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Abstract

Dynamin 2 (DNM2) mutations cause autosomal dominant centronuclear myopathy (CNM), a rare form of congenital myopathy, and intermediate and axonal forms of Charcot-Marie-Tooth disease (CMT), a peripheral neuropathy. DNM2 is a large GTPase mainly involved in membrane trafficking through its function in the formation and release of nascent vesicles from biological membranes. DNM2 participates in clathrin-dependent and clathrin-independent endocytosis and intracellular membrane trafficking (from endosomes and Golgi apparatus). Recent studies have also implicated DNM2 in exocytosis. DNM2 belongs to the machinery responsible for the formation of vesicles and regulates the cytoskeleton providing intracellular vesicle transport. In addition, DNM2 tightly interacts with, and is involved in the regulation of actin and microtubule networks, independent from membrane trafficking processes. We summarize here the molecular, biochemical and functional data on DNM2 and discuss the possible pathophysiological mechanisms via which DNM2 mutations can lead to two distinct neuromuscular disorders.

Key Words: dynamin 2, centronuclear myopathy, Charcot-Marie-Tooth neuropathy, endocytosis, cytoskeleton.

Dynamin 2 (DNM2) belongs to a superfamily of large GTPases, including three classical dynamins and several dynamin-like proteins, which are involved in a wide range of cell functions [1]. The importance of DNM2 was emphasized in 2005 with the demonstration of *DNM2* gene mutations causing two distinct human diseases [2, 3]. Our purpose is to review the molecular and functional data on DNM2 to highlight the possible pathophysiological hypotheses in DNM2-related diseases. Knowledge of the dynamins, especially of their molecular and biochemical properties, mainly comes from numerous studies of dynamin 1 (DNM1) [4]. However, we have focused this review on DNM2 since several studies have clearly demonstrated notable differences between DNM1 and DNM2 [5-9]. Moreover, DNM1 and DNM2 seem to be involved in different membrane trafficking processes in cells expressing the two proteins [10, 11].

DNM2 gene organization and isoforms

DNM2, one of three classical dynamins, was initially identified in rat liver and brain cDNA libraries [5, 12]. A human homologue was thereafter identified by screening of a fibroblast library [13]. The human transcript (3.6 kilobases) is ubiquitously expressed, with higher abundance in heart and skeletal muscle [13]. Human DNM2 is encoded by the *DNM2* gene located on the short arm of chromosome 19 (19p13.2). The gene is composed of 22 exons in a 114-kilobase region. Four isoforms are expressed by the *DNM2* gene using a combination of two alternative splice sites (Figure 1A). Isoforms 1, 2, 3 and 4 are also known as isoforms aa, ba, ab and bb, respectively [14]. Exons 10 and 10bis have the same length (139 base pairs encoding the amino-acids 399-445 in the middle domain) and are alternatively spliced. In addition, the exon 13bis (12-base pair length) can be spliced leading to the translation of proteins of 866 or 870 amino-acids (Figure 1) without or with the GEIL

sequence at position 516-519 in the C-terminal part of the middle domain. The expression of the four isoforms have been assessed in a panel of rat tissues including brain, heart, kidney, liver, lung, pancreas and testis [14]. The human tissue expression pattern is unknown, but we have shown expression of the four isoforms in skeletal muscle and peripheral nerve [15]. Specific functions of these isoforms will be discussed below.

DNM2 structure and regulation

The 98 kDa DNM2 is a large GTPase composed of a N-terminal GTPase domain, a middle domain, a pleckstrin homology domain (PH), a GTPase effector domain (GED) and a C-terminal proline rich domain (PRD) (Figure 1B). The catalytic GTPase domain is responsible for GTP binding and hydrolysis, whereas the middle domain is involved in DNM2 self-assembly [16] and in GTP hydrolysis-induced conformational change of the protein [17]. The PH domain interacts with membrane phosphoinositides and therefore involved in the targeting of dynamin to plasma or Golgi membranes [18]. Klein and collaborators reported that the DNM2-PH domain displays phosphoinositide binding affinity following the order; PI4,5P2 ≈ PI3,4,5P3 ≈ PI3,4P2 > PI4P ≈ PI3P, and DNM2 oligomerization appears crucial for high affinity [19]. The GED probably participates in the self-assembly of DNM2 and acts as a GTPase-activating protein (GAP) [20]. The PRD contains multiple Src homology 3 (SH3) binding motifs and mediates multiple protein-protein interactions (Table 1).

In vitro at high ionic strength, DNM2 is in monomer-tetramer equilibrium. At low ionic strength, DNM2 self-assembles into higher order aggregates leading to a drastic increase in GTPase activity [21, 22]. Microtubules or phospholipid vesicles, especially those containing PI4,5P2, also induce self-assembly and increase DNM2 GTPase activity [6, 21,

23]. Purified from baculovirus, GTP-bound and GDP-bound monomer DNM2 has Kd values of 13.2 and 7.1 μM, respectively, with GTPase activity of 37 nmol/mg/min. When in an oligomeric state, the GTPase activity of DNM2 markedly increased and Kd values decreased [24]. DNM2 basal activity appears 10-fold higher than for DNM1 probably due to the greater propensity of DNM2 to self-assemble and a higher affinity for GTP (Km=12 μM) [21]. When compared to small GTPases, DNM2 exhibits a relatively low affinity for GTP but high intrinsic rates of GTP hydrolysis.

DNM2 activity is regulated by post-translational modifications. DNM2 becomes phosphorylated on Tyr231 (middle domain) and Tyr597 (PH domain) through Src-mediated phosphorylation, leading to its association with caveolin and thus albumin endocytosis [25]. Similarly, IL5-induced DNM2 phosphorylation leads to redistribution of DNM2 within endocytic vesicles and is required for IL5 receptor internalization [26]. In contrast, dopamine leads to the dephosphorylation of DNM2 by increasing protein phosphatase 2A activity, necessary for dopamine-induced Na^+K^+-ATPase endocytosis [27]. S-nitrosylation of Cys86 (GTPase domain) and Cys607 (PH domain) by nitric oxide (NO) increases DNM2 GTPase activity and endocytosis [28]. Finally, regulation of DNM2 by proteolysis under pathological condition has also been reported [29]. Sever et al. identified a cathepsin L cleavage site at positions 355-360 in the middle domain (Figure 1B). In a mouse model of kidney disease, cathepsin L induction leads to the cleavage of the cytoplasmic DNM2 and then actin reorganization in renal podocytes, filtration impairment and proteinuria [29]. It remains to be determined whether such proteolytic regulation occurs in other tissues.

Phosphorylation of DNM1 by protein kinase C (PKC) increases Ca^{2+} binding to the protein, which in turn inhibits DNM1 stimulated GTPase activity [30]. Although DNM2 is not a substrate of PKC and does not bind Ca^{2+} [5] it was demonstrated that Ca^{2+} also inhibits

DNM2 GTPase activity (IC_{50}=150µM) and receptor mediated endocytosis in Hela cells [31]. This may have physiological importance in excitable cells like neurons and muscle fibers.

It is still largely unknown how the expression of DMN2 is regulated. In rat, DNM2 is up-regulated during normal pancreatic development after birth [32] but not in the liver [12] or the brain [33]. In mouse, treatment with opioid agonist results in increased DNM2 protein content in the spinal cord [34, 35] whereas opioid antagonist decreases DNM2 abundance [35, 36]. These changes in the level of DNM2 expression are inversely correlated with opioid receptor density at the plasma membrane, suggestive of feed-back regulation. A similar type of regulation has also been described in brain [37].

DNM2 function

1. *Endocytosis*. DNM2 has been implicated in the formation of clathrin-coated pits [21]. In the cytosol, DNM2 forms a complex with sorting nexin 9 (SNX9) and fructose-1,6-bisphosphate aldolase [38, 39]. Phosphorylation of SNX9 releases aldolase from the SNX9-DNM2 complex which is now competent for membrane targeting [39, 40]. DNM2 anchorage to the membrane occurs via interaction with PI4,5P2 membrane phosphoinositide [41] and BAR (Bin1/Amphiphysin/RVS167) domain proteins; amphiphysin 1, amphiphysin 2, and SNX9 (Table 1) in curved sites of the membranes. Subsequently, DNM2 co-localizes with clathrin before and during the internalization of the coated vesicle [7]. This suggests that DNM2 may play a role not only in the release, but also in the first steps of vesicle formation, as recently shown in the turnover of intermediates during the maturation of clathrin-coated pits [42]. During this process, DNM2 forms an oligomer helical structure around the neck of the nascent vesicles [21] and GTP hydrolysis is associated with the release of the vesicles.

DNM2 is also involved in clathrin-independent endocytosis by its role in the formation of the phagosomes in macrophages and Sertoli cells [43, 44] and caveolae in hepatocytes and endothelial cells [45, 46]. Predescu et al. described a protein complex, including DNM2, intersectin and SNAP-23, that was important for the fission and internalization of caveolae [47]. In caveolae, DNM2 also interacts with endothelial nitric-oxide synthase (eNOS) in bovine aortic endothelial cells [48] where DNM2 may regulate eNOS activation and the NO signalling cascade [48-51]. DNM2 also participates in coat-independent endocytosis processes, i.e. micropinocytosis and macropinocytosis, by which fluid droplets and specific membrane components are internalized [52, 53].

Altogether, these data demonstrate a role for DNM2 in clathrin- and caveolae-dependent endocytosis as well as coat-independent endocytosis, and in regulating several important cellular processes including signal transduction, cholesterol homeostasis, plasma membrane composition and turnover, cell migration and entry of pathogens.

2. *Intracellular membrane trafficking*. DNM2 targets to the Golgi apparatus where it is predominantly localized in the trans-Golgi network (TGN) [54]. Anti-DNM2 antibody injection and over-expression of DNM2 mutants impair vesicle formation from the TGN [55-57]. Association of DNM2 with cortactin, by an arf-1 and actin-dependent mechanism, and with syndapin 2, is required for trafficking of nascent vesicles from the TGN [58, 59]. DNM2 is also found at the clathrin-coated buds of early endosomes [60] and in late endosomes in Hela cells, located to the tubulo-vesicular appendices [61]. In these two cases, interfering DNM2 mutant impairs the recycling of components from the endosomal system towards the plasma membrane or TGN [60, 61]. These data highlight the role of DNM2 in the secretory pathway and in the sorting of cell components from the Golgi apparatus and endosomal compartment.

3. *Exocytosis*. In neuroendocrine cells, monomers of DNM2 are associated with the membrane of secretory chromaffin granules in a complex including syntaxin, a member of the exocytosis machinery [62]. DNM2 also interacts and co-localizes with complexin I, a SNARE regulatory protein [63]. Therefore, DNM2 may participate in endocytosis-exocytosis coupling as suggested in mouse pancreatic β-cells [64]. However, a role for DNM2 in exocytosis alone has been reported. During cell-mediated killing by natural killer (NK) cells, DNM2 co-localizes with lytic granules after NK cell activation, and is required for fusion of the granules with the plasma membrane [65]. Similarly in macrophages, focal exocytosis is blocked after anti-DNM2 antibody microinjection [66] and DNM2 GTPase activity regulates the fusion of secretory vesicles at the plasma membrane [67]. Further studies will be necessary to precisely identify the molecular role played by DNM2 in the exocytosis machinery.

4. *Actin network*. Actin-based dynamic processes are crucial for late stage endocytosis and vesicle formation, and DNM2 interacts with several actin-binding proteins. Direct interactions have been identified with Abp1 (actin-binding protein 1) [68] and cortactin [69, 70]. Abp1 is a Src kinase which provides a physical bridge between the endocytosis machinery and the cortical actin network, and cortactin is a component of the clathrin-mediated endocytosis machinery [71]. In addition, DNM2 is a component of actin-based motile vesicles (actin comets) [72, 73] which provide transport through the cytoplasm for vesicles formed by the Golgi apparatus or plasma membrane. Expression of DNM2-K44A mutant, defective for the GTPase activity, strongly reduces the number, length and velocity of the comets [72, 73].

Interaction between DNM2 and the actin cytoskeleton may have another cytoskeletal role such as in the formation of membrane tubules and protrusions. Furthermore, a recent study showed the crucial function played by the DNM2-cortactin complex in the global

organisation and remodelling of the actomyosin cytoskeleton [74]. In addition, DNM2 is present in cortical ruffles and lamellipodia, both important in cell migration [14, 69]. The supramolecular complex including DNM2, cortactin and Arp2/3 mediates the reorganization of actin allowing lamellipodia formation at the leading edge of migrating cells [75]. Disruption of DNM2 function by DNM2-K44A mutant or small interfering RNA (siRNA), inhibits the formation of lamellipodia [76]. Similarly, under PDGF stimulation, DNM2 is concentrated within the leading ruffles of migrating fibroblasts where it co-localizes with cortactin [69]. To allow cell migration, DNM2 participates in disassembly of focal adhesions, as well as β-integrin internalization at the rear of the cell [77, 78]. Additionally, DNM2 is enriched in specialized membrane protrusions such as podosomes and invadipodia. Podosomes represent attachment sites between cells and substratum [79] and invadipodia are focalized matrix degradation sites [80]. Inhibition of DNM2 diminishes the amount of such structures [80]. It has also been shown that DNM2 regulates the formation of actin stress-fibers by interaction with the cell surface heparin sulphate proteoglygan syndecan-4 [81] Expression of DNM2-mutant, truncated for the PRD domain mediating interaction with cortactin, increases the number of actin-stress fibers, which is associated with abnormal cell shape [69].

5. *Microtubule network and MTOC*. It is noteworthy that the first isolated dynamin, i.e. DNM1, was initially reported as a microtubule associated protein [4]. A similar binding property has been evidenced for DNM2 *in vitro* [21] and the binding region was located to the PRD [6, 82]. More recently, it was shown that down-regulation of DNM2 by siRNA increases the amount of acetylated tubulin, a more stable form of tubulin in microtubules, and reduces their growing capacity [83], suggesting that DNM2 may regulate the polymerization-depolymerization equilibrium of microtubules. Through its interaction with microtubules,

DNM2 appears involved in Golgi apparatus cohesion [83]. Moreover, DNM2 has been identified as a component of the centrosome, the main microtubule organizing center (MTOC), where it binds to γ-tubulin [84]. The centrosome consists of a pair of centrioles embedded in a filamentous pericentriolar matrix, where γ-tubulin is essential for microtubule nucleation. The function played by DNM2 at the centrosome is still unknown, but DNM2 silencing by siRNA suggests a role in centrosome splitting [84]. Likewise, participation of DNM2 in all the phases of mitosis has also been reported. DNM2 is detected in the 2 MTOC during early prophase, along the mitotic spindle during metaphase and in the spindle midzone region during anaphase and early telophase [85]. Thereafter, DNM2 is accumulated at the intracellular bridge where the final separation occurs. The time required for separation of the two daughter cells is longer in DNM2 knock-out cells [53]. Taken together, these data suggest that DNM2 may regulate microtubule-dependent processes by acting on microtubule dynamics and organisation.

6. *Apoptosis*. In order to establish a stable Hela cell line over-expressing DNM2 isoform 2, Fish et al. have reported a significant cell toxicity in dividing cells [86]. The cytotoxicity occurred via induction of apoptosis by a p53-dependent mechanism. Similar results were gained in vascular smooth muscle cells [87]. The capacity to trigger apoptosis appears DNM2-specific as DNM1 over-expression does not induce apoptosis [86]. The GTPase domain of DNM2 is crucial to induce apoptosis [88]. Besides, a point mutation (p.I684K) in the DNM2 GED enhances the apoptosis induction by the wild-type DNM2 suggesting that GED negatively regulates this DNM2 function [88]. Mitochondria are key actors in apoptosis and, interestingly, DNM2 has been detected in isolated mitochondria from bovine lymphoblastoid BL-3 cells [89]. However, to our knowledge, such localization has not been reported in other cell lines or tissues. DNM2 also regulates the apoptosis-inducing Fas-Fas

ligand pathway by facilitating the transport of Fas from the trans-Golgi network to the plasma membrane [90].

7. *Specific functions of DNM2 isoforms*. In a cultured rat epithelial cell line (clone 9), both DNM2 isoforms 1 and 3 show punctuate labeling of clathrin heavy chain-positive or -negative structures, but only isoform 1, with the GEIL sequence in the middle domain, appears located to the Golgi apparatus [14]. These data suggest a role for the GEIL sequence in targeting to the Golgi apparatus. However, cell-type specificity probably exists, as isoforms without the GEIL sequence were also shown to be targeted to the Golgi apparatus in MDCK cells [56], 3T3L1 adipocytes [91] and fibroblastoid-like cells derived from mouse embryonic stem cells [53]. Nevertheless, this possible differential localization argues for distinct functions. Indeed, in clone 9 cells, the K44A mutants of isoforms 2 and 4 are able to inhibit fluid-phase endocytosis, whereas the mutant forms of isoforms 1 and 3 do not [52], and are more potent inhibitors of clathrin-mediated endocytosis. Similarly in a hepatocyte cell line, the K44A-isoform 1 inhibits caveolae-dependent internalization, but not the other K44A mutant isoforms [92]. In fibroblastoid-like cells derived from mouse embryonic stem cells, isoforms 2 and 4 are the most efficient at rescuing export from the Golgi in DNM2 knock-out cells [53]. Altogether, these data suggest a preferential involvement of isoforms 1 and 3 in clathrin- and caveolae-dependent endocytosis, whereas isoforms 2 and 4 participate in uncoated endocytosis and trafficking from the Golgi apparatus. However, cell-type specificity also occurs as the four isoforms exhibit a similar subcellular distribution in 3T3L1 adipocytes and dominant negative mutants of each isoform similarly affect basal and insulin-stimulated GLUT4 trafficking [91].

DNM2 and human disease

Mutations in the *DNM2* gene cause rare forms of the Charcot-Marie-Tooth peripheral neuropathy (CMT) [2, 93-96] and autosomal dominant centronuclear myopathy (CNM) [3, 15, 97-99]. The 19 reported heterozygous mutations affect only the Middle domain, the PH domain and the GED (figure 1B). DNM2-related CNM is a slowly progressive congenital myopathy characterized by frequent centrally located nuclei in muscle fibers. The most common clinical features are delayed motor milestones, facial and generalized muscle weakness, ptosis and ophthalmoplegia [100]. Nevertheless, the severity of DNM2-CNM is variable, ranging from severe neonatal to mild late-onset forms. DNM2-CMT is a peripheral neuropathy characterized by progressive muscle weakness and atrophy. *DNM2* mutations can cause axonal CMT (CMT2) and dominant intermediate CMT (DI-CMT-B). In CMT2, the nerve conduction velocity is usually normal (> 38 m/s for the median nerve, which represents the cut-off value between the demyelinating CMT1 and the axonal CMT2). In the group of rare patients affected by DI-CMT-B, the nerve conduction velocity values are intermediate (between 25 and 45 m/s). In some CMT patients, neuropathy is associated with neutropenia [2, 94, 96] but this association has not been described in DNM2-CNM patients. Clinical overlap could exist in some patients, but the majority of patients are affected by a tissue-specific disorder targeting either skeletal muscle or peripheral nerve [94, 99, 100]. Among the 19 distinct *DNM2* mutations identified to date, there are no mutations common to the two disorders and no mutations in the regions of variation due to alternative splicing. No clear genotype-phenotype relationship can be generated, except for the *de novo* mutations located in the C-terminal part of the PH domain, which are all associated with a severe neonatal CNM phenotype [98]. In these patients, the phenotype progressively improves, suggesting compensatory mechanisms. Of note is that the CMT-mutation G358R, is located in the

cathepsin L cleavage site [29] and therefore may impair the regulation of DNM2 by proteolysis.

More recently, the *DNM2* gene has been described as a susceptibility gene for late-onset Alzheimer disease [101], and DNM2 expression was subsequently found to be decreased in the brains of late-onset Alzheimer patients [102]. Cognitive impairments have been reported in some CNM patients harboring the p.E368Q [97], p.R465W [103; Family 1] and p.R369Q [103; Families 2 and 3] DNM2 mutations. Future studies will be necessary to determine the prevalence of central nervous system involvement in DNM2-related diseases.

Pathophysiological hypotheses

1. *Membrane trafficking and signaling pathway hypothesis*. In addition to the *DNM2* mutations in autosomal dominant CNM, mutations in the *BIN1* gene encoding amphiphysin 2, a partner of DNM2 in the endocytic process, can cause the autosomal recessive form of the disease [104]. This suggests that endocytic impairment is implicated in the pathophysiological mechanisms of autosomal CNM. Indeed, impairment of clathrin-mediated endocytosis was also reported in cultured cells expressing CNM- or CMT-DNM2 mutants [2, 15, 83]. The crucial question which remains to be explored is how a defect in clathrin-mediated endocytosis can alter the cell function. Endocytosis (via clathrin-coated vesicles, caveolae or uncoated vesicles) regulates fundamental processes including nutrient uptake, membrane composition and turnover, cell adhesion or migration, pathogen entry, and signaling of G protein-coupled receptors, tyrosine kinase receptors or channels [105-107]. Thus, DNM2 mutations may have a large spectrum of functional consequences. On one hand, DNM2 mutations may lead to a decrease in receptor stimulated signaling as shown for the MAPK ERK1/2 pathway [15, 108]. On the other hand, DNM2 mutations may lead to a prolonged

half-life of various proteins at the cell surface due to a defect in protein removal, similar to that as suggested for the EnaC sodium channel [109, 110], KCNQ1 potassium channel subunits [111] or the GLUT4 glucose transporter [91, 112-114]. A deregulation of glucose transport in patients with DNM2 mutations could have a strong impact on muscle fibers given their high glucose consumption. Of note, the microtubule network plays an important role in GLUT4 trafficking [115] and a decrease in muscle weight has been reported for a transgenic mouse over-expressing GLUT4 [116].

To date, the impact of disease-associated DNM2 mutants on other membrane trafficking processes in which DNM2 is involved, especially in endosomal and Golgi pathways, has not been studied. In cells over-expressing the K44A-DNM2 mutant, an impairment in the trafficking from the Golgi apparatus has been reported [117, 118]. We cannot exclude a participation of these pathways in the pathomechanisms of DNM2-related disorders.

2. *Cytoskeleton*. In DNM2-CNM, the majority of patients harbour a mutation in the middle domain, which is essential for the centrosomal localization of DNM2 and for its interaction with γ-tubulin [84]. Previous results in skin fibroblasts indicate that transfected GFP-DNM2-mutants failed to be correctly targeted to the centrosome, suggesting that DNM2 mutations might cause CNM by interfering with centrosome function [3]. In addition, CMT-related DNM2 mutants can disorganize the microtubule cytoskeleton [2] and one particular CMT-mutant was shown to impair microtubule-dependent membrane transport [83]. During skeletal muscle differentiation a profound reorganization of the microtubule network occurs, changing from a classical network centered on the juxtanuclear centrosome in myoblasts, to a longitudinal organization along the axis of differentiated myotubes [119]. One can hypothesize that mutated-DNM2 can impair functions associated with this specific

cytoskeleton reorganization. In addition to their roles in intracellular trafficking, the microtubule and actin networks regulate cellular architecture including nuclear positioning [120, 121]. Thus, cytoskeletal impairment may be implicated in the abnormal central location of the nuclei in the muscle fibers in CNM. In CMT, DNM2 mutations could also induce a destabilization of the microtubule network leading to abnormal axonal transport and protein trafficking, a pathophysiological mechanism described previously in various forms of CMT [122].

Concluding remarks and open questions

Given the numerous distinct functions in which the ubiquitously expressed DNM2 is involved, the identification of pathophysiological mechanisms will be a challenge. The phenotypes encountered in CNM and CMT patients are probably due to impairment of the various functions of the protein. DNM2 is engaged in numerous protein-protein interactions (Table 1) but the relevance of these interactions in skeletal muscle and the nervous system, is largely unexplored. For example, DNM2 interacts with β-catenin in rat testis in relation to the maintenance of the blood-testis barrier integrity [123] but no data are available on this interaction in skeletal muscle where β-catenin plays an important role [124, 125]. Another essential unresolved question is whether each particular mutation can similarly affect the functions of the four DNM2 isoforms. Finally, whereas some data emerge on the impact of disease-related DNM2 mutations on the microtubule network, their impact on the actin cytoskeleton is totally unknown. Future developments and characterization of animal models will certainly be useful to better determine the main functions of DNM2 *in vivo,* especially in skeletal muscle and peripheral nerves where membrane trafficking displays different characteristics depending to the length of the cells.

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Abbreviations

PI4,5P2: phophatidylinositol 4,5-bisphosphate

PI3,4,5P3: phophatidylinositol 3,4,5-triphosphate

PI3,4P2: phophatidylinositol 3,4-bisphosphate

PI4P: phophatidylinositol 4-monophosphate

PI3P: phophatidylinositol 3-monophosphate

LPA: lysophosphatidic acid

GLUT4: glucose transporter 4

TGN: Trans-Golgi network

BAR: Bin1/Amphiphysin/RVS167

Legends

Figure 1: *DNM2* gene organization and mutations.

A. Schematic organization of the human DNM2 gene showing alternative splicing. Asterisks indicate the seven exons in which disease associated mutations have been identified. Exons were colored relative to the encoded protein domain illustrated in B. The combination of the two alternative splice sites leads to the translation of four DNM2 isoforms. B. Schematic representation of DNM2 showing the five protein domains and the position of the 19 disease-associated mutations. CMT-mutations are indicated in green and CNM-mutations in red. The two regions of variation (at positions 399-445 and 516-519) between the four isoforms were indicated in the middle domain by black lines. In black are indicated the sites of post-translational modifications (phosphorylation, nitrosylation and cathepsin L cleavage). In blue are indicated the DNM2 constructs with point mutations or small deletions overexpressed *in vitro* [2, 15, 29, 79, 83, 88, 126, 159].

Figure 2: DNM2 cellular functions

Representation of the multiple cellular localizations reported for DNM2 (in red). EE: early endosome. LE: late endosome.

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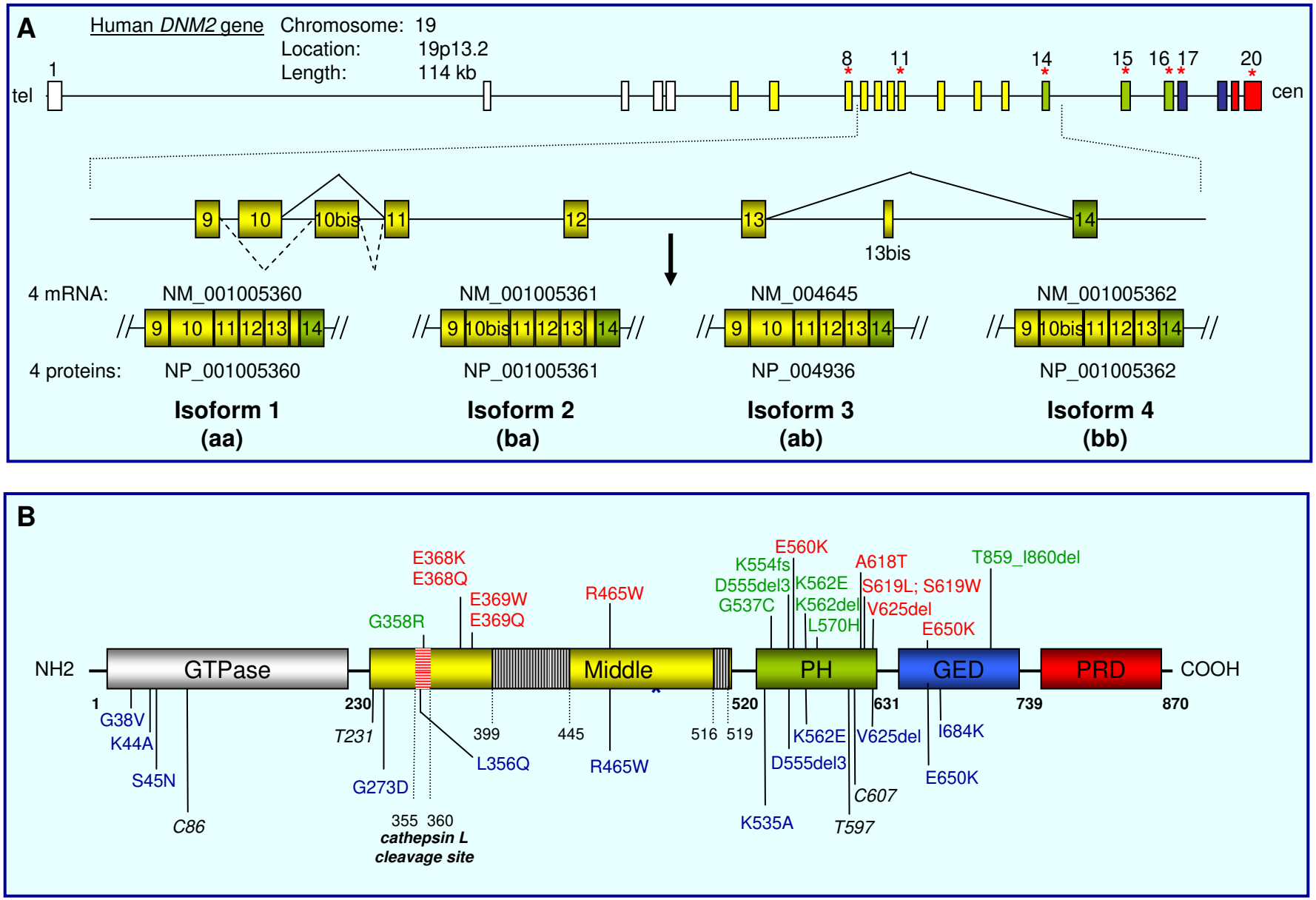
Table 1: Direct or indirect interactions with DNM2

Name	OMIM	Site of interaction in DNM2	Function	Reference
Abp1	610106	nd	Endocytosis-actin bridge	[68, 127]
Amphiphysin 1	600418	PRD	Membrane trafficking	[18, 128]
Amphiphysin 2	601248	PRD	Membrane trafficking	[18, 129]
Annexin VI	114070	nd	Membrane trafficking	[130]
Aquaporin 2	107777	nd	Aquaporin trafficking	[131]
Arc	612461	PH	AMPA receptor trafficking	[132]
CAP	605264	nd	Actin remodelling during endocytosis	[133]
β-catenin	116806	nd	Blood-testis barrier integrity	[123]
Caveolin-1	601047	nd	endocytosis	[92, 134, 135]
CBL	165360	nd	Actin remodelling during endocytosis	[136]
CIP4	604504	nd	GLUT4 trafficking	[137]
Complexin I	605032	nd	Acrosome formation and/or exocytosis	[63]
Complexin II	605033	nd	Acrosome formation and/or exocytosis	[63]
cortactin	164765	PRD	Actin assembly - Endocytosis-	[69, 70, 135]
c-Src	124095	PRD	Cell signalling and membrane trafficking	[138]
eNOS	163729	nd	NO production - Cell signalling	[48, 139]
Ese1	602191	PRD	Endocytosis	[140]
FAK	600758	PRD	Focal adhesion disassembly	[77]
FBP17	606191	PRD	Actin reorganization during endocytosis	[141, 142]
Grb2	108355	PRD	Receptor internalization and signalling	[138, 143, 144]
IL-5Rα	147851	nd	IL-5 signaling pathway and trafficking	[26]
Jak2	147796	nd	IL-5 signaling pathway	[26]
JAM-A	605721	nd	Blood-testis barrier integrity	[123]
Kalirin 12	604605	GTPase	Membrane trafficking	[145]
KDR	191306	nd	Receptor signaling and expression	[146]
LYN	165120	PRD	IL-5 signaling pathway	[26, 138]
MLK2	600137	PRD	Actin (filipodia and membrane ruffles)	[147]
Myosin 1E	601479	PRD	Receptor-mediated endocytosis	[148]
N-cadherin	114020	nd	Blood-testis barrier integrity	[123]
Nef	-	MD / GED	HIV-1 entry	[149]
Nostrin	607496	PRD	eNOS trafficking	[150]
N-WASp	605056	nd	Actin remodelling	[75]
Occludin	602876	nd	Blood-testis barrier integrity	[123]
p85	171833	PRD		[138]
PDEγ	180073	nd	Cell signalling	[151]
PLCγ	172420	PRD		[138]
PLD2	602384	nd	Cell signalling	[152]
Shank 1	604999	PRD	Postsynaptic membrane turnover	[153]
Pyk2	601212	nd	Podosome dynamics	[154]
Shank 2	603290	PRD	Postsynaptic membrane turnover	[153]
SNX9	605952	PRD	Membrane remodelling – actin dynamics	[38, 155]
SNX18	-	PRD	Endosomal trafficking	[155]
SNX30	-	PRD	Membrane trafficking?	[155]

Syndapin 2	604960	PRD	Vesicle formation from the TGN	[59]
Syndecan-4	600017	PH	Actin stress-fibers and focal adhesion sites	[81]
Tks5/FISH	-	PRD	Cell signalling	[156]
TULA	605736	nd	EGFR trafficking	[157]
Vav1	164875	PRD	T cell activation by actin remodelling	[158]
β-tubulin	191130	PRD		[18]
γ-adaptin	603533	PRD		[18]
γ-tubulin	191135	MD	Centrosome cohesion	[84]
ZO1	601009	nd	Blood-testis barrier integrity	[123]

Abp1: actin binding protein. CAP: CBL associated protein. CBL: Cas-Br-M murine ecotropic retroviral transforming sequence homolog. CIP4: cdc42 interacting protein-4. eNOS: endothelial nitric-oxide synthase. Ese1: EH domain and SH3 regulator of endocytosis 1. FAK: focal adhesion kinase. FBP17: Formin-binding Protein 17. Grb2: growth factor receptor-bound protein 2. IL-5Rα: α subunit of the interleukin 5 receptor. Jak2: Janus kinase 2. JAM-A: junctional adhesion molecule A. KDR: kinase insert domain receptor also known as Vascular endothelial growth factor receptor-2. MLK2: mixed-lineage kinase 2. Nef: accessory protein of the HIV-1. N-WASp: Wiskott Aldrich syndrome protein. PDEγ: inhibitory γ subunits of the retinal cGMP phosphodiesterase. PLCγ: Phospholipase C gamma 1. PLD2: phospholipase D2. SNX9: sorting nexin 9. Tks5/FISH: tyrosine kinase substrate 5/five SH3 domains. TULA: Cbl- and ubiquitin-interacting protein T-cell ubiquitin ligand. Vav1: Rho family guanine nucleotide exchange factor Vav1. ZO1: Zonula occludens 1.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cen
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9
10
10bis
11
12
13
13bis
14
4 mRNA:
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NM_001005361
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NM_001005362
4 proteins:
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NP_004936
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(aa)
Isoform 2
(ba)
Isoform 3
(ab)
Isoform 4
(bb)
B
NH2
GTPase
Middle
PH
GED
PRD
COOH
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739
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G38V
K44A
S45N
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T231
G273D
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cathepsin L
cleavage site
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445
R465W
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K535A
K562E
D555del3
T597
V625del
C607
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E650K
E368K
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G358R
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E369Q
R465W
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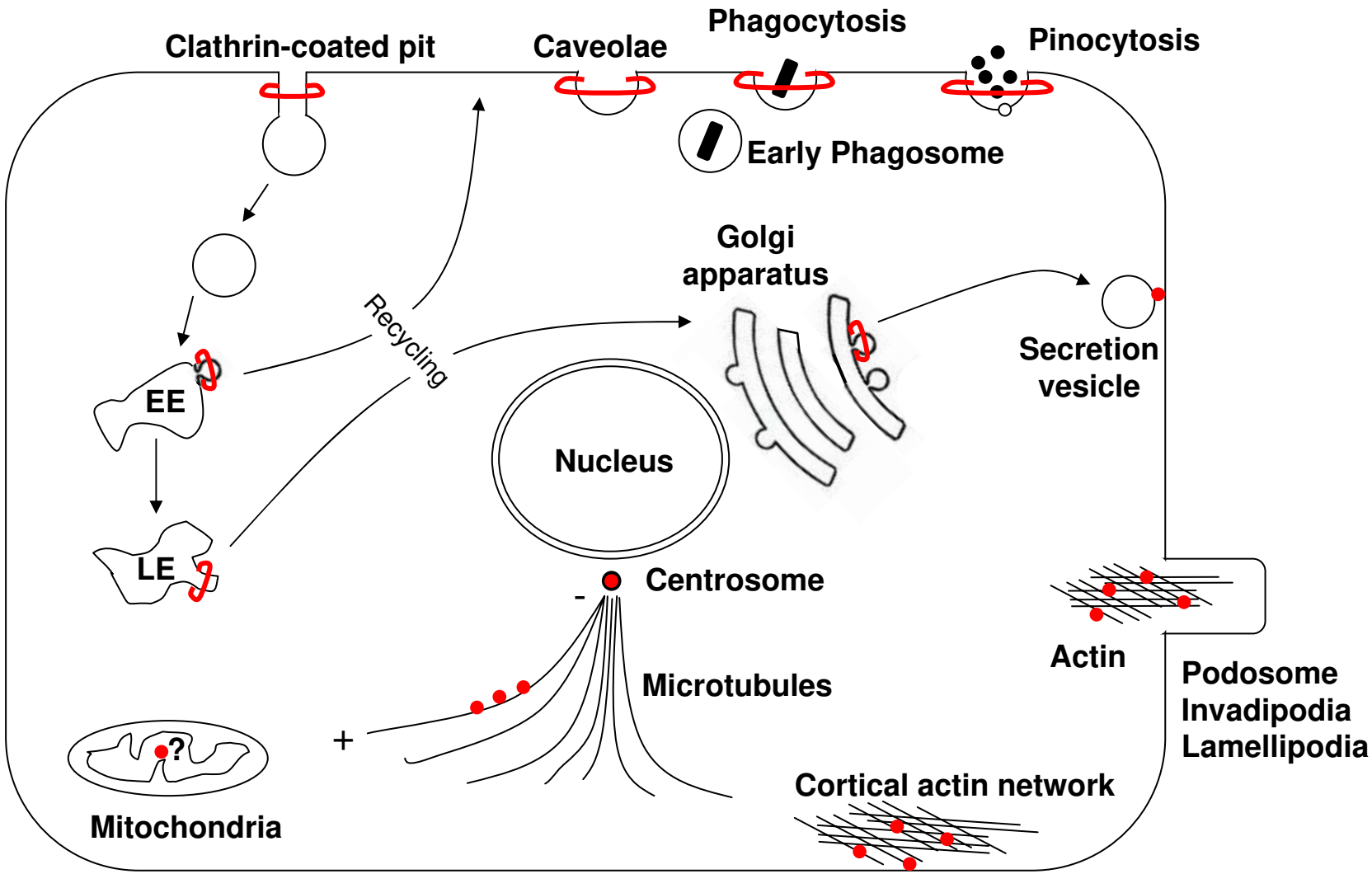
Clathrin-coated pit
Caveolae
Phagocytosis
Pinocytosis
Early Phagosome
Golgi apparatus
Recycling
Secretion vesicle
EE
LE
Nucleus
Centrosome
-
+
Microtubules
Actin
Podosome
Invadipodia
Lamellipodia
?
Mitochondria
Cortical actin network