I Introduction

Cellular compartmentalization is a characteristic feature of the eukaryotic cells. Membrane-bound compartments like endoplasmic reticulum, Golgi apparatus, endosomes and lysosomes, as well as plasma membrane domains require controlled transport of proteins and lipids to fulfill their individual functions. This transport is mediated by transport vesicles that were first characterized in 1975 by Palade (Palade, 1975). In the following years it became evident that plasma membrane domains and membrane-bound intracellular organelles maintain their protein and lipid homeostasis by regulated vesicular transport pathways and that secretory proteins are exported by vesicular exocytic carriers (reviewed by Rogers and Gelfand, 2000; Tooze, 1998).

Secretory and transmembrane proteins are synthesized by ribosomes bound to the rough endoplasmic reticulum (rER). The growing polypeptide chains are injected into a protein channel that leads soluble proteins into the lumen of the ER whereas membrane proteins leave the channel laterally and become inserted into the ER membrane. After initial glycosylation steps and chaperone-assisted folding, both protein classes accumulate at ER exit sites where they are concentrated and sorted into transport vesicles. Active sorting of proteins discriminates cargo proteins form unfolded and ER-resident proteins that are excluded from the vesicles (Stevens and Argon, 1999). The vesicles move along microtubule (MT) to the intermediate compartment and finally reach to the Golgi cisternae where cargo proteins are further modified by glycoprotein trimming enzymes and glycosyltransferases (Munro, 1998). At trans-Golgi network (TGN), proteins are sorted and packed into several classes of vesicles that deliver them to endosomes, lysosomes or to the plasma membrane (reviewed by Ikonen and Simons, 1998; Farquhar and Palade, 1998; Tooze et al., 1998) (Figure 1). By fusion of transport vesicles with their acceptor membrane, membrane proteins and lipids are merged into the acceptor membrane whereas soluble proteins are injected into the lumen of the organelle or, in case of the plasma membrane, are secreted into the extracellular space. This transport pathway, which carries newly synthesized proteins from the ER to their final destinations, was given the term anterograde pathway (Figure 1, route 1-10). To keep cell organelles in an equilibrium, equal amounts of proteins and lipids have to move in reverse directions, which are collectively specified as retrograde pathways (Bannykh et al., 1998) (Figure 1, route 11-

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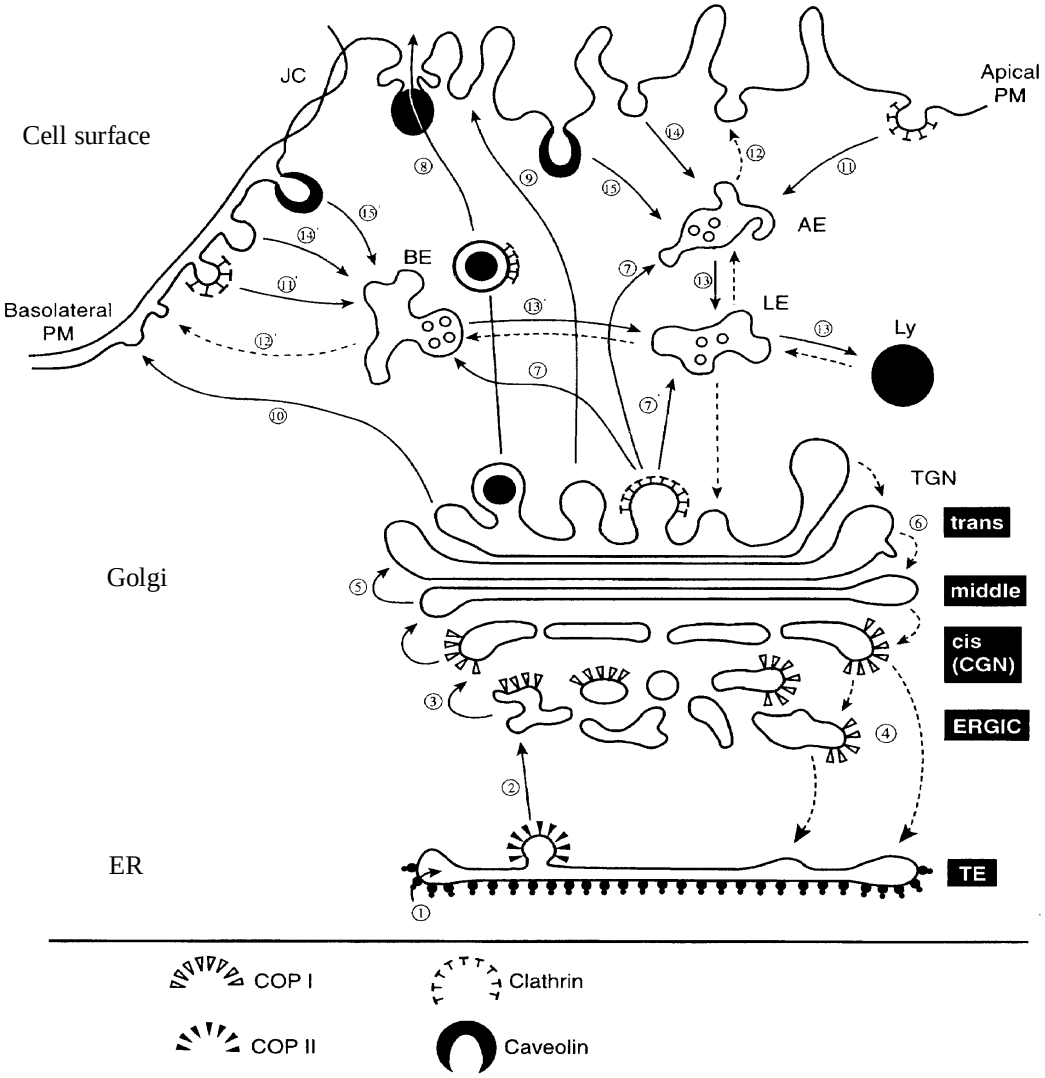

Figure 1. Diagram depicting the major routes of vesiclular traffic along the exocytic (1-10) and endocytic (11-15) pathaways (Farquhar and Palade, 1998). *Exocytic pathway*: Secretory proteins, membrane glycoproteins and lysosomal enzymes are synhesized on polyribosomes and are translocated to the ER (1) where they undergo cotranslational and post-translocational processing (e.g. disulfide bond formation, glycosylation, trimming, and oligomeriza-tion in some cases). They exit the ER via COP II vesicle (2) that shuttle them to the ER-Golgi intermediate compartment (ERGIC). From there they are transported to the cis-Golgi network via COP I-coated vesicles (3). COP I-coated vesicles also function in retrograde transport from the cis-Golgi and ERGIC to the ER (4). Subsequently the proteins either traverse the Golgi cisternae one by one via vesicluar carries (5) or transport occurs by cisternae progression (maturation). Retrograde transport is also assumed to take place between the stacked cisternae (6). Sorting occures in the trans cisternae or TGN: Lysosomal enzymes bind to M6P receptors, are packaged into clathrin-coated vesicles in the TGN, and are delivered either to early (7) or late (7´) endosomes. Membrane and secretory proteins are also sorted in the TGN and delivered by exocytosis along the regulated secretory pathway via secretion granules (8) or along the constitutive pathway (9). In polarized secretory cells there is a separate pathway for delivery of vesicles to the basolateral domain (10). *Endocytic pathway*: Several endocytic pathways have also been charted. The best characterized is receptor-mediated endocytosis via clathrin-coated vesicles budding from either the apical domain of the plasma membrane (PM)(11) and directed to apical early endososmes (AE) or from the basolateral PM domain (11´) and directed to basal early endosimes (BE). Recent evidence indicates that there is also exchange between apicla and basal endosomes. Many receptors (LDL, transferrin) recycle back to the plasma membrane from early endosomes (12), where many ligands are transported from early to late endosomes (LE) to reach lysosomes (Ly)(13). Other pathways include uptake in non-clathrin coated vesicles(14) or caveolae (15).

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15). A main retrograde transport pathway starts with endocytosis (Figure 1, route 11) of membrane proteins, membrane lipids and extracellular proteins at the plasma membrane (McClure and Robinson, 1996). The formed early endosomes are converted - after sorting of some proteins and receptors into a recycling compartment (Figure 1, route 12) - into late endosomes (Figure 1, route 13) from which their cargo is transported to lysosomes, TGN or ER.

Vesicle formation in all transport pathways has to be regulated quantitatively to synchronize anterograde and retrograde transport. Each vesicle class has to be addressed specifically at the donor membrane to find its proper destination and to fuse with the acceptor membrane. For both constitutive and regualted anterograde transport of proteins, the TGN plays a key role and vesicle formation at the TGN was therefore studied in detail.

1.1 Vesicular transport of newly synthesized proteins between the TGN and the plasma membrane

Once the newly synthesized proteins become glycosylated and eventually folded under formation of disulfide bonds, they exit the ER via COP II vesicles and traffic to the ER-Golgi intermediate compartment (ERGIC). From the ERGIC, proteins were transported via COP I vesicles to the cis-Golgi network (reviewed by Bannykh et al., 1998; Nickel et al., 1998) (Figure 1, route 1-3) and traverse the Golgi apparatus to the trans-cisternae. On the way, protein glycosyl side chains are trimmed, additional glycosylation, sulfation and phosphorylation occurs, and the proteins finally reach the trans-most Golgi cisternae (Figure 1, route 5) where the TGN emerged. The individual protein classes are sorted and packaged into distinct populations of vesicles that are subsequently transported to specific destinations. Thus the TGN serves as the main sorting station in the anterograde pathway (Traub and Kornfeld, 1997). Although the total diversity of TGN-derived vesicles is presently unknown, three main routes have been characterized. First, clathrin-coated vesicles carrying lysosomal enzymes bound to mannose-6-phosphate receptors (M6PR) are destined for lysosomes via early or late endosomes (Klumperman et al., 1998) (Figure 1, route 7 and 7´). Second, constitutive secretory vesicles send both membrane and secretory proteins to the plasma membrane (Figure 1, route 9). Third, heparansulfat proteoglycan (HSPG)-containing vesicles that are formed constitutively at the TGN deliver HSPG to the plasma membrane (Nickel et al., 1994). In polarized cells, two distinct types of the TGN-derived vesicles trafficking either to the apical or to the basolateral domain (Figure 1, route

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10) of the plasma membrane have been characterized and first information on the composition of both vesicle types has been obtained (Shevchenko et al., 1997).

In regulated secretory cells such as cells of endocrine and exocrine glands and in neurons, additional populations of immature secreotory granules are formed at the TGN and subsequently maturate to secretory granules containing regulated secretory proteins or hormones (Thiele and Huttner, 1998) (Figure 1, route 8). The granules are stored near the cell surface and their contents are discharged by fusion of granules with the plasma membrane upon external stimulation of the secretory cell. In addition to the transport of newly synthesized proteins, the intracellular membrane transport via vesicles, vesicular tubules or strings composed a basic mechanism to change the shape of the cell and to transform plasma membrane domains specifically for cell movement and signal transduction between neighbouring cells (Roth and Sternweis, 1997; Ceresa and Schmid, 2000; Corvera and Czech, 1998). Hence, the vesicle traffic pathways require precise regulation to keep the effectivity and the specificity.

1.2 Vesicular transport

Vesicular traffic comprises three main steps: vesicle formation at donor membrane, vesicle transport between membrane compartments and fusion of vesicle with acceptor membrane.

1.2.1 Formation of vesicles at donor membrane

Vesicles were formed at special sites of donor membrane where cargo proteins have been concentrated by binding sorting receptors, which also recruit coat proteins to, the cytoplasmic phase of the donor membrane (Kirchhausen et al., 1997). Sorting receptors located on the donor membrane can recognize sorting signals on the cargo proteins and help package them into specific transport vesicles. At Golgi, for example, mannose-6 phosphate (M6P) residues were specifically added to the N-linked oligosaccharides of lysosomal hydrolases which can be recognized by M6P receptor (M6PR) on the TGN membrane and were sorted into special vesicles destined for endosomes (Brown and Farquhar, 1984). The protein coat is thought to change membrane curvature and may induce the formation of coated buds by physically deforming a membrane microdomain and sequestering selected membrane proteins into the emerging vesicles.

The formation of clathrin-coated vesicles has been extensively studied and is often used as a paradigm for the molecular organization of vesicle formation (reviewed by Le Borgne and Hoflack, 1998). The basic component of the clathrin coat is the clathrin triskelion

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consisting of three heavy clathrin chains (190 KD) and three light clathrin chains (35 KD) (Smith and Pearse, 1999). The anchor points for the triskelion at vesicle membranes are heterotetrameric adaptor protein complexes (AP) that are essential for assembly of clathrin coats (Hirst and Robinson, 1998). The clathrin triskelions form a polyhedral lattice that is believed to provide the driving force for formation of membrane buds. Recruited APs have three functions. First, they link clathrin to the membrane by interaction with membrane lipids. Second, they interact with the cytoplasmic domain of sorting receptors (cargo recepor), concentrating and selecting cargo into clathrin-coated vesicles. Third, adaptor complexes recruit other essential molecules involved in vesicle formation, such as SH3-containing protein amphiphysin and the GTPase dynamin. TGN-derived clathrin coated vesicles contain AP-1 complexes, consisting of β1- and γ–adaptin, as well as μ1- and σ1-subunits. AP-1 complexes have been implicated in sorting of newly synthesized lysosomal enzymes into M6PR coated vesicles which destined for early endosomes. Clathrin coated vesicles at the plasma membrane contain the structurally related AP-2 complex of α- and β- adaptin as well as μ2- and σ2-subunits. AP-2 facilitates the receptor- mediated endocytosis of ligands, such as low-density lipoproteins (LDL) and transferrin (Tfn). Recently, two additional types of adaptor complexes, AP-3 and AP-4, have been identified. AP-3 is composed of δ- (160 kD) and β3A- (120 kD) adaptins associated with the μ3- (47 kD) and σ3- (22 kD) subunits and was located to the Golgi or to endosomes (Dell'Angelica et al., 1997). In analogy, AP-4 contains ε- , β4-, μ4- and σ4- subunits (Hirst et al., 1999) and is exclusively recruited to the TGN (Dell'Angelica et al., 1999). Both AP-3 and AP-4 are ubiquitously expressed and are involved in recognizing and sorting of cargo proteins with tyrosine-based motifs (Odorizzi et al., 1998). But whether or not protein sorting mediated by AP-3 or AP-4 depends on clathrin coats is currently not clear.

At the plasma membrane, recruitment of coat proteins from the cytosol proceeds in three steps. First, AP-2 proteins are attached in an ATP- and GTP-independent reaction by binding to appropriate membrane sites. Bound AP-2 complexes then mediate the binding of clathrin triskelions to form a planar clathrin lattice on the cytoplasmic side of the plasma membrane. In the third step, the planar lattice invaginates aided by other proteins like synaptajanin, amphiphysin and dynamin to form a coated bud.

Recruitment of clathrin coats to the TGN is also required for vesicle formation, but *in vitro* binding studies indicate that AP-1 binding to the Golgi is regulated by the GTPase ARF1

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in form of the GTP complex (Zhu et al., 1998). ARF1·GTP initiates clathrin-coat assembly by generating high-affinity membrane-binding sites for the AP-1 adaptor complex (Traub et al., 1993). Other proteins such as M6PR, which is sorted into coated buds, and some docking proteins have been suggested to be partners of ARF·GTP in generating the AP-1-docking sites (Simon et al., 1996; Happe and Weidman, 1998).

At later stage, the bud is deeply invaginated and the neck of bud is constricted until membrane scission occures and a closed vesicle is formed. Among the proteins being required for this step, dynamin, a member of a large GTPase family, was identified. Overexpression of a defective mutant form (Dyn-K44A) in HeLa cells blocked vesicle formation and resulted in formation of accumulated long tubules at local plasma membrane (Damke et al., 1994). Similar structures were also observed in nerve termini when GTP-γ-S was used to block the GTP hydrolysis by dynamin (Takel et al., 1995). Also in this case, coated pit assembly, invagination, and the recruitment of receptors into coated pits were not affected, suggesting that dynamin acts at late stage of vesicle formation (Kirchhausen, 1998).

1.2.2 Transport of vesicles

If vesicles are formed near to the acceptor membrane, they may freely diffuse to reach their destination. When they have to travel over a long distance, they move along microtubules by means of dynein (Gilbert and Sloboda, 1989; Schroeder et al., 1990) or other motor proteins (Kreitzer et al., 2000). Using GFP-labeled vesicular stomatitis virus G protein (VSVG) that moves from TGN to plasma membrane, rhodamine-labeled MT was found to be used as the track for VSVG-containing vesicles, and the post-Golgi carriers are capable of switching between MT tracks (Toomre et al., 1999). In PC12 cells, GFP-labeled chromogranin B (hCgB) is sorted into immature secretory granules that move bidirectionally along MT tracks. Disruption of MT retarded the delivery of hCgB to the plasma membrane (Wacker et al., 1997). Also, transport pathway between ER and Golgi are blocked by disassembly of MT (Sciaky et al., 1997; Lippincott and Cole, 1995). In addition to MT, other proteins of cytoskeleton, such as spectrin and ankyrin-like proteins have an impact on vesicle transport. They form a meshwork at cytoplasmic face of the plasma membrane or associate with Golgi and pre-Golgi membranes (Devarajan et al., 1996) and may interact with vesicles. Myosin/p200 is recently found to be involved in the assembly of basolateral transport vesicles carrying VSVG from the TGN of polarized

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MDCK cells (Musch et al., 1997), suggesting that the actin cytoskeleton also participates in the process of vesicle transport.

1.2.3 Fusion of vesicles with acceptor membrane

Vesicles deliver their cargo to the final destinations through fusion with the target membrane. Vesicle fusion is preceded by a tethering/docking step to immobilize vesicles near the acceptor membrane (reviewed by Waters and Pfeffer, 1999). Tethering proteins are usually large and contain high content of coiled-coil structures, for example, p115 in mammalian cells or Uso1 in yeast. (Sapperstein et al., 1996). ER-derived vesicles are tethered to the cis-Golgi cisternae through interactions between p115 and the Golgi receptors GM130 and giantin (Shorter and Warren, 1999). In addition, the TRAPP complex with a molecular weight of about 800 kD stably associates with the Golgi and is needed for docking of ER-derived vesicles at the Golgi in yeast (Sacher et al., 1998). Recently, the exocyst complex, which associates with secretory vesicles, has been found to dock vesicles to the sites of exocytosis on the plasma membrane through interaction with Sec3p (Guo et al., 1999). It is assumed that tethering factors may collaborate with Rabs and SNAREs to increase targeting specificity in secretory pathway.

In a second step, docked vesicles fuse with the acceptor membrane by forming SNARE complexes that contain, as exemplified for synaptic vesicles, VAMP2, SNAP-25 and syntaxin (Hay and Scheller, 1997). The two membrane compartments are brought in close contact by complex formation between SNAREs on the vesicle (v-SNAREs) and SNAREs on the target membrane (t-SNAREs). The high affinity between v-SNAREs and t-SNAREs, indicated by the resistance of the complexes to 0.1% SDS, may generate enough force to drive membrane fusion. The initially identified complex of SNARE proteins consist of synaptobrevin 2 (VAMP2) as a v-SNARE (Sollner et al., 1993), syntaxin (Walch et al., 1995), as well as SNAP-25 as t-SNAREs (Chapman et al., 1994). Later, more putative v- and t-SNARES have been identified and shown to be required for specific vesicular transport between ER and Golgi, as well as between Golgi and plasma membrane (Nichols and Pelham, 1998). However, recent studies have shown that SNAREs alone may not be sufficient for vesicle fusion: other partners like synaptotagmin, a calcium sensing synaptic vesicle protein, may also contribute to vesicle fusion (Schiavo et al., 1997). Importantly, interaction between SNAREs was tightly regulated by SNARE protectors that prevent uncontrolled fusion. n-Sec1/Munc-18, for example, can bind directly and tightly to

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the t-SNARE syntaxin-1A at the presynaptic plasma membrane and blocks association with v-SNAREs (VAMP or SNAP-25)(Pevsner et al., 1994a).

1.3 Regulation of vesicular transport

Maintenance of bi-directional traffic of vesicles requires effective and precise regulation. A wealth of evidences suggest that key components are small GTPases, heterotrimeric G proteins and membrane lipids, e.g,, phosphoinositides.

1.3.1 Small GTPase

Of the six families of small GTPases, Arf/Sar, Rab(Ypt) and Rho(including Rho, Rac and CDC-42) are the most common regulators of membrane traffic.

Arf GTPase family

Arf was first recognized by its ability to support ADP-ribosylation of trimeric G proteins and was therefore called ADP-ribosylation factor or Arf (reviewed by Chavrier and Goud, 1999; Schimmoller et al., 1997). Arfs are activated by binding of GTP and regulated by guanine nucleotide exchange factors (GEFs), which catalyze the release of GDP and the binding of GTP. GTPase activating proteins (GAPs) reduce the stability of the GTP form. GAPs activate the inherent GTPase of small G-proteins which is, otherwise, very low. Convertion from the active GTP-bound form into the inactive GDP form is triggered by hydrolysis of GTP. The Arf family consists of 6 different Arf proteins (Arf-1 to Arf-6) that associate with intracellular membranes (reviewed by Donaldson and Klausner, 1994). Arf-1 controls the clathrin coat adaptor AP-1 and the nonclathrin coat COP I, whereas Sar1p controls the nonclathrin coat COP II. In the early secretory pathway between ER and Golgi, Arf-1 recruits a cytosolic coat protein complex named COP I onto membranes as a key step in the formation of transport vesicles (Zhang et al., 1994). Arf-1 localizes at Golgi membrane through interaction with γ-adaptin and enhance the release of immature secretory vesicles from the TGN of PC12 cells and of endocrine cells (Austin et al., 2000; Dittie et al., 1996). In addition, membrane association of AP-3 is also regulated by Arf-1(Ooi et al., 1998). Formation of COP II vesicles starts with the recruitment of Sar1p (a Arf related small GTPase) to the ER membrane and conversion to its GTP-bound form by a GEF (Sec12p) (d'Enfert et al., 1991). Besides Arf-1, Arf-6 regulates the endocytic (D'Souza et al., 1995) and the exocytic pathway (Caumont et al., 1998), perhaps by interacting with heterotrimeric G proteins.

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Rab GTPase family

Rab GTPases represent a family of over 40 proteins that are localized to membranes of vesicles and organelles (reviewed by Chavrier and Goud, 1999; Novick and Zerial, 1997). Rab proteins have been primarily implicated in vesicle docking as regulartors of SNARE pairing. However, recent findings suggest that the function of Rab proteins can go beyond this role. They are believed to determine the specificity of membrane transport by controlling vesicle fusion with the appropriate target membrane.

Rab proteins play roles in multiple processes of vesicular traffic. Rab11, for example, functions in both recycling of endosomes and sorting of post-Golgi secretory vesicles (Mammoto et al., 1999). Rab1a, Rab1b and Rab2 participate in the vesicle transport between ER and Golgi (Peter et al., 1994). Rab 6 and Rab 8 regulate vesicle transport from the Golgi to the plasma membrane (Martinez et al., 1994; Huber et al., 1993). In the endocytic pathway, Rab4a, Rab5 and Rab15 associate with early endosomes and play a role in recycling of receptors from early endosomes to the cell surface. Studies on Rab9 indicated a role in vesicle transport from endosome to the TGN.

Rab GTPases interconvert between inactive GDP-bound form and active GTP-bound form. GTP binding causes a conformational change, enabling Rab proteins to recruit docking factors from the cytosol onto membranes. Rab5, for example, catalyses both homotypic endosome fusion and the fusion of newly formed endocytic vesicles with early endosomes through interaction with the tethering protein Rabaptin-5 (Vitale et al., 1998). Rab5-GTP also interacts with EEA-1 which is an early endosome associated protein that may form coiled-coil structures. Another member of the Rab family, Sec4p, interacts with the exocyst complex and is required for the delivery of Golgi-derived secretory vesicles to the plasma membrane in yeast (Guo et al., 1999). The yeast Ypt1 interacts with tethering protein p115/Uso1 and is required for vesicle docking during ER-Golgi transport (Cao et al., 1998a). These evidences reveal a broad involvement of Rab GTPases in intracellular membrane trafficking

Rho GTPase family

Rho GTPases play dynamic roles in the regulation of the actin cytoskeleton, and may indirectly control cell adhesion, motility and shape. In addition, they also participate in membrane traffic (reviewed by Ellis and Mellor, 2000; Tanaka and Takai, 1998). Activated form of RhoA and Rac are able to block internalization of transferrin receptor (Lamaze et

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al., 1996). Activation of RhoA also increases the number of caveolae at plasma membrane (Senda et al., 1997). RhoB regulates endocytic traffic of EGF receptor by retarding the progress of vesicles to lysosome. Another member of the family RhoD is localized to the endosome derived vesicles and regulates the rate of vesicle moving along cytoskeletal tracks (Murphy et al., 1996). Recently, Cdc42 has been shown to regulate polarized endocytosis at the basolateral membrane of mammalian cells (Kroschewski et al., 1999). The data suggest that many pathways of vesicular traffic are regulated by Rho proteins.

1.3.2 Trimeric G proteins

Heterotrimeric G proteins were originally found at the plasma membrane where they transmit signals from seven transmembrane domain receptors to intracellular effectors (reviewed by Ferguson and Caron, 1998). The first evidence for the involvement of Gα subunits in vesicule transport was obtained by inhibiting intra-Golgi transport *in vitro* with AlF_4^- that activated heterotrimeric G protein but not small GTPases (Melancon et al., 1989). Recent reports show that inhibitory trimeric G proteins localize to intracellular membranes, including the Golgi apparatus. Overexpression of Gαi-3 inhibits constitutive secretion of HSPG (Stow et al., 1991). It appears that trimeric G proteins are implicated in control of both exocytic (Stow and Heimann, 1998) and endocytic vesicular traffic (Bunemann and Hosey, 1999). They regulate vesicule transport from ER to Golgi (Schwaninger et al., 1992), budding of vesicles from the TGN destined for the apical and basolateral domains of the plasma membrane (Pimplikar and Simons, 1993; Barr et al., 1991), fusion of vesicles with endosomes (Lenhard et al., 1994) and transcytosis (Bomsel and Mostov, 1993). It has been demonstrated that dynamin interacts (*in vitro*) physically with G-protein βγ-subunits, which results in an inhibition of dynamin GTPase activity (Liu et al., 1997). Thus, dynamin may be targeted to the particular sites during receptor-mediated endocytosis by sharing βγ-subunits with the α subunit. Additionally, other G-protein subunits (GαI-2, Gαs, G-αq, G-βγ, Gαxl) have been found to function in sorting and packing of Golgi vesicles (Hidalgo et al., 1995; Maier et al., 1995; De et al., 1998). However, most results were indirect and based on AlF_4^- or GTPγS-mediated inhibitions. The molecular mechanism by which G-proteins control vesicle formation has not been elucidated yet.

1.3.3 Phosphoinositides

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Phospholipids, which consist of phosphatidylserine, phosphacholine, sphingomyelin, phosphatidylethanolamine and phosphatidylinositol, are the most abundant lipids of biological membranes and have a potential impact on vesicle formation. Among these phospholipids, phosphatidylinositol plays a important role (Corvera et al., 1999). Phosphorylation of its inositol head-group at single or multiple sites results in formation of a number of derivatives, such as phosphatidylinositol 3-phosphate [PI(3)P], phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2], phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3] and so on. It is generally believed that a local increase in the concentration of specific phosphoinositides is a precondition for endocytosis and regulated exocytosis (reviewed by Kobayashi et al., 1998; Roth and Sternweis, 1997). Recent evidence suggested that specific phosphatidylinositides regulate the activity of proteins that have diverse functions in membrane flux. These proteins include dynamin (Lin et al., 1997; Lee et al., 1999; Klein et al., 1998), clathrin-associated AP-2 complex (Jost et al., 1998), and proteins that stimulate guanine nucleotide exchange of Arf (Kahn et al., 1996). The PH domain of dynamin has been identified to bind directly to phosphoinositides by which the GTPase activity was stimulated (Zheng et al., 1996). PI(4,5)P_2 and [PI(4)P] have been shown to be the preferable partners for PH domain of dynamin (Zheng et al., 1996), whereas other forms of phosphatidylinositides show a very low affinity. The adaptor protein complex AP-2 binds PI(4,5)P_2, PI(3)P and inositol phosphates (IP). Phosphatidylinositol binding regulates AP-2 self-assembly and the formation of AP-2 complex with clathrin or with peptides containing endocytic motifs (Jost et al., 1998). Arf proteins were found to bind PI(4,5)P_2 specifically through a PH domain, which increased the rate of GDP dissociation and stabilized the nucleotide-free form of the protein. Because binding of PI(4,5)P_2 is required for Arf to stimulate phospholipase D (PLD) activity and to interact with GAP, PI(4,5)P_2 may act as a cofactor in one or more Arf pathways (Kahn et al., 1996).

Although the mechanism by which phospholipids regulates vesicle formation has not been clarified, it is assumed that the highly charged phosphoinositides could selectively recruit or activate key proteins for vesicle formation. Changes of membrane composition may influence the membrane curvature since head groups of phosphoinositides need more space as provided on the convex site to form the cytoplasmic phase of a vesicle bud, whereas phosphatidic acid, for example, has a very small head group consisting of a phosphate residue and may accumulate on the concave membrane side. Therefore, Sec14, the yeast

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homologue of the phosphatidylinositol transfer protein, may support post-Golgi secretory traffic by maintaining the appropriate phospholipid composition in the Golgi membranes (Cockcroft, 1998). In addition, lipid modification by PtdIns-3 kinase (PI3K) (Domin et al., 2000), Vps34p (Stack et al., 1995), inositol 5'-phosphatase—synaptojamin (Li et al., 1997), PLC and PLD may directly support binding of proteins to the specific phosphoinositides (Terui et al., 1994).

1.4 The dynamin family

Dynamin and their relatives form a superfamily of large GTPases (Table 1) characterized by a molecular weight between 70 and 100 kD. Dynamin contains the GTPase domain (McClure and Robinson, 1996) and other regulator or modulator domains (Figure 2). This protein family displayed several functions in intracellular membrane transport (reviewed by Damke, 1996; McClure and Robinson, 1996; McNiven, 1998) and maintenance of morphology of organelles (Smirnova et al., 1998; Pitts et al., 1999).

Table 1. Members of dynamin family

Subfamily	Identified members	Main functions	Refs
Dynamin	Dynamin 1,2 ,3	Vesicle traffic	[25, 16, 29]
Vps1p/Drp1	Vps1p, Drp1, Dnm1p, DLP1/Dymple/DVLP	Vesicle traffic, maintenance of the morphology of ER and mitochondria	[152, 213, 269] [274, 204, 90]
Mgm1	Mgm1, Mgm1p, Msp1p	Control mitochondrial distribution	[6, 202]
Mx	Mx1 MxA	Anti-RNA viruses	[158, 49
PDL/ADL1	PDL/ADL1(from plants)	Form new cell walls	[67, 157]

Dynamin was initially identified from bovine brain based on its ability to bind microtubules in a nucleotide-dependent manner (Shpetner and Vallee, 1989). Molecular cloning of the rat brain dynamin identified it as a GTP-binding protein (Obar et al., 1990). Further functional characterization of dynamins was finally initiated by the observation that a *Drosophila* fly carrying a "*shibire*^{ts}" mutant gene, which is homologous to the mammalian dynamin, showed a temperature-sensitive paralytic phenotype (Chen et al., 1991; van-der Bliek and Meyerowitz, 1991). The mutant flies are unable to move above the permissive temperature but are completely viable at lower temperature. Ultrastructural analysis found a rapid loss of synaptic vesicles and accumulation of deep invaginations at

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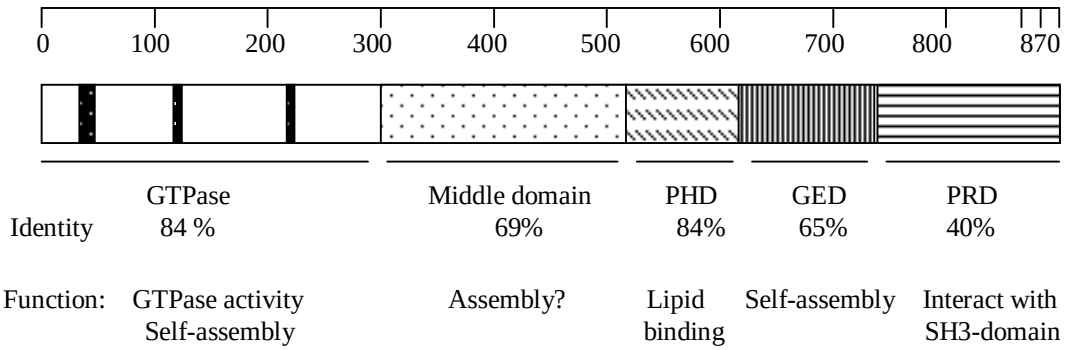

Figure 2. **Domain structure of dynamin.** Dynamin is composed of an N-terminal GTPase domain (1-300), a middle domain (301-520), a pleckstrin homology domain (521-622), a GTPase effector domain (623-745) and a C-terminal proline-rich domain (746-870). Percent identities among rat dynamin 1, 2 and 3 isoforms are shown at the amino acid level. Putative functions of individual domain are listed below the figure.

the synaptic membrane, which suggested a deficiency in recycling of synaptic vesicles (Poodry and Edgar, 1979; Kosaka and Ikeda, 1983; Koenig and Ikeda, 1989). Since the *shibire* gene was found to be a homologue to mammalian dynamin, dynamin could be correlated with synaptic transmission and finally with the formation of synaptic vesicles. Further studies in the last decade have produced a wealth of evidences that dynamins play important roles in endocytosis (Kessell et al., 1989; Koenig and Ikeda, 1990; Tsuruhara et al., 1990). In addition to the recycling of synaptic vesicles, receptor-mediated endocytosis in numerous cell types depends on dynamin, such as uptake of transferrin receptors (Vallee et al., 1993; Altschuler et al., 1998), EGF receptors (Vieira et al., 1996), β2 adrenergic receptors (Zhang et al., 1996), glucose transporter GLUT-4 (Omata et al., 1997) (Volchuk et al., 1998; Kao et al., 2000) and opioid receptors (Chu et al., 1997). Recently, dynamin has been shown to be required for the internalization of caveolae (Henley et al., 1998; Oh et al., 1998)-- a process independent of clathrin and membrane receptor.

While *Drosophila* has only a single dynamin gene, mammalian cells express three dynamin isoforms in a tissue specific manner (Sontag et al., 1994). Dynamin I is exclusively expressed in neuronal tissues, whereas dynamin II is ubiquitously expressed, and dynamin III is predominantly expressed in testis and to a lesser extent in neurons and lung (Table 2). Moreover, each isoform contains several spliced variants (Figure 3). Unlike dynamin I, which acts dominantly in endocytosis in brain, dynamin II has an impact on

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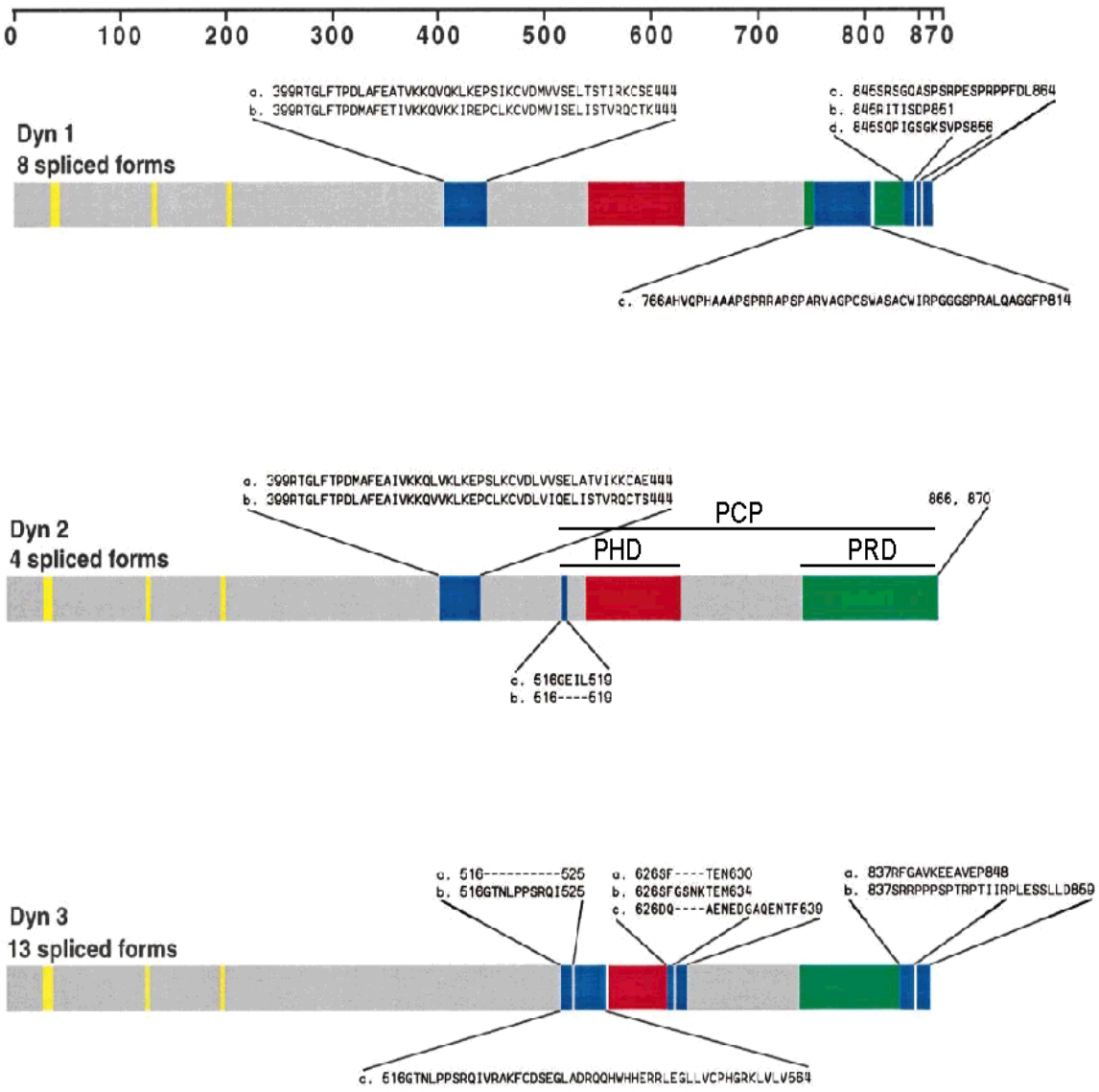

Figure 3. Schematic illustration of the dynamin spliced variants (Cao et al., 1998). The three known dynamin gene products are diagrammed and the corresponding alternatively spliced sites are indicated. The splicing regions are colored as blue. Dyn 1 and Dyn 2 each have two splicing regions, whereas Dyn 3 has three splicing regions. Yellow color represents the GTP-binding sites; red and green colors represent PHD and PRD, respectively. The dynamin II domain PHD, PRD and PCP studied in this work are indicated in the diagram of Dyn 2.

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both endocytic and exocytic vesicle traffic (McNiven et al., 2000). It has been found that the formation of exocytic vesicles at the Golgi membrane depends on dynamin II *in vitro* (Jones et al., 1998), and vesicle transport between endosomes and Golgi may be mediated via dynamin II-dependent mechanism (Llorente et al., 1998). Recent reports showed that dynamin II participated in formation of podosomes (Ochoa et al., 2000) and the process of apoptosis (Fish et al., 2000). Therefore, dynamin II may have more functions than originally predicted.

Table 2. Rat dynamin isoforms and their tissue expression

Isoform	number of variants	Brain	Heart	Kidney	Liver	Lung	Pancreas	Testis
Dyn-1	8	+	-	-	-	-	-	-
Dyn-2	4	+	+	+	+	+	+	+
Dyn-3	13	+/-	+/-	-	-	+	-	+/-

Note: +/- means only some variants of dynamin 3 expressed in indicated tissues

Studies of subcellular distribution in transfected HeLa cells revealed that overexpressed dynamin I and dynamin II closely associate with clathrin-coated vesicles in the cytoplasm as well as with coated buds at the plasma membrane (Damke et al., 1994; Herskovits et al., 1993a). However, differences in the subcellular distribution of spliced variants have been observed. For example, EGFP-tagged dynamin I (ab) and dynamin II (ab) are exclusively localized to the clathrin-coated pits at plasma membrane, while EGFP-tagged dynamin I (bb) and dynamin II (aa) associate with clathrin-coated buds at both the Golgi apparatus and plasma membrane (Cao et al., 1998b; Jones et al., 1998). Moreover, dynamin 3 isoforms also display a differential distribution (Cao et al., 1998b). The reason for the different localization is obscure. It is speculated that the difference in sequences of isoforms and spliced variants may affect the affinity for membrane lipids or protein partners and may also regulate the distribution of dynamins within the cell. The observation that spliced variants exist for each isoform and display heterogenous subcellular distribution has lead to the hypothesis that different isoforms and spliced variants of dynamin may participate in vesicle formation at distinct intracellular locations (Urrutia et al., 1997; Kozlov, 1999).

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1.4.1 Domain structure of dynamins

By sequence comparison, five distinct protein domains (Figure. 2) can be recognized. These are the amino-terminal GTPase domain, the middle domain, the pleckstrin-homology domain (PHD), the putative coiled-coil or GTPase effector domain (GED) and the carboxyl-terminal proline-rich domain (PRD) (Warnock and Schmid, 1996).

Sequence homology analysis of dynamin isoforms shows a different extent of identity between the corresponding domains of dynamin I, II and III. The N-terminal GTPase domains share as high as 84% identity, the middle domains 69%, the PHDs 84% and the GEDs 65%, while the PRDs only share 40% identity (Schmid et al., 1998) (Figure 2). The higher variability of sequences in the PRDs may reflect functional difference between dynamin isoforms.

The GTPase domain

This domain encompasses the N-terminal 300 residues and is highly conserved within the dynamin family (Warnock and Schmid, 1996). It is characterized by three consensus motifs that are required for GTP binding. The three motifs GXXXGKS, DXXG and TKXD form a tridimensional GTP binding structure which in Ras consists of six β sheets interconnected by five α helices (Kjeldgaard et al., 1996). It is presumed that the GTPase domains of dynamins are arranged in a similar fashion.

Two functions of the dynamin GTPase domain have been suggested: first, binding of GTP may alter the conformation of dynamin and may stimulate complex formation with other proteins during vesicle formation, or may expose binding sites for membrane lipids which mediate the attachment of dynamin to functional sites (Sweitzer and Hinshaw, 1998). Second, GTP hydrolysis is certainly needed to recycle dynamin between the inactive form and the active form (Sever et al., 2000). Replacement of the conserved lysine 44 located in the first GTP binding motif with alanine resulted in the dominant defective mutant (dyn-K44A) which lost the ability to bind and hydrolyze the GTP and therefore prevented the internalization of receptors at plasma membrane (Herskovits et al., 1993a; Damke et al., 1994). Morphological study of HeLa cells expressing the dyn-K44A demonstrated an accumulation of elongated tubular and multi-vesicular structures that were very similar to those observed in nerve termini of *shibire*^{ts} flies at nonpermissive temperature (Damke et al., 1994; Baba et al., 1999). This observation, combined with the finding that dynamin I can form ring structures *in vitro* (Hinshaw and Schmid, 1995; Sweitzer and Hinshaw,

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1998) and *in vivo* (Takel et al., 1995), was taken as argument to suggest that dynamin acts like a pinchase during vesicle formation (Hinshaw, 1999; Schmid et al., 1998).

The intrinsic GTPase activity differs between dynamin isoforms: the ubiquitous dynamin 2 has a higher intrinsic GTPase activity than dynamin 1 (Warnock et al., 1997). In addition, the GTPase activity is regulated by binding of lipids or proteins. Negatively charged phospholipids (Tuma et al., 1993; Lin and Gilman, 1996) and SH3-containing proteins, such as Grb2 (Gout et al., 1993) (Herskovits et al., 1993b) and PLC-γ (Scaife et al., 1994), stimulate GTPase activity of dynamin. In addtion to GTP binding and hydrolysis, the GTPase domain may direct dynamin I to distinct subcellular sites as observed in neurons of Caenorhabditis elegans (Labrousse et al., 1998).

The middle domain

The middle domain encompasses residues 301-520 of dynamin and lacks sequence homology to any known structural domain. This domain is, at present, the least characterized one. Based on proteolytic degradation studies, the middle domain has been further divided into an N-terminal half being more conserved (92%) in comparison to the C-terminal half (73%) (Muhlberg et al., 1997). Notably, all alternative splicing sites of the three dynamin isoforms are located within the C-terminal half (Figure 3). However, up to now the exact function of this domain is unknown.

The pleckstrin-homology domain (PHD)

The PHD of dynamin, encompassing residues 521-622, was identified by comparison with other domains being homologous to platelet pleckstrin (Haslam et al., 1993). The PHD of dynamin I was shown to mediate the interaction with βγ subunits of heterotrimeric G-proteins (Liu et al., 1997). This interaction inhibited the GTPase activity *in vitro* and may therefore stabilize the active GTP form of dynamin.

Structural analysis revealed that PHD of dynamin has only limited sequence homology to other known PHDs, however, they all share the well-established structure that consists of a seven-stranded β sandwich and a C-terminal α helix (Ferguson et al., 1994; Timm et al., 1994a). Mutation studies provided evidence that the three variable loops between the β sheets that contain basic residues are responsible for binding of negatively charged lipids. It was therefore suggested that dynamins bind to the membranes via the PHD and become assembled at functional sites (Downing et al., 1994).

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Recently, the ability of PHD to bind phosphoinositides, preferentially [PI(4,5)P_2] and [PI(4)P] (Artalejo et al., 1997; Klein et al., 1998; Barylko et al., 1998) was demonstrated. Binding of phospholipids stimulates the GTPase activity of dynamin (Lin et al., 1997). A PHD truncated dynamin I or a dynamin I with a point mutant in PHD, both of which failed to interact with [PI(4,5)P_2], inhibited endocytosis when expressed in cells (Achiriloaie et al., 1999; Lee et al., 1999; Vallis et al., 1999).

Although PHDs of dynamin I and dynamin II are more than 80% identical, they may differentially regulate dynamin function in endocytosis. Introduction of isolated PHD of dynamin I into cells at concentrations as low as 1 µM completely suppressed the rapid endocytosis, while PHDs from the closely related dynamin-2 were ineffective as inhibitors, even at high concentrations (Artalejo et al., 1997). This suggests that PHDs may contribute to the specificity of individual isoforms of dynamins in distinct endocytotic processes.

The GTPase effector domain (GED)

The residues 623-745 intersected between PHD and PRD have recently been defined as the GTPase effector domain or GED (Muhlberg et al., 1997). This region is predicted to contain α helices to form coiled-coil structures and may mediates protein-protein interaction or domain-domain interaction within the dynamin molecules. The GED has been shown to interact with the GTPase domain and to be required for efficient GTPase activity (Muhlberg et al., 1997; Schmid, 1997). GED may also interact with each other and may thereby promote dynamin self-assembly (Okamoto et al., 1999a). Incubation of a proteolytic fragment containing the GED with the GTPase domain fragment gave rise to a stimulated GTPase activity (Muhlberg et al., 1997). This finding led to the speculation that the GED may function like a GTPase activating protein (GAP) which stimulates the N-terminal GTPase domain of dynamin.

The proline-rich domain (PRD)

The PRD is located at the C-terminus and encompasses the residues 746-870 of dynamin I or II (Obar et al., 1990; Diatloff et al., 1995). The PRD is characterized by several proline-rich sequences (Figure 4) which mediate the interaction of dynamin with SH3-containing proteins. The binding sites for SH3 domains were identified on the basis of two consensus motifs, RXPXXP and PXXPXR (Tan et al., 1999; Feng et al., 1994). Binding of dynamin I to SH3 domains of amphiphysin I (Grabs et al., 1997) and amphiphysin II (Owen et al., 1998), both of which are concentrated in brain tissue, has been observed. In addition,

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dynamin I and dynamin II are able to bind the SH3 domain of growth factor receptor-bound protein 2 (Grb2)—an adaptor protein involving in Ras signaling during tyrosine-phosphorylation of receptors (Yoon et al., 1997; McPherson et al., 1994). Amphiphysin has been shown to bind specifically to the sequence PSRPNR which is conserved in all PRD domains of dynamin isoforms, whereas Grb2 binds to the neighbouring sequence PQVPSR (Figure 4). Other SH3-containing proteins such as endophilin/SH3P4/SH3GL (Ringstad et al., 1999; Ringstad et al., 1997), profilin I and II (Witke et al., 1998; Dong et al., 2000a), syndapin/pacsin (Qualmann et al., 1999; Qualmann and Kelly, 2000), PLC-γ (Seedorf et al., 1994), c-src and Fyn (Gout et al., 1993), Ese1/Eps15 (Sengar et al., 1999), intersectin (Okamoto et al., 1999b; Yamabhai et al., 1998; Pucharcos et al., 2000) and DAP160 (Guipponi et al., 1998) have also been found to interact with dynamin via PRD.

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                                                        c-src/Fyn
dyn1-PRD        STPMPPPVDDSWLQVQSVAGRRSPTSSPTPQRR--APAVPPARPGS--RGPAPGPP----
dyn2-PRD        STPVPPPVDDTWLQNT--------SSHSPTPQRRPVSSVHPPGRPPAVRGPTPGPPLIPM
dyn3-PRD        STPAPPPVDDSWLQHS-----RRSPPPSPTTQRRL-TLSAPLPRPASSRGPAPAIP----
                *** ******:***          .. ***.***  :   *  ** : ***:*. *

                        PLC-γ/p85-PI3K        Grb2/Amphiphysin
dyn1-PRD        PAGSALGG-APPVPSRPG------ASPDPHGPPPQVPSRPNRAPP----GVPSRSGQASP
dyn2-PRD        PVGATSSFSAPPIPSRPGP-QNVFANNDPFSAPPQIPSRPARIPPGIPPGVPSRRAPAAP
dyn3-PRD        SPGPHSG--APPVPFRPGPLPPFPNSSDSYGAPPQVPSRPTRAPP----SVPSRRPPPSP
                  *.  .  ***:* ***       . *.:..***:**** * **    .****   .:*

dyn1-PRD        SRPESPRPPFDL---
dyn2-PRD        SRPTIIRPAEPSLLD
dyn3-PRD        TRPTIIRPLESSLLD
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Figure 4. Comparison of sequences of proline-rich domains of dynamin 1, 2 and 3. Sequence aligment of the PRD from rat dynamin 1, 2 and 3 isoform was analyzed using CLUSTALW program. Amino acids in bold indicate proline residues. The value of residue identity were marked with ´´*´´ under the aligned sequences. Boxed sequences indicate the SH3 binding sites identified for dynamin I.

Interactions between SH3 domain-containing proteins and the PRD may direct dynamin to the coated buds where it functions *in vivo* (Shpetner et al., 1996; Shupliakov et al., 1997). Expression of truncated form of dynamin I lacking the PRD failed to accumulate the mutant dynamin I at clathrin coated pits (Okamoto et al., 1997). Accordingly, binding of SH3 domains is an effective way to stimulate the GTPase activity of dynamin (Gout et al., 1993). For example, recombinant Grb2 and the SH3 domain of c- Src was found to stimulate GTPase activity of dynamin I *in vitro* (Herskovits et al., 1993b). Therefore, it is

hypothesized that PRD plays an important role in targeting and activating of dynamin during vesicle formation.

The PRD of dynamin I can also be phosphorylated by protein kinase C (Robinson et al., 1993), MAP kinase (ERK2) (Earnest et al., 1996) and cdc2 kinase (Hosoya et al., 1994). Phosphorylation of dynamin I regulated the binding to amphiphysin (Slepnev et al., 1998; Liu et al., 1994a) and to the phospholipids (Powell et al., 2000), while dephosphorylation of dynamin 1 by calcineurin caused a decrease in its GTPase activity (Liu et al., 1994b). Recently, it was showed that Grb2-bound dynamin-II can be tyrosine-phosphorylated in response to LPA stimulation. This suggests that dynamin II might be a downstream signal protein in cascade activation of the Ras-MAP kinase. However, the detailed biological meaning is not clear. Although several proteins were identified to bind the PRD of dynamin I *in vitro*, considerable less data are available on binding partners for dynamin II. The low sequence identity between PRDs indicates that binding proteins may differ between dynamin isoforms.

1.4.2 The role of dynamin in endocytosis

Expression of GTPase defective dynamin mutants in mammalian cells revealed a direct and specific role of dynamin in endocytosis (McClure and Robinson, 1996; Damke, 1996). The common used form is the K44A mutant. The mutation abolishes the ability of dynamin I or II to bind and hydrolyze GTP and thus results in a dominant defective phenotype. (van der Bliek et al., 1993). Mammalian cells expressing dyn-1 (K44A) showed elongated invaginations at the plasma membrane which are similar to these observed in nerve termini of mutant *Drosophila* when exposed to nonpermissive temperature (Damke et al., 1994; Herskovits et al., 1993a). This suggests that the role of dynamin is not restricted to formation of synaptic vesicles but is required for endocytosis (van der Bliek et al., 1993). In fact, dynamin has been confirmed to participate in clathrin mediated endocytosis in neuronal or non-neuronal cells (McNiven, 1998; Schmid et al., 1998). Especially, the role of dynamin in endocytosis has been further proved since overexpression of SH3 domain of amphiphysin, a major binding protein for dynamin in brain, inhibited the uptake of transferrin by fibroblasts. Because non-neuronal cells do not express dynamin I, indirect evidence was obtained that the ubiquitously expressed dynamin II may also function in endocytosis. Besides clathrin-mediated endocytosis, several other endocytic mechanisms require dynamin II, such as internalization of caveolae of small (50-

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60 nm) flask-shaped invaginations associated with lipid microdomains on the plasma membrane (Henley et al., 1998; Oh et al., 1998; Schnitzer et al., 1996), as well as macrophage phagocytosis (Gold et al., 1999; Gold et al., 2000).

Extensive study of dynamin I in endocytosis revealed more functional details. Dynamin was believed to act at a late stage of vesicle formation—possibly vesicle detachment or pinching. First, the collar-like structure around the neck of constricted buds in mutant *Drosophila* and the elongated membrane invaginations observed in dyn-K44A transfected HeLa cells showed a block of vesicle release from the membrane, whereas early processes, like formation of coated pits or invagination of membranes, were not impaired. Second, purified dynamin could self assemble into rings or spirals either at low salt conditions without any nucleotide (Hinshaw and Schmid, 1995; Warnock et al., 1996) or at physiological salt conditions in the presence of GDP and metallofluorides (BeF or AlF) (Carr and Hinshaw, 1997). These structures resemble the collar structure seen in the nerve termini of the mutant *Drosophila.* And third, dynamin alone has been shown to be sufficient to deform the liposomes into tubules (Sweitzer and Hinshaw, 1998; Takei et al., 1998). These findings are interpreted by a model which explains the putative function of dynamin in endocytosis (Hinshaw and Schmid, 1995; Sweitzer and Hinshaw, 1998; Schmid et al., 1998)(Figure 5). Endocytosis is suggested to proceed in four steps: (1) recruitment and assembling of coat proteins to coated pits, (2) invagination of the coated membrane, (3) constriction of the vesicle neck, and (4) detachment of the vesicles. It is supposed that dynamin acts like a ”scissor enzyme” to pinch off the mature vesicles from donor membranes. Once the vesicle form, most coated proteins and dynamin proteins disassembled and detached from the vesicles, allowing the vesicle to travel and fuse with the target membrane. Coat proteins and dynamin may return to the cytoplasm from which they will be recruited for the next cycle of vesicle formation.

1.4.3 Function of dynamin II in the formation of post-Golgi vesicles

In comparison to the roles of dynamin in clathrin-mediated endocytosis which have been well-established, functions of dynamin in other membrane traffic pathways were less studied. As mammalian cells express three dynamin isoforms in a tissue specific pattern and each of these isoforms has mutiple spliced variants (Figure 3), it has been suggested that distinct isoforms and spliced variants may be targeted to different intracellular locations where they could support the formation of distinct vesicles (Urrutia et al., 1997). In accordance with this hypothesis, dynamin II has been shown to colocalize with the

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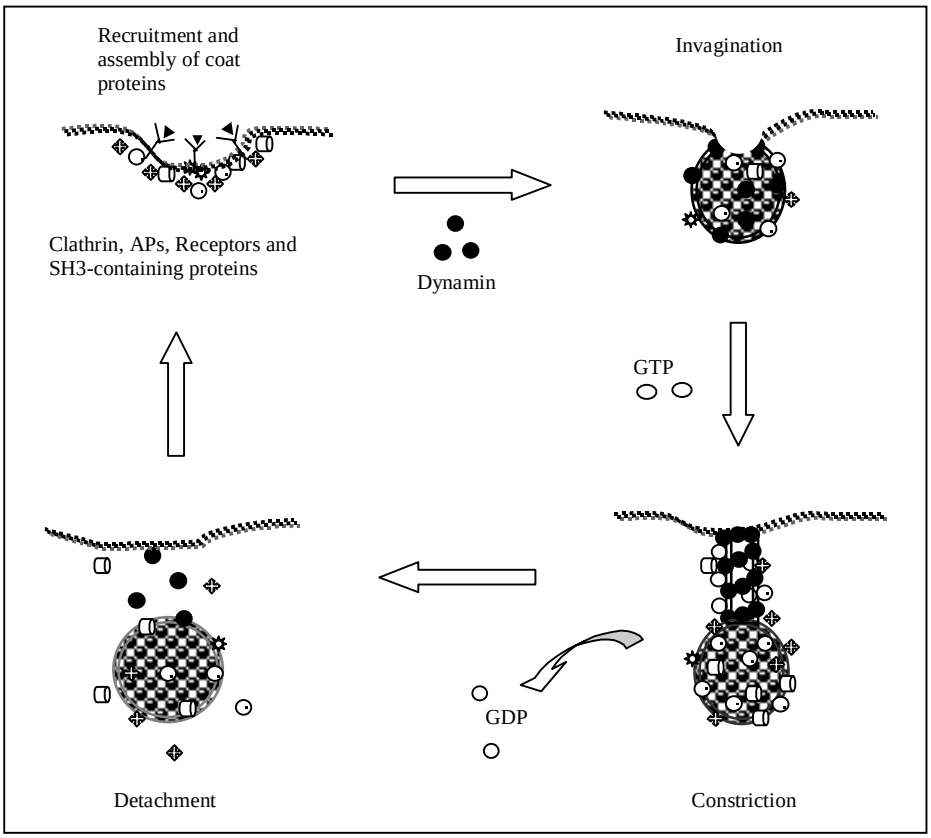

Figure 5. A working model for dynamin function in clathrin-mediated endocytosis. Dynamin is targeted to coated pits by interactions with α-adaptin of AP-2 and SH3-domain-containing proteins (e.g., amphiphysin). GTP binding triggers the assembly of dynamin into spiral collars at the necks. Vesicle fission is initiated by hydrolysis of GTP by dynamin.

TGN-specific marker--TGN38 by immunoelectron microscopy (Maier et al., 1996). In addition, a GFP-fused dynamin II colocalized with clathrin-coated vesicles at the Golgi complex (Cao et al., 1998b). This suggested a possible role of dynamin II in the formation of post-Golgi vesicles. Later, additional evidence was obtained by use of a cell-free budding assay in which the formation of both constitutive exocytic and clathrin-coated vesicles were strongly inhibited by addition of a dynamin II specific antibody or by immunodepletion of dynamin II from the cytosol. In the same case, the inhibited budding efficiency was restored upon re-addition of purified dynamin (Jones et al., 1998). A recent report demonstrated that Golgi structures were disrupted by overexpression of Dyn-2

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(K44A) in mammalian cells. Electron microscopy of transfected cells revealed large numbers of Golgi stacks comprised of highly tubulated cisternae and an extraordinary number of coated vesicle buds (Cao et al., 2000). Cells expressing mutant dynamin and GFP-tagged VSVG demonstrated a marked retention (8- to 11-fold) of the nascent viral G-protein in the Golgi compared to control cells, which suggested an inhibition of post-Golgi vesicle transport (Cao et al., 2000). These *in vivo* and *in vitro* observations opened a new aspect that dynamin II may play a role in the formation of exocytic vesicles at the TGN. Nevertheless, there are also reports that dynamin is not essential for vesicle formation at the TGN *in vivo* (Damke et al., 1994). In accordance, while both dyn-1 (K44A) and dyn-2 (K44A) were potent inhibitors of receptor-mediated endocytosis, neither mutant directly affected vesicle budding from the TGN or other transport pathways, which include the constitutive transport of newly sythesized TfnR from the ER through early Golgi compartments to the plasma membrane and transport of newly sythesized polymeric Ig receptor (pIgR) to the basolateral domain (Altschuler et al., 1998). This may deny an impact of dynamin I or dynamin II on the formation of post-Golgi vesicles. Therefore, the precise role of dynamin II at the Golgi membrane remains controversial.

1.5 Aim of the study

At the start of this study in 1997, major contributions had been published on structure and functions of brain specific dynamin I, whereas little was known on the structure and function of dynamin II - the ubiquitously expressed isoform. The general sequence identity between dynamin I and dynamin II implied a possible role of dynamin II in vesicular traffic, which was subsequently demonstrated by the finding that cell endocytosis was inhibited by overexpression of dyn-2 (K44A) as well as dyn-1 (K44A) (Altschuler et al., 1998). Notably, the evidence that dynamin II localized to the Golgi apparatus (Maier et al., 1996) promoted us to speculate that dynamin II may also function in vesicle formation at the TGN. To study putative functions of dynamin II and its domains in post-Golgi vesicle transport, we postulated that recombinant domains preserve their structure and specific function and may allow us to investigate the impact of individual domains on vesicle formation. As a result, direct evidence should be obtained to understand the roles of individual domains and of dynamin II in vesicle formation at the TGN.

1.5.1 Functions of dynamin II domain PHD and PRD in vesicle formation at the TGN

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In contrast to the PHD of dynamin I (Ferguson et al., 1994; Timm et al., 1994a), no information was available on the structure of PHD of dynamin II. In this study, the PHD of dynamin II is to be expressed as a recombinant protein and isolated to study its structure and function. Although the PRD of dynamin I has been expressed by fusion to GST, only the binding proteins had been patterned (Slepnev et al., 1998). Major difference in primary structures between PRDs of dynamin I and dynamin II indicates that structure and function may differ considerably. Functional activities of purified domain proteins are to be determined by protein and membrane binding assays as well as by measuring vesicle formation at isolated Golgi cisternae or cellular protein secretion. The combination of data should provide direct evidence for the function of dynamin II and its domains at the TGN.

1.5.2 Subcellular localization of dynamin II domains and endogenous dynamin II

Using EGFP-fusion proteins and immunofluoresence microscopy or subcellular fractionation, the distribution of dynamin II, as well as that of individual domains, will be studied to elucidate the roles of domains in targeting dynamin II to the Golgi apparatus or to the plasma membrane. In addition, *in vivo* colocalization of dynamin II and its putative binding proteins can be investigated.

1.5.3 Binding proteins of dynamin II domains

The investigation of binding proteins common to dynamins or specific for dynamin II will provide further information on the mechanism(s) by which dynamin II effects vesicle transport. For this purpose, binding proteins of brain or HepG2 cell extract will be examined by binding to purified and immobilized domain proteins. After protein identification, useful information on the complexity of dynamin II functions in vesicle formation may be obtained.

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