closely corresponds to the larger and more abundant of the two dynamin II mRNAs we observed (Fig. 6). cDNA cloning has revealed that the two size classes of dynamin I mRNAs correspond to two different C termini. The RNA blots reveal a clear tissue-specific expression of the two mRNAs of dynamin I, with non- or paraneuronal tissues such as adrenal gland or PC12 cells expressing only the larger mRNA (Fig. 6B). Both forms of dynamin are subject to alternative splicing, with each dynamin exhibiting at least four protein forms. The functional significance of the alternative splicing is unclear. The conservation of the usage of alternative exons for a sequence in the middle of the dynamins in both dynamin genes, and the sequence conservation of these exons, suggests the presence of an evolutionary pressure to retain this alternative splicing after the two dynamin genes diverged. Furthermore, the C-terminal alternative splicing of dynamin I is regulated since brain expresses much higher levels of the shorter mRNA than the longer mRNA, whereas in PC12 cells, adrenal gland and other peripheral tissues only the longer mRNAs are observed, although at low levels. Together these observations support a functional role of alternative splicing in dynamins that will have to be tested upon identification of their biochemical targets.

Analysis of the sequences of dynamins and their differential splicing suggests a distinct domain structure for these proteins (Fig. 5). The N-terminal halves of all dynamins are highly homologous to each other (>80% identity) and contain a GTP binding sequence motif. They are followed by the alternatively spliced middle region and a second well conserved region. At the C terminus, an unusual sequence of 92–97 amino acids is observed that contains 28–30% proline residues and ends in the variable tails of the different dynamins (Figs. 5 and 6). The conserved N-terminal domains of dynamins are homologous to a series of proteins with a variety of functions: VPS1/SPO1 (Rothman et al., 1990; Yeh et al., 1991), Mgm1 (Jones and Fangman, 1992), and Mx proteins (Staeheli et al., 1985). The homologies between these proteins extend beyond their actual GTP binding domains, suggesting that their functional similarities are not restricted to GTP binding. However, each of these proteins has distinct C-terminal domains that are not homologous between different proteins. Studies using mutants of dynamin and of VPS1 have demonstrated that the unique C-terminal domains of these proteins are functionally important (Herskovits et al., 1993; Vater et al., 1992). This suggests a model whereby the function of a common N-terminal domain in these proteins may be differentially specified by distinct C-terminal domains. It is likely that the C-terminal domains of dynamins will have a major role in the selection of their biochemical targets during their endocytotic function.

Protein kinase C phosphorylates dynamin I on one or two serine residues close to the C terminus of the protein and thereby stimulates dynamin GTPase activity (Robinson et al., 1993). Negatively charged phospholipids and microtubules also appear to bind dynamin at the C terminus and this binding also stimulates dynamin GTPase activity (Shepner and Vallee, 1992; Maeda et al., 1992; Tuma et al., 1993). These observations indicate that the N-terminal GTPase activity of dynamin I is under the control of its C terminus. Interestingly, the C terminus is the least conserved domain between dynamins (Fig. 5), suggesting that a similar GTPase activity in different dynamins may be under different regulatory control. This seems to be indeed the case since dynamin II expressed in COS cells is not subject to protein kinase C phosphorylation whereas dynamin I is (Fig. 7), suggesting that dynamin II is not subject to the same phosphorylation control as dynamin I.

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REFERENCES

Chen, M. S., Obar, R. A., Schroeder, C. C., Austin, T. W., and Poodry, C. A. (1991) Nature 351, 585–586

Fairs, K., Trent, F., Tepper, J. M., and Bonder, E. M. (1992) Proc. Natl Acad. Sci.
Expression and Regulation of Multiple Dynamins

Herskovits, J. S., Burgess, C. C., Obar, R. A., and Vallee, R. B. (1993) J. Cell Biol. 122, 565-576
Johnston, P. A., Jahn, R., and Südhof, T. C. (1989a) J. Biol. Chem. 264, 1268-1273
Johnston, P. A., Cameron, P. L., Stukenbrok, H., Jahn, R., De Camilli, P., and Südhof, T. C. (1989b) EMBO J. 8, 2663-2672
Jones, B. A., and Fangman, W. L. (1992) Genes & Dev. 6, 380-389
Kessell, I., Holst, B. D., and Roth, T. F. (1989) Proc. Natl. Acad. U. S. A. 86, 4966-4972
Kosaka, T., and Ikeda, K. (1983a) J. Neurobiol. 14, 207-225
Kosaka, T., and Ikeda, K. (1983b) J. Cell Biol. 97, 499-507
Kozak, M. (1991) J. Cell Biol. 115, 887-903
Laemmli, U. K. (1970) Nature 227, 680-685
Maeda, K., Natake, T., Noda, Y., Sato-Yoshitake, R., and Hirokawa, N. (1992) Mol. Biol. Cell 3, 1181-1194
Masur, S. K., Kim, Y.-T., and Wu, C.-F. (1990) J. Neurogenet. 6, 191-206
McMahon, H. T., Ushkaryov, Y. A., Edelmann, L., Link, E., Binz, T., Niemann, T., Jahn, R., and Südhof, T. C. (1993) Nature 364, 346-349
Mignery, G. A., Newton, C. L., Archer, B. T., and Südhof, T. C. (1990) J. Biol. Chem. 265, 12873-12885
Nakata, T., Iwamoto, A., Noda, Y., Takemura, R., and Yoshikura, H. (1991) Neuron 7, 461-469
Obar, R. A., Collins, C. A., Hammarback, J. A., Shpetner, H. S., and Vallee, R. B. (1990) Nature 347, 256-261
Obar, R., Shpetner, H. S., and Vallee, R. B. (1991) J. Cell Sci. 14, 143-145
Podory, C. A., and Edgar, L. (1979) J. Cell Biol. 81, 520-527
Robinson, P. J., Hauptman, R., Loeneng, W., and Dunkley, P. R. (1987) J. Neurochem. 48, 187-195
Robinson, P. J., Sontag, J.-M., Liu, J.-P., Fykse, E. M., Slaughter, C. A., and Südhof, T. C. (1993) Nature 365, 163-166
Rothman, J. H., Raymond, C. K., Gilbert, T., O'Hara, P. J., and Stevens, T. H. (1990) Cell 61, 1631-1674
Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Shpetner, H. S., and Vallee, R. B. (1989) Cell 59, 421-432
Shpetner, H. S., and Südhof, R. B. (1992) Nature 358, 733-735
Sibra, T. S., Bogonez, E., and Nicholas, D. G. (1995) J. Biol. Chem. 270, 1983-1989
Staeheli, P., Haller, O., Boll, W., Lindemann, J., and Weisemann, C. (1986) Cell 44, 147-158
Südhof, T. C., Baumert, M., Perin, M. S., and Jahn, R. (1989) Neuron 2, 1475-1481
Tuma, P. L., Stachniak, M. C., and Collins, C. A. (1993) J. Biol. Chem. 268, 17240-17246
Ushkaryov, Y. A., Petrenko, A. G., Geppert, M., and Südhof, T. C. (1992) Science 257, 59-66
Vallee, R. B. (1992) J. Muscle Res. Cell Motil. 13, 199-207
van der Bliek, A. M., and Meyerowitz, E. M. (1991) Nature 351, 411-414
van der Bliek, A. M., Edelmann, T. E., Damke, H., Tisdale, E. J., Meyerowitz, E. M., and Schmid, S. L. (1993) J. Cell Biol. 122, 553-563
Vater, C. A., Raymond, C. K., Ekana, K., Howald-Stevenson, I., and Stevens, T. H. (1992) J. Cell Biol. 119, 773-786
Yoh, E., Driscoll, R., Colteyra, Oliva, A., and Bloom, K. (1991) Nature 349, 713-715