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DYNAMIN: CHARACTERISTICS, MECHANISM OF ACTION AND FUNCTION

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Abstract: Dynamin - a member of the GTP-ase protein family - is essential for many intracellular membrane trafficking events in multiple endocytic processes. The unique biochemical features of dynamin - especially its propensity to assemble - enable severing the nascent vesicles from the membrane.

The mechanism of dynamin's action is still a subject of debate - whether it functions as a mechanochemical enzyme or a regulatory GTPase. The GTPase domain of dynamin contains three GTP-binding motifs. This domain is very conservative across the species, including that recently cloned by us in the unicellular eukaryote *Paramecium*. Dynamin interacts with a number of partners such as endophilin and proteins involved in coordination of endocytosis with motor molecules. A growing body of evidence indicates that dynamin and dynamin-related proteins are involved both in pathology and protection against human diseases. The most interesting are dynamin-like Mx proteins exhibiting antiviral activity.

Key Words: Dynamin and Dynamin-Related Proteins, Endocytosis, Actin Cytoskeleton, Membrane Vesicles, Antiviral Activity

INTRODUCTION

Dynamin is a 100-kDa GTP-ase, which belongs to a large family of related proteins implicated in a variety of clathrin-dependent and clathrin-independent vesicle trafficking events on both endocytic and secretory pathways such as: receptor-mediated endocytosis, synaptic vesicle recycling, internalization of caveolae, trafficking in and out of Golgi, phagocytosis and maintenance of mitochondrial morphology [1,2].

Dynamin interacts with a number of partners, some of which are involved in coordination of endocytosis with motor molecules. Other dynamin-interacting

partners include proteins of endocytic machinery (α -adaptin and amphiphysin), and signaling and lipid interacting molecules: synpatojanin and endophilin [3]. Formation of coated pit - preceding coated vesicle generation - is facilitated by endophilin [4,5]. Endophilin is a SH3 domain-containing protein, which has acyltransferase activity. It catalyzes the conversion of lysophosphatidic acid (LPA) - an inverted-cone-shaped lipid - to phosphatidic acid (PA) - a cone-shaped lipid. Such an enzymatic process results in the changing of the membrane lipids' shape. This is a crucial step for the adopting of an inward curvature of the inner leaflet of the plasma membrane, thus enabling its invagination and formation of pit/vesicle. Endophilin acts as an "effector" of dynamin [4,5]. Compounds that are internalized in a dynamin-dependent pathway include receptors, nutrients, toxins, viruses, hormone transporters and channels [2]. Dynamin may also be engaged in signaling through its interaction with: phospholipase C γ , Grb 2, PI 3-kinase and G proteins [6].

ESSENTIAL DOMAINS OF DYNAMIN

Dynamin is a multidomain protein composed of [2,3,7,8]:

- an N-terminal GTP-binding domain, which contains three, very conservative across the species, GTP-binding motifs;
- a middle domain, which has a coiled-coil region thought to be involved in dynamin assembly;
- a pleckstrin homology domain (PHD) involved in phosphoinositides binding enabling membrane localization of dynamin;
- a GTP-ase effector domain (GED), an oligomerization domain, essential for inter- or intramolecular protein interactions; it is also an internal GAP (GTP-ase activating protein) for dynamin, since the isolated domain causes activation of dynamin GTP-ase;
- a C-terminal proline-rich domain (PRD), absent in other related proteins, recognized by several SH3 domain-containing proteins (like amphiphysin and endophilin) [2,3,7,8].

DYNAMIN ISOFORMS

Three isoforms of dynamin were identified in mammals, which generate more than 25 possible spliced variants expressed in a tissue-specific manner, and several dynamin-related proteins in different species [3].

The phylogenetic tree of dynamins has recently been extended by the dynamin homologue in the unicellular eukaryote *Paramecium*: a gene fragment (GenBank Acc. #AF351193) recently identified by us reveals 74% similarity to human dynamin 2 mRNA. The deduced amino acid sequence shows 61.1% homology in a 175 amino acid overlap to the N-terminal region of human, mouse and rat dynamin 2. The cloned gene fragment encodes the conservative region of the GTP-ase domain including all the three GTP-binding motifs: QSAGKSS, DLPG

and TKLD [9,10]. We also identified the *Paramecium* dynamin immunoanalogue that is involved in receptor-mediated endocytosis of transferrin [11]. This marker enters the endosomal compartment as previously shown by us with a 3-D reconstruction in confocal microscopy during the studies on its effect on fluid phase uptake [12].

GTP-ASE ACTIVITY OF DYNAMIN

The biochemical properties of dynamin are unusual for a member of the GTPase superfamily [12,13]: it has a low affinity for GTP ($K_M \sim 10\text{-}25 \mu\text{M}$), a high basal ($\sim 8\text{-}30 \times 10^{-3} \text{ s}^{-1}$) and a very high stimulated rate of GTP hydrolysis ($1\text{-}5 \text{ s}^{-1}$). In addition, the rate of GTP association ($7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) and GDP dissociation (93 s^{-1}) is very rapid in comparison to the classical GTPase - ras ($1.4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $3 \times 10^5 \text{ s}^{-1}$, respectively). Thus GTP hydrolysis is a rate-limiting step in dynamin's GTPase cycle, and it takes 0.5-2 min in comparison to 60 min in ras proteins [13,14].

MODELS OF DYNAMIN ACTION

Dynamin has the ability to pinch off membrane vesicles. There is a controversy in the literature whether dynamin acts as a mechanochemical enzyme ("pinchase") [3] or performs its function as a regulatory GTPase [14-16].

Dynamin exists as a homotetramer and has a propensity to self-assemble into complex polymers forming helical ring structures and spirals around the neck of the invaginated coated pit [15]. In this process a GED domain on one dynamin molecule interacts with a GED from another one. This interaction requires Lys694. Self-assembly is an initial step of dynamin's action in severing the vesicles [2,3,14].

A unique feature of dynamin is an internal GAP (GTPase activating protein) in the GED domain that undergoes activation as a result of self-assembly. The arginin finger located in the GED at R725 had been thought to be indispensable for the GTPase stimulation based on the experiments with GED mutants of dynamin 1 [14]. However, Marks *et al.* [17] obtained an opposite result, that the GED does not contribute to the catalytic site of dynamin. Their conclusions are in agreement with the most recent data of Eccleston *et al.* [18] on the equivalent dynamin 2 mutant.

Different models for the dynamin's function as a mechanoenzyme - using the GTPase hydrolysis to generate the force required for cutting off the plasma membrane vesicles - were proposed [2,3,14]. The first model - a molecular "garrote"- implies that upon targeting to nascent coated pit and binding of GTP, dynamin undergoes a conformational change resulting in its dissociation from the clathrin cage and promotion of self-assembly into a helical collar constricting the neck of the forming vesicle [2,14]. The model of dynamin's action as a molecular "spring" suggests that GTP-bound dynamin self-assembles into spirals

around the neck of the invaginated membrane and then upon GTP hydrolysis adopts a new GDP-bound conformation. This loosens the spiral (increased pitch), enabling the release of a vesicle [2,14]. The third model of dynamin's action - as a molecular "ratchet" - is based on interactions between GED and other regions of the dynamin molecule. Constriction is due to a ratcheting of one rung of the dynamin spiral along the adjacent rung. GTP hydrolysis causes a release and reassociation of dynamin domains thus tightening the collar around the neck of the forming vesicle [14,19].

There are many studies pointing out that dynamin may act as a regulatory GTPase that recruits some downstream effectors [14-16]. This was concluded from the data on GED mutants (R725A and K694A) which were in conflict with mechanochemical models. The K694A mutant had decreased ability to self-assemble without affecting the acceleration of GTPase activity. This mutation resulted in an increased rate of coated vesicle formation. In contrast, the R725A mutant, which had an impaired stimulated rate of GTP hydrolysis and no defect in self-assembly, showed a slower overall rate of coated vesicle formation - presumably due to inhibition of membrane fission. Overexpression of both mutants caused, surprisingly, stimulation of early events of receptor-mediated endocytosis. According to Sever *et al.* [14-16], these observations suggest that stimulated GTP hydrolysis inactivates dynamin by triggering disassembly, which is important for membrane fission. On the other hand, efficient assembly is not necessary for vesicle budding. Moreover, there is a possibility that other factors (a putative dynamin guanine dissociation inhibitor) may regulate dynamin's GTPase cycle *in vivo* [14].

Commenting these results, Kirchhausen [20] suggested that increased endocytosis may be due to formation of a deeper dynamin spiral as a result of the delay in GTP hydrolysis, which increases the number of binding sites for downstream effectors or prolongs their activation [20].

Marks *et al.* [17] analyzed the effect of several point mutations in and around GTP-binding motifs of dynamin to understand the mechanism of its action. They also examined the conformation of two GED mutants, previously studied by Sever *et al.* [15,16]. They concluded that both an efficient GTP hydrolysis and a resulting conformational change are required for dynamin's function in endocytosis.

Other results were obtained by Janzen *et al.* [21], who analyzed the Lys612 mutant of the monomeric MxA protein - a dynamin-related interferon-induced GTPase with antiviral activity. This mutation (in the GED domain) resulted in lack of oligomerization and GTPase activity. They showed that MxA monomers could inhibit virus replication in infected cells, so GTP hydrolysis does not seem to be required for antiviral activity *in vivo*. They concluded that GTP binding is necessary and sufficient for viral recognition by the MxA protein, and self-assembly is not needed for its activity [21].

Self-assembly and subsequent increase in GTPase activity of dynamin in endocytosis of ligand-activated receptors are promoted by c-Src-mediated

tyrosine phosphorylation [22]. Phosphorylation occurs on Tyr597 and may induce a conformational change of dynamin, which favors self-assembly. This phosphorylation is not required for constitutive endocytosis of recycling receptors. Neuronal dynamin 1 is also phosphorylated on a serine residue by protein kinase C in intact synaptosomes. This stimulates its GTP-ase activity, increases binding to calcium, but blocks binding to phospholipids [22].

INTERACTION OF DYNAMIN WITH ACTIN CYTOSKELETON

Actin cytoskeleton coordinates vesicle formation and motility at different steps and dynamin plays an important role in this process [23]. Dynamin interacts with different partners including profilin and three SH3-domain containing proteins: syndapin, cortactin and Abp1 [23-27].

Profilin, which promotes actin polymerization, binds directly to dynamin 1 through the ZPPX consensus motif (Z = proline, glycine, alanine and occasionally a charged amino acid, X = a hydrophobic residue) [24]. Syndapin is involved in actin rearrangement: SdpI links endocytosis and cytoskeletal dynamics in mature nerve terminals, whereas SdpII performs a similar role in other cell types [25]. Cortactin activates nucleation of actin filaments. It binds to F-actin through a series of six 37-amino acid tandem repeats [26]. Abp1 activates the Arp2/3 complex – a regulator of the actin polymerization downstream of many receptors. It binds F-actin through its N-terminal actin-depolymerizing factor homology (ADH) domain [27]. Dynamin seems to play an essential role in the formation of actin "comet tails" and movement of the living cells [23].

ROLE OF DYNAMIN AND DYNAMIN RELATED PROTEINS IN PATHOLOGY AND PROTECTION AGAINST HUMAN DISEASES

Dynamin and dynamin-like proteins have focused the interest of pathologists. Many viruses including hantaviruses, influenza, adenovirus and Semliki F viruses may invade the cells by the dynamin-dependent endocytic pathway [2, 28], whereas some dynamin-related proteins have been found to exert antiviral activity [29, 30].

Hantaviruses are etiologic agents for two severe human illnesses, hemorrhagic fever with the renal syndrome (HFRS) and the hantavirus pulmonary syndrome. HFRS is caused by infection with Hantaan, Seoul, Dobrava/Belgrade and Puumala hantaviruses [28].

These RNA viruses are transmitted to man through inhalation or ingestion of virus-contaminated rodent excreta [31]. Mite transmission including certain species of gamasid mites and chigger mites has also been identified [32]. A high annual incidence of HFRS was observed in China (reaching 115,985 cases in 1985) and the total number of cases was 1,256,431 from 1950 to 1997, with 44,304 death cases. Almost 70 species of vertebrates harbor hantavirus antigen

or antibodies, but the main reservoir hosts are *Apodemus agrarius* and *Rattus norvegicus* [32].

The most recent studies of Jin *et al.* on HeLa cells indicate that cytosolic GTP-ase active dynamin is critical for the entry of the Hantaan virus [28]. Overexpression of a dominant-negative dynamin mutant (K44A) inhibits Hantaan virus internalization, and compounds that block clathrin - but not caveolae-dependent endocytosis - also reduce Hantaan virus infectivity [28].

On the other hand, some human dynamin-related proteins (MxA and hGBP1), which are induced by type I interferons, display antiviral activity. MxA seems to block the replication of stomatitis and influenza viruses [29,30]. The most recent data indicate that MxA prevents the transport of incoming viral nucleocapsids to the cell nucleus where viral transcription takes place [33].

Mutations in dynamin-like proteins might cause human diseases. The best known is autosomal dominant optic atrophy (DOA). Histopathological and electrophysiological studies suggest that the underlying defect is a retinal ganglion cell degeneration caused by mutations in a mitochondrial dynamin-related GTP-ase - OPA1. This protein is involved in the formation and maintenance of the mitochondrial network. Up to now 62 mutations, localized mainly in the N-terminal GTPase domain, have been identified [34].

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