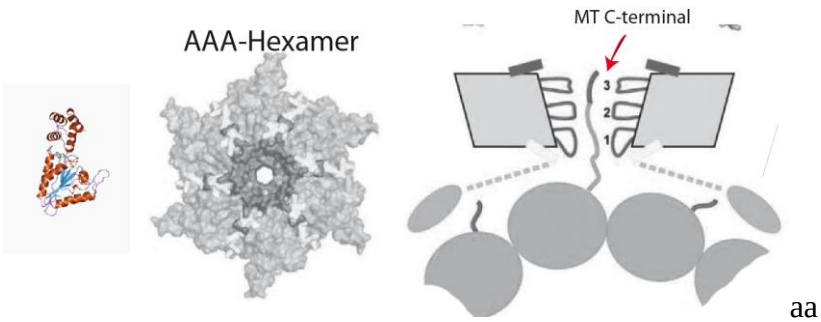
Glossary to the Lecture Note "The shaping of the ER"

AAA-family of ATPases: Members of the AAA family are found in all organisms AAA-ATPases are often coupled with scaffolding proteins which serve the formation of motors, such as the dynein motor. AAA-ATPases can transform ATP hydrolysis directly in mechanical forces. These mechanical nanomachines mediate numerous mechanical processes, such as DNA replication, degradation of proteins modified by ubiquitin, membrane fusion, microtubule severing (discussed in this Chapter). The monomers form discs with wedge-like cross section (see left side of image below) and tend to from hexamers with a central pore. The right side of the figure below shows the current model of the microtubule cleavage. It is assumed that the C-terminal of tubulin is pulled through the hole after ATP-hydrolysis, resulting in their denaturation and the cleavage of the tubules [Morin-Leisk 2011].The Figure was reproduced from [Morin-Leisk 2011] for this lecture.



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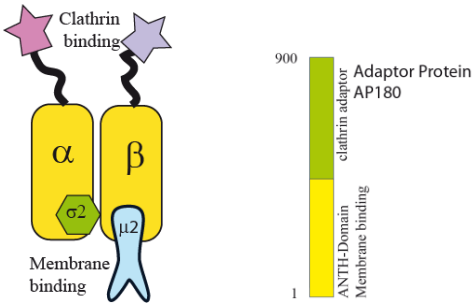
Adaptor AP-X; COP-Y and AP180: Cooperation of AP-2 and AP180

The adaptors AP-2 (see image below) recruit clathrin to membranes (often together with the AP180) and simultaneously bind specifically to the cargo, such as transferrin receptors. The vesicle transport between the ER and the Golgi apparatus is mediated by the adaptors COP-I and COP-II.



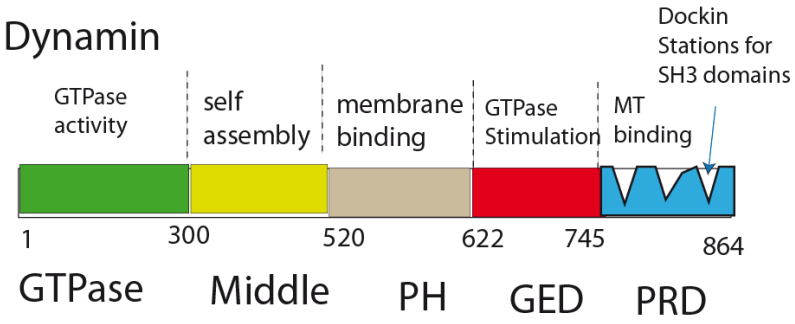
The adaptor 180 is particularly abundant in the brain. It exhibits a membrane binding domain (μ2) exhibiting a pleckstrin homology domain AP180 is a protein that plays an important role in clathrin-mediated endocytosis of synaptic vesicles. It is capable of simultaneously binding both membrane lipids (via an ANTH domain) and clathrin and is therefore thought to recruit clathrin to the membrane of newly invaginating vesicles. **To 180 affinity:** The binding constant of the ANTH domain is relatively week (K_d= 24μM). Therefore the coupling to the triskelion clathrin is essential to increase the binding affinity. The cooperation between AP-2 and AP180 serves the control of membrane curvature. AP180 plus clathrin does not induce substatial curvature of lipid monolayers containing 10 % of PIP2 while together with AP- 2 it generates vesicles of defined size. It may thus serve the control of the membrane curvature.

Structure and function of AP-2 [Edeling 2006]. AP-2 is the major adaptor which couples clathrin to the plasma membrane. The central body composed of γ and ß domains is common for AP-1 and AP-2 . For the activation of the adaptor it has to be activated as follows. In the resting state the μ2-domain binds strongly to the ß trunk, rendering the adaptor inactive. This link must be broken for activation. It is assumed that the activation and binding to the transferrin receptor occurs by phosphorylation of a threonine group. It results in the uncovering of the binding site for the cargo.
A second important role of the AP core structure is the membrane binding by binding to phosphoinositides including PI(3,4,5) P3. Interestingly the PI-3-Kinase that generates PIP3 is activated by clathrin. The counteracting phosphatase synaptojanin uncoupled the AP2 adaptor form the membrane. The membrane binding is enforced by electrostatic binding of a basic segment to acidic lipids.

[Edeling 2006] Edelin et al.(2006) Life of clathrin coat: insights from clathrin and AP structures Nature Rev Molec, Cell Biol.7:32-.

Axonopathy is a disorder disrupting the normal functioning of the axons. The dysfunction increases with the distance from the center toward the periphery. One example is Hereditary Spastic Paraplegia (HSP) resulting in progressive spasticity and weakness of the leg and hip muscles.

Dynamin consists of five domains with different functions.



The GTPase domain (residues 1-299**)** has some similarities with other small GTPases (such as Rac). It has a high GTPase activity (up to 20sec^{-1}) but low affinity (10-100 µM) for GTP. Under conditions promoting dynamin dimer formation the GTPasde activity is increased by a factor of 15. The aggregation is further promoted by binding to microtubules via its C-terminal, which is rich in proline and basic amino acids. Finally, the GTPase activity is 12fold accelerated by phosphorylation via protein kinase C (PKC).

The middle domain (residues 300-520) is divided into the C- and N-terminal half. The latter mediates the dynamin-dynamin assembly.

The pleckstrin homology(PH)-domain: (521-520) favors binding top PIP2 (PI(4,5)P2). It is essential for the membrane binding of dynamin. The binding to membranes increases the GTPase activity. Interestingly the lipid binding affinity is enhanced if the dynamin forms tetramers.

The GTPase effector Domain (GED; residues 623-745): The purpose of this domain is to increase the GTPase activity. Its effect is largest if dynamin forms tetramers. The GED domain plays the same role as the guanine activation proteins (GAP) which are responsible for the efficiency of small GTPases (Rac). The GAP accelerates the turnover of the GTPase activity of small GTPases since the life time of GTP-GTPase is very long.

Proline-rich Domain including SH3-binding domain: This C-terminal domain is rich in basic amino acids and proline. It contains several binding domains for functional proteins exposing SH3 homology domains. The SH3 domains serve the recruitment of numerous proteins such as (i) Grb2, which is universally involved in signal transduction or cell communication; (ii) the phospholipase Cγ which plays a key role for the generation of the second messenger PIP_3; (iii) The BAR-like protein amphyphysin.

Regulation of dynamin-self-assembly: Under physiological conditions dynamin exists in a monomer-tetramer equilibrium and tetramers can assemble in higher oligomersforming ring- and spiral-like structures. These structures require the help of BAR-like proteins. Moreover the interaction of the GED-domain with the GTPase segment is a prerequisite for spiral formation.

Regulation of membrane docking of dynamin: Dynamin can directly couple to membranes containing negatively charged lipids, in particular PIP2. According to the present state of our knowledge, the membrane binding requires in addition amphyphysin or endophilin, that is two proteins exposing the BAR domain. Purified dynamin binds to membrane containing

anionic lipids and the lipid forms tubes with helical assemblies of dynamin exhibiting a pitch of 13 nm. The tube diameter is 50 nm (similar to the width of the neck of coated pits) and the inner diameter is 20 nm. Addition of GTP retracts the diameter of the tubes to an outer diameter of 40 nm and an inner width of 10 nm. The distance between the dynamin molecules ie reduced from 13 to 10 nm. These observations suggest that dynamin is a force generating GTPase.

References: Hinshaw , J.et al. (2000) Dynamin and its role for membrane fission. Annu. Rev Cell Dev. Biol.16: 483-519

Dynamin: This multifunctional complex including GTPase function consists of five domains with different functions.

1. ***The GTPase domain (residues 1-299)*** has some similarities with other small GTPases (such as Rac). However , in contrast to small GTPases it has a high GTPase activity (up to 20/sec) but low affinity (10-100 µM) for GTP. Under conditions promoting dynamin dimer formation the GTPase activity is increased by a factor of 15. The aggregation is promoted by binding to microtubules via its C-terminal, which is rich in proline and basic amino acids. The activity of the GTPase is 12fold accelerated by phosphorylation via protein kinase C (PKC).

The middle domain (residues 300-520) is divided into the C- and N-terminal half. The latter mediates the dynamin-dynamin assembly.into dimers

The pleckstrin homology-(PH-) domain**:** (521-520) favours binding PI(4,5)P2 (abbreviated as top PIP2 below). It is essential for the membrane binding of dynamin. The binding to membranes increases the GTPase activity. Most importantly the membrane binding of dynamin is enhanced if it forms tetramers.

The GTPase effector Domain (GED) (residues 623-745). In the active state of dynamin it forms a complex with the GTPase domain. The purpose of this complex is to increase the GTPase activity. Its effect is largest if dynamin forms tetramers. It appears that the GED domain plays the same role as the guanine activation proteins (GAP) for small GTPases which are responsible for the efficiency of this family of GTPases (Rac). The GAP enhance the GTPase activity of small GTPases since the life time of GTP-GTPase is very slow, while that of the GTPase domain if dynamin is fast (see above).

Proline-rich Domain including SH3-binding domain: This C-terminal domain is rich in basic amino acids and proline. It contains several binding domains for functional proteins exposing SH3 homology domains. The proline rich domains serve the recruitment of numerous proteins such as (i) Grb2, which is universally involved in signal transduction or cell communication; (ii) the phospholipase Cgamma which plays a key role for the generation of the second messenger PIP_3; (iii) The BAR-like protein amphyphysin.

On the regulation of dynamin-self-assembly: Under physiological conditions dynamin exists in a monomer-tetramer equilibrium. The tetramers can assemble further into higher oligomers which exhibit ring- and spiral-spring like structures. The interaction of the GED-domain with the GTPase segment is a prerequisite for spiral formation. These structures require the help of BAR-like proteins.

Regulation of membrane docking of Dynamin: Dynamin can directly couple to membranes containing negatively charged lipids, in particular PIP2. According to the present state of our knowledge, the membrane binding is strongly enhances if dynamin is coupled to endophilin, that is a protein exposing a BAR domain (see BAR domain). Coupling is mediated by binding of the SH3 homology domain of endophilin to the proline rich N-terminal of dynamin.

Purified dynamin binds to membrane containing anionic lipids and the lipid forms tubes with helical assemblies of dynamin exhibiting a pitch of 13 nm. The tube diameter is 50 nm (similar to the width of the neck of coated pits) and the inner diameter is 20 nm. Addition of GTP retracts the diameter of the tubes to an outer diameter of 40 nm and an inner width of 10 nm. The distance between the dynamin molecules ie reduced from 13 to 10 nm. These observations suggest that dynamin is a force generating GTPase.
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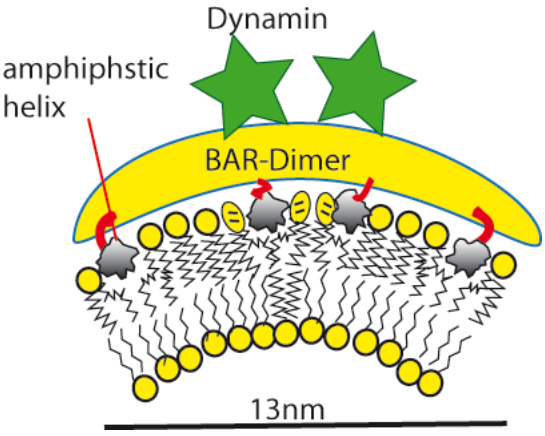
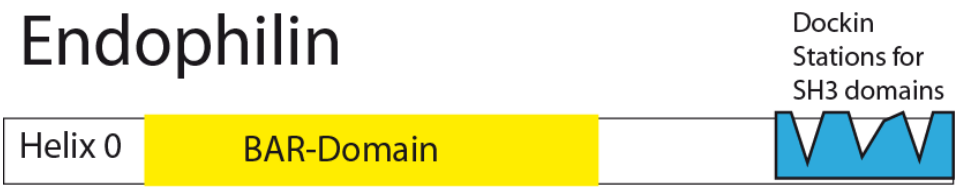
BAR domains (see also Endophilin): Banana shaped proteins which are connected to protein complexes involved in budding and fission of transport vesicles from the plasma membrane, Golg and the ER organelles. Examples of BAR-domain carrying proteins are amphiphysin, endophilin (see image below). The BAR-domain forms dimers which can sense or induce highly curved membranes (curvatures of the order of 1/20 nm^{-1}). The membrane anchoring is generally enforced by binding to phosphoinositides (such as PIP2) and other charged lipids such as phosphatidylserine. The membrane binding is mediated by two short helices attached to the core of the BAR domain via flexible peptide segments (structure see Gallop EMBO J. **25:** 2898–2910 (2006), see also [Sackmann 1996] .



Endophilin A major protein involved in recycling of synaptic vesicles via coated pits. It localizes at the neck of coated pits, connecting the bud to the plasma membrane, together with the GTPase dynamin. Endophilin and dynamin together form spiral like assemblies and can thus extend the neck into nanotubes. The tubes are narrower if both endophilin and dynamin are bound.



The binding is mediated by coupling of the SH3 domain of endophilin to the proline rich domain of dynamin. The experiments by Sundborger et al suggest that fission of vesicles is impeded when the hydrolysis of the GTP is constituently activated by using a non-hydrolysable isoform of GTP, namely GTP-gamma. (see Sundborger, A. et al. (2010]) Regulation of Synaptic Vesicle Budding and Dynamin Function by an EHD ATPase J. Neurosci.**31:** 13972-13980).

Sec61: Member of endoplasmic reticulum (ER) membrane proteins which acts as translocator of nascent proteins. The protein forms pores through the ER and mediates the translocation of nascent proteins, protruding from the ribosomes, to the lumen of the ER (see Kapitel 9 of Textbook).