Dymple, a Novel Dynamin-like High Molecular Weight GTPase Lacking a Proline-rich Carboxyl-terminal Domain in Mammalian Cells*

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We have cloned human dymple, a novel dynamin family member. The full-length cDNA sequence encodes a protein composed of 736 amino acids with a molecular mass of 80 kDa. This amino acid sequence most resembles yeast DNM1P and VPS1P. Dymple lacks a proline-rich carboxyl-terminal domain through which dynamin binds to SH3 domains to be activated. Northern blot analysis revealed two transcript sizes of 2.5 and 4.2 kilobases with alternative polyadenylation at the highest levels in brain, skeletal muscle, and testis. It was further established that there are three patterns of alternative splicing producing in-frame deletions in the coding sequence of dymple in a tissue-specific manner. Since overexpressed, wild-type dymple exhibited a punctate perinuclear cytoplasmic pattern, whereas an amino-terminal deletion mutant formed large aggregates bounded by a trans-Golgi network marker. Since dynamin participates in clathrin-mediated endocytosis through a well-characterized mechanism, the existence of a dynamin-like molecule in each specific vesicle transport pathway has been predicted. Our findings suggest that dymple may be the first example of such a subfamily in mammalian cells other than dynamin itself, although its precise role and membrane localization remain to be resolved.

Dynamin was originally isolated from cow brain because of its ability to cross-link and bundle microtubules in vitro in a nucleotide-dependent manner (1). Its extensive sequence similarity with the interferon-inducible Mx proteins (2) and the yeast VPS1 (3) gene product revealed the existence of a superfamily of dynamin-related high molecular weight GTP-binding proteins (4). A variety of recently found proteins has been classified into this family (5).

Different forms of Mx, first identified as a murine gene that confers selective resistance to influenza viruses related to interferon (IFN-α/β), have been cloned in vertebrates, including a nuclear protein murine Mx1 (6, 7) and cytoplasmic examples human MxA and MxB (8). The yeast VPS1, one of more than 50 genes that are required for soluble vacuolar protein sorting (9), encodes an Mx-like protein (3) shown to play a direct role in the trafficking of vacuolar and Golgi membrane proteins from the late Golgi apparatus to the prevacuolar compartment (10). VPS1 was also found to be the same gene as SPO15 in mutated yeasts not able to sporulate because of a defect in meiotic spindle pole separation (11). Two dynamin family genes have been identified in yeast: MGM1 required for mitochondrial genome maintenance (12) and DNM1, involved in endosomal trafficking (13). Both the primary sequence and function of Mgm1p are least similar to those of other dynamin family members. Although DNM1P shares high sequence homology with VPS1P, its function is distinct, since it participates in endocytosis at a novel step after internalization and before delivery to the vacuole (13). In plant cells, the dynamin-like protein phragmosplastin is associated with the formation of cell plates, disc-like membrane-bound structures, by functions of Golgi-derived vesicles (14).

Dynamin has been isolated from Caenorhabditis elegans, Drosophila, and a number of mammals with almost 68% identity across species and appears to function in vesicular transport during endocytosis elucidated from analysis of the shibire phenotype of Drosophila in which a temperature-sensitive mutation renders the flies paralyzed because of an inability to recycle synaptic vesicles at nerve terminals (15, 16). In mammals, dynamin seems to exist as at least two distinctive isoforms, brain-specific dynamin I (17–19) and ubiquitous dynamin II (20, 21). The most pronounced diversity between the sequences of dynamin isoforms and other dynamin-related proteins resides in the carboxyl-terminal span, where a basic proline-rich domain exists. Through this region, dynamin GTPase activity is stimulated by various factors in vitro such as the binding of microtubules and SH3 domains (22), acidic phospholipids (23), or phosphorylation by protein kinase C (19, 24).

Recent findings have shown that interaction of the dynamin proline-rich carboxyl-terminal domain with SH3 domains is responsible for in vivo function in clathrin-mediated endocytosis (25–27). Electron microscopic studies revealed that the necks of invaginated clathrin-coated pits are collared by dynamin oligomers and that pinching-off of vesicles requires dynamin GTPase activity (28, 29). Although clathrin-coated vesicles originate from both the plasma membrane and TGN1 membrane, respectively, specified by their adaptor complex AP2 and AP1, dynamin has been shown to be specifically associated with endocytic clathrin-coated vesicles at the plasma

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1 The abbreviations used are: TGN, trans-Golgi network; mAb and pAb, monoclonal and polyclonal antibodies, respectively; AP, alkaline phosphatase; GST, glutathione S-transferase; HA, hemagglutinin; RACE, rapid amplification of cDNA ends; PBS, phosphate-buffered saline; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pairs; PK domain, pleckstrin homology.
membrane (30, 31). Within the emerging superfamily of high molecular weight GTPases, VPS1P and DNM1P in yeast cells resemble dynamin in their involvement with protein sorting. Thus dynamin/VPS1P-like molecules also might be expected to exist in vertebrates.

Human autoantibodies have been proven to be useful reagents in elucidating the structure and function of eukaryotic cellular components. Several nuclear and cytoplasmic proteins have been identified as autoimmune antigens (32), and recently we isolated a novel mitogen-activated protein kinase kinase, MAPKK6, using serum from a Behçet patient (33). In the present study, we aimed to identify the antigen targeted by the serum of a scleroderma patient and screened by a HeLa cell cDNA library. We obtained a human cDNA clone for dymple, a dynamin family member lacking a proline-rich carboxy-terminal domain that exhibits overall homology to yeast VPS1P and DNM1P. Primary characterization of this novel gene was also performed.

Experimental Procedures

Materials—Autoimmune serum 114 was taken from a scleroderma patient. mAb and rabbit serum 114 were purchased from Sigma, and anti-hemagglutinin (HA) mAb was a gift from Boehringer Mannheim. Alkaline phosphatase (AP)-conjugated goat anti-human IgG, fluorescein isothiocyanate-conjugated goat anti-mouse IgG, and fluorescein isothiocyanate-conjugated goat anti-human IgG were obtained from Zymed Laboratories Inc. (San Francisco, CA). Texas red-conjugated goat anti-mouse IgG and Texas red-conjugated goat anti-human IgG were obtained from Southern Biotechnologies (Birmingham, AL). The mouse m1 plasmid (pMXMN12) (34) was a kind gift from Dr. K. Nagata (Tokyo Institute of Technology).

Immunoscreening and cDNA Sequencing—The UNI-ZAP HeLa cDNA expression library was screened with serum 114 by standard procedures as described previously (33) with more than 500,000 plaques included in the first screening. The 20 positive clones obtained were then subcloned into pBlueScriptII SK− with the in vitro excision system (Stratagene, La Jolla, CA). Inserted cDNA sequences were determined by the dye termination method with an ABI 373S DNA sequence (Perkin-Elmer).

5′-RACE—Using oligo-dDNA 5′-TATCATCAGGGGTTACCGC-3′ (1096–1077 bp) and 5′-GCTGCTGATGTTGCCATATC-3′ (698–679 bp), respectively, as gene-specific primers 1 and 2, 5′-RACE was carried out following manufacturer protocol (Life Technologies, Inc.). A 700-bp major band was recovered from 1% SeaKem agarose gels and subcloned into a pCRII TA cloning vector (CLONTECH, Palo Alto, CA). The insert cDNA sequences were determined by the dye termination method with the ABI 373S DNA sequence (Perkin-Elmer).

Preparation of Vectors, Recombinant Proteins, and Polyclonal Antibody—The dymple and murine M1x full-length insertions were amplified as Sall-NotI fragments having a Sall site before the initial codon and a NotI site after the stop codon and subcloned into the mammalian transfection vector pME-HA (33) or the bacterial expression vector pGEX 5X-3 (Pharmacia Biotech Inc.). Insertion and batch purification of recombinant dymple and murine M1x GST fusion proteins were performed as detailed in manufacturer protocols. The amino-terminal deletion mutant N246 was created by the deletion of the HinII fragment (1–245 amino acids) of the dymple insert in pBlueScriptII SK− and subcloned as a XhoI-NotI fragment into the Sall-NotI site of the pME-HA vector. A site-directed point mutation was introduced into the dymple cDNA at serine 39 to give isoleucine, and the resultant clone S39I was subcloned into the pME-HA vector. For polyclonal antibody preparation, the coding sequence of 570 amino acids to the carboxy terminus (736 amino acids) of dymple were amplified as a BamHI-PstI fragment and subcloned into the pQE31 vector (Qiagen, Chatsworth, CA). His-tagged protein was purified in the native condition following GTase assays. The assays of GTase activity were performed at 37 °C for 1 h in 25 μl of reaction mixture containing 50 mM Tris-HCl (pH 8.0), 5 mM MgCl2, 0.1 mM dithiothreitol, 130 μM cold GTP, 13 μM [α32P]GTP (1 μCi, 3,000 Ci/mmol) (Amersham, Buckinghamshire, UK), and 0.1 μg of purified GST fusion proteins. Aliquots (1 μl each) of the reaction products were resolved by chromatography on polyethyleneimine cellulose plates in 1.6 M LiCl (34).

RESULTS

Isolation and Characterization of a Novel Dynamin-like Gene, Dymple—More than 300,000 plaques of UNI-ZAP HeLa cell cultures at 37 °C in Dulbecco’s modified Eagle’s medium (Nissui, Tokyo, Japan) were used for transfection with the DEAE-dextran method as described previously (33). Plasmid DNA for transfection was prepared by the cesium chloride ultracentrifugation method. The DNA was grown to 40–60% confluence, washed once with PBS, and incubated in the transfection medium (80 μl of 20 mg/ml DEAE-dextran + 20 μg of plasmid DNA/μl of serum-free medium) for 4 h. Cells were then incubated in 10% MeSO/PBS for 1 min, washed with PBS, and re-fed in complete medium until the assay.

Western Blotting—Approximately 48 h after transfection, COS7 cells were washed three times with ice-cold PBS, collected, and lysed in radioimmuno precipitation buffer (10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium deoxycholate, 0.1 μM EDTA, 0.1 mM dithiothreitol, 5 μM tunicamycin inhibitor, 5 μg/ml leupeptin) at 4 °C. 10 μl aliquots of 1-ml lysed samples were resolved on SDS-polyacrylamide (10%) gels followed by transfer to nitrocellulose membranes. For visualization, blots were blocked at room temperature for 1 h with 5% skim milk, PBS, briefly rinsed with Tween 20/TBS, and then incubated at 4 °C overnight with anti-HA mAb at a dilution of 1/1,000 or with human serum 114 at a dilution of 1/200 in 1% bovine serum albumin, PBS and then washed four times each for 15 min with Tween 20/TBS. Bound antibodies were detected using AP-conjugated goat anti-mouse IgG or anti-human IgG.

Immunolocalization—For immunofluorescence, anti-dymple pAb and anti-γ-adaptin mAb were diluted with 1% bovine serum albumin, PBS to 1/2,000 and 1/100, respectively. Fluorescent second antibodies were used at 1/100 dilution. COS7 cells were plated out on coverslips, transfected, and cultured for 36 h. They were then washed twice with PBS, fixed with ice-cold methanol for 4 min at 4 °C, and washed twice with PBS. The coverslips were then incubated at room temperature for 1 h with primary antibody, washed twice with PBS for 5 min, incubated at room temperature for 30 min with fluorescent (Texas red or fluorescein isothiocyanate)-conjugated second antibody, and washed twice again with PBS for 5 min. The coverslips were then dried, mounted onto glass slides, and viewed by MRC-1024 confocal microscopy (Bio-RAD).

Northern Blot Analysis—A 607-bp fragment (1564–2171 bp) of dymple cDNA was amplified by PCR labeled with [32P]dCTP using a random priming kit (Boehringer Mannheim). Labeled probe was hybridized to multiple tissue Northern blot in ExpressHyb hybridization solution (CLONTECH). As a control, the same multiple tissue Northern blot was rehybridized with the 2.0-kb β-actin cDNA control probe supplied with the kit. The blot was exposed to an imaging plate overnight and analyzed with a BAS-2000 Image Analyzer (Fujix, Tokyo, Japan).

Reverse-transcriptase (RT)-PCR—Total RNA from ICR mouse brain, skeletal muscle, and testis was used for transcriptions. RT reactions were conducted at 70 °C for 5 min, 42 °C for 40 min, and 70 °C for 15 min. To terminate the reaction, 0.5 μl of RNase H (Toyobo, Tokyo, Japan) was added to the reaction mixture, followed by incubation for 15 min at 55 °C. The primers used were 5′-TTAGTGGCAGGTTGACTG-3′ and 5′-GGAGGCAATAGCGGTAG-3′. A 1-μl aliquot of RT reaction solution was used for the amplification of dymple cDNA by PCR cycling in a 40-μl reaction containing 1× EX-Tooq buffer (Takara; Tokyo, Japan), 10 pmol of each primer, 0.2 mM dNTPs, and 0.3 μl of EX-Tooq polymerase (Takara). The PCR involved denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min for a total of 38 cycles. Amplified products were analyzed by agarose gel electrophoresis and ethidium bromide staining. The cDNA fragments were cloned into the pGEM-T TA cloning vector (Promega, Madison, WI).

In Situ Hybridization—A 607-bp fragment was labeled by RT-PCR from mouse brain as described above and subcloned into the pGEM-T TA cloning vector (Promega). A digitonin-labeled antisense RNA probe was produced by in vitro transcription with SP6 RNA polymerase using the Neol-linearized plasmid as the template, and a sense RNA probe was made with Spel digest and T7 RNA polymerase using the same plasmid. The preparation of samples and hybridization were performed as described previously (35).
Fig. 1. Primary structures of dymple. A diagrammatic representation of the structures of the dymple mRNA (top) and the immunoscreened cDNA clones (below). The open box indicates the coding sequence, and the stippled boxes show the in-frame deletions found in clones 14 and 31.
cDNA expression library was screened with serum 114 taken from a scleroderma patient to obtain 20 positive clones. Partial sequence analysis showed seven of these to be overlapping (03, 04, 14, 31, 33, 51, and 61 in Fig. 1A). These clones had significant homology with dynamin family GTPases in the 5' sequence. To obtain an extended 5' sequence, 5'RACE was carried out. The obtained 699-bp-length fragment (D1) had a 308-bp overlap with clone 04 and extended 391 bp beyond its 5' end. D1 and 04 were ligated using an internal HindIII site, resulting in a full-length clone (D104, 2524 bp) with an open reading frame of 2208 bp (736 amino acids). The initiation codon was found 84 bp downstream of the in-frame stop codon. A putative polyadenylation signal (AATAAA) was found at bp 2502 of this clone.

A homology search in the Genbank™ data base revealed that the deduced amino acid sequence of the clone shares high homology with dynamin family GTPases, especially yeast DNM1P and VPS1P (Fig. 1B). The sequence similarity was pronounced in the amino-terminal half around the three conserved GTP binding domains (indicated as 1, 2, and 3 in Fig. 1B) but less so in the carboxyl-terminal remainder. The most remarkable difference from known dynamin isoforms was found to be the lack of proline-rich carboxyl-terminal domain, so we named this clone dymple for dynamin family member proline-rich carboxyl-terminal domain less. This carboxyl-terminal region is known to regulate dynamin GTPase activity among the dynamin superfamily members. The divergence in the PH domain was even greater, dymple most resembling yeast DNM1P, but actually no sequence similarity was evident with the primary homology searches.

To examine tissue-specific expression of dymple mRNA, human multiple tissue Northern blots were hybridized with the carboxyl terminus of clone 04 (Fig. 1A) as a specific probe. As shown in Fig. 2 (upper panel), dymple demonstrated two major transcript sizes of 2.5 and 4.2 kb. Ubiquitous expression of both of them at low level was found with high level, the smaller one proved to be abundant in the testis. Because the middle size of the 3' sequence in multiple overlapping cDNA clones (clone 14 and 51 in Fig. 1A) appeared to arise from mis-anneling of the oligo-(dT) primer during the library synthesis, the two transcript sizes correspond well to the alternative polyadenylation

The consensus GTP binding motifs (I, II, III) adot enclose by vertical lines. The corresponding region used as the probe for the Northern blot analysis and the in situ hybridization experiment are indicated below the mRNA (A), represents a putative polyadenylation signal. B, comparison of the amino acid sequences of human dymple, dynamins I and II, and yeast DNM1P and VPS1P. Dashes represent gaps inserted to facilitate alignment. Identical residues to those in dymple are indicated by reverse-contrasted letters. The locations of consensus GTP binding motifs (I, II, III) are indicated by double underlining. Alternative splicing sites in dymple between the (> ) and (< ) and in dynamin I underlined with ($) are also indicated. The PH domain of dynamin I is designated as ($) for consensus and ( ) for spacer regions. The membrane targeting motif in dynamin I (25) is underlined with (#!), and the binding site for SH3 domain (PXXPPPXXPX) is indicated. C, multiple alignment of the amino acid sequences between the first and second consensus of the GTP binding domain. Region 2 of the self-assembly motif found in the Mx family of proteins (7) corresponds to the variable region in the dynamin superfamily, whereas region 1 of the same motif is highly conserved. Note that each subfamily has a typical sequence pattern and that dymple and the C. elegans clone T12E12.4 have identical spacing sizes. The indicated sequences are L07807 (human h) dynamin I), L36983 (human dynamin II), L40588 (yeast DNM1), M33315 (yeast VPS1), M12279 (mouse (m) Mx1), M30817 (human MxA), M30818 (human MxB), U61944 (C. elegans cosmids T12E12).
found in the cDNA clones described above. The 111-bp in-frame deletion in the coding sequence (described below) could not be analyzed in this experiment.

HA-tagged dymple and mouse Mx1 were transiently expressed in COS7 cells, and the expressed proteins were detected with anti-HA mAb and the human serum 114 on nitrocellulose membranes. Both gave bands at 80 kDa, corresponding to their deduced amino acid sequences (Fig. 3). Only dymple was visualized by the human serum 114, suggesting that this recognizes a nonconserved region of dynamin-related proteins.

Because high molecular weight GTPases are known to have relatively high basal GTP hydrolysis activity (5, 34, 38), bacterially expressed GST fusion dymple was purified, and its GTPase activity was examined. Like the murine Mx1 protein, dymple could hydrolyze GTP without additive modifications or coactivators (Fig. 4).

Intracellular Localization of Dymple—The anti-dymple pAb raised against a carboxyl-terminal 166-amino acid peptide of human dymple allowed investigation of the distribution of wild-type and mutant forms by confocal beam scanning laser fluorescence microscopy (Fig. 5). The wild type, a form with a point mutation in the first consensus GTP binding domain and an amino-terminal deletion mutant lacking the entire GTP binding domain were transiently expressed in COS7 cells and detected with the anti-dymple pAb. To address the question of whether the TGN counterpart of dynamin is dymple, cells were double-labeled with γ-adaptin, a component of the AP1 complex (39).

Overexpressed wild-type dymple localized to the perinuclear zone with a punctate cytoplasmic pattern, but neither interference nor obvious overlapping were observed with γ-adaptin, as seen in a similar experiment with dynamin (30). A serine 39 to isoleucine mutation in the first consensus GTP binding domain (S39I) resulted in decreased signal in the perinuclear region with no alteration in the localization of γ-adaptin. The amino-terminal deletion mutant (N246) lacking the entire GTP binding domain, however, made large aggregates surrounded by γ-adaptin-labeling with quite little overlapping, although weak uniform cytoplasmic staining also remained. An analogous dynamin mutant (N272 construct) also forms aggregates but throughout the cytoplasm (30). Brefeldin A treatment altered the localization of γ-adaptin as shown previously (39), but no changes in the staining pattern for the overexpressed wild-type and dymple mutants were detected when used in the present study (data not shown). Endogenous dymple protein in COS7 cells can be recognized with this pAb in Western blots (data not shown) but was not visualized in immunofluorescence at the applied dilution.

Tissue-specific Splicing and Distribution of Dymple—We found two types of “in-frame deletions” within multiple overlapping cDNA clones. As indicated in Fig. 1B, clones 14 and 31 had 87- and 111-bp deletions, resulting in 29- and 37-amino acid deletions, respectively. This was also proved by RT-PCR analysis of the carboxyl terminus of dymple (1564–2171 bp) using mRNA prepared from murine brain, testis, and skeletal muscle (Fig. 6A). Sequence analysis of the cDNAs recovered from the major product bands (a 607-bp band for brain, a 529-bp band for testis, and a 496-bp band for skeletal muscle) revealed their deduced amino acid sequences to be almost identical to the corresponding parts of clones 04, 14, and 31, respectively, with exactly the same splicing patterns (summarized in Fig. 6B and C). These results indicate that these variants are caused by natural 3′ alternative splicing and are
not a cDNA synthesis artifact, strongly suggesting the existence of tissue-specific alternative splicing. Dynamin isoforms also have alternative exon use of the middle region that is identical in both dynamin I and II, whereas these two have alternative termination and an in-frame deletion of 4 amino acids, respectively (21, 40), although the sites and patterns of alternative splicing are different between dymple and dynamin isoforms.

Cell- and site-specific expression of dymple was examined using murine brain sections with the murine brain-form fragment of dymple as the probe. Abundant expression of dymple mRNA was noted in the cerebellum and in the several regions of the cerebrum and diencephalon (Fig. 7A). Prominent signals were observed in the cerebellar Purkinje cells (Fig. 7B) and in the pontile giant neurons (Fig. 7C).

DISCUSSION

In the present study, we isolated a novel GTPase clone, dymple, from a HeLa cell cDNA library by immunoscreening with serum from an autoimmune patient. The epitope recognized by the antiserum was concluded to be located in the carboxyl terminus (460–736 amino acids), since all immunopositive clones overlapped in this region (Fig. 1A). Its low level of sequence conservation as compared with dynamin superfamily members (5) presumably contributed to the observed specificity of the serum.

The dynamin superfamily is subdivided into groups, each named for a prototypic member: dynamin, VPS1P, Mx, and Mgm1p (13). The primary sequence structure of dymple indicated that it should be classified into the DNM1P/VPS1P subgroup because of the much closer similarity than with the Mx family proteins or the yeast Mgm1p. Furthermore, a BLAST search of the full-length amino acid sequence of dymple scored slightly higher against the yeast DNM1P and VPS1P than human dynamin I. Both dymple and DNM1P/VPS1P lack the proline-rich carboxyl-terminal domain, which is the most obvious difference between dynamin isoforms and other related proteins found so far.

Comparison of the sequence revealed two regions with least homology and thus, most likely to be responsible for specific function. The diversity of carboxyl-terminal halves especially around the region corresponding to the PH domain of dynamin is very pronounced, as noted elsewhere (5). This also applies to dymple, since in this respect it resembles neither known members of the dynamin superfamily nor other sequences in the GenbankTM data base. In the sequence after the first consensus of the GTP binding domain (Fig. 1C), which corresponds to domain 2 of the self-assembling motif in mouse Mx1 (Fig. 7 in Ref. 7), the yeast DNM1P/VPS1P possess larger spacer regions than dynamin or Mx family proteins (13). Dymple also has a unique sequence here, although its length differs from those of DNM1P/VPS1P or dynamin. The functional significance of these differences still remains to be elucidated, but the identical spacing size among dymple and the C. elegans clone T12E12.4 (Fig. 1C) suggests that these variable lengths may characterize each of the dynamin superfamily subgroups.

Recent studies have revealed multiple regions that target dynamin to both clathrin-coated and non-clathrin-coated plasma membrane elements (25). The sequence PAVP-PARP94 in rat dynamin is one expected binding site for SH3 domains necessary for colocalization with clathrin on coated pits, and the sequence ALKEALSIIDIN746 is involved in membrane binding. The latter sequence motif corresponds to...
the extreme carboxyl terminus of the dymple and DNM1P/ VPS1P sequences. Up to 100 amino acids upstream of this motif, but not the additional upstream sequence where dynamin PH domain resides, is sufficient for membrane binding in the case of dynamin and appears to be highly conserved among dynamin family members including dymple (Fig. 1B), implying that dymple might target membrane elements other than clathrin-coated plasma membranes.

An immunofluorescence study revealed that dymple and dynamin might have similar characteristics for cycling between the soluble and membrane-bound states and common functional property among amino-terminal GTP binding and carboxyl-terminal putative domains for protein interaction. Dynamin has been found to be abundant in the cytosolic pool, with binding sites at the plasma membrane being saturable; thus, mutant forms exert dominant interference (30). In our experiment, wild-type dymple was also found to be distributed like dynamin, conceivably for analogous reasons, but amino-terminal deletion resulted in aggregation at different places for the two proteins: inside areas of TGN with dymple and throughout the cytoplasm in the dynamin case. The different accumulation patterns may reflect variation in the membrane localization targeted by specific binding factors, although there remains a possibility that the large aggregates were merely caused by inclusion into some other structure. Both in vitro and in vivo interactions between dynamin and AP2 complex were confirmed, and expression of mutant dynamin was shown to alter the distribution of α-adaptin (30). In the case of dymple, neither interference nor obvious overlapping with γ-adaptin were observed, so clathrin-coated TGN membranes can be dismissed as possible targets.

The expression pattern of dymple is reminiscent of the tissue specificity of dynamin isoforms in rat (40). Dynamin I is exclusively expressed in neuronal tissues (17, 18), whereas dynamin II is reported to be ubiquitously expressed (20, 21), and dynamin III is testis-specific (41), each having different alternative splicing patterns. This suggests that the similarities in tissue distribution patterns may reflect a functional relationship between dymple and dynamin isoforms. Although it is possible that we found a ubiquitous type and that another tissue-specific isoform exists, the same dymple gene is strongly expressed in both neurons and testis. On the other hand, we demonstrated tissue-specific alternative splicing patterns within the coding sequence of dymple, resulting in an in-frame deletion (Fig. 6B). The physiological significance of this phenomenon is unknown, but an attractive speculation is that such variation causes differential specificity of interaction. Interestingly, there are conceivable proline-rich sequence motifs around the deletion (Fig. 6C).

Since tubulation and vesiculation seem to be innate properties of the intracellular membranes responsible for protein transport (42), it is tempting to speculate that each of the transport pathways utilize dynamin-related molecules. Previously, the potential association of a dynamin-like protein with the Golgi apparatus in mammalian cells was discussed (43). Immunofluorescence, subcellular fractionation, and electron microscopy have demonstrated that pAbs raised against three different peptides from the amino-terminal-conserved domain of dynamin labeled the Golgi complex. These peptide sequences are generally conserved in dymple, with LTLVDLPGMTKV sequences in the second GTP binding motif being identical. The corresponding pAb MC12 was found to recognize an additional faint 80–90-kDa protein in rat brain and cultured human fibroblasts other than the 100-kDa major band of dynamin (Fig. 2 in Ref. 43), which seems to be consistent with our findings. It is likely that different anti-dynamin antibodies give apparent various staining patterns in immunofluorescence studies, so our result with overexpressed dymple (Fig. 5) would not be inconsistent with previous data whether the Golgi-associated dynamin-like protein includes dymple or not. Further electron microscopic analysis is needed to determine the precise localization, especially whether the Golgi is involved.

Dynamin participates in the budding of plasma membrane-derived clathrin-coated vesicles with the AP2 complex but not
TGN-derived ones associated with AP1 (31). Thus the most likely position where dynamin-like molecules might exist is the clathrin-coated pit at the TGN membrane. Dymple, however, cannot be a simple TGN counterpart of dynamin for the reasons outlined above. This would likely require a dynamin isofrom with a proline-rich carboxyl-terminal domain, since the latter appears necessary for the mechanism of AP2 complex recruitment of dynamin to clathrin-coated plasma membranes (26).

Furthermore, the sequences are similar between α-adaptin and γ-adaptin (39), subunits of AP2 and AP1 complexes that seem to serve for the specificity of adaptor complexes for the plasma membrane and TGN, respectively. Dymple does not complement VPS1 mutation in yeast cells,2 so it can also not be a cognate homologue of yeast VPS1P. The available data thus indicate that the protein transport in a manner similar to dynamin, functional properties of this protein.

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