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## **Role of dynamin 2 in the disassembly of focal adhesions**

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**Abstract**

Dynamin 2 (DNM2) is involved in endocytosis and intracellular membrane trafficking through its function in vesicle formation from distinct membrane compartments. During the last decade, several studies pointed out an important role of DNM2-dependent trafficking in turnover of focal adhesions which represent a physical link between the extracellular matrix and the intracellular actin cytoskeleton, and a platform for several signaling pathways. Here, we review the involvement of DNM2 in structural and functional aspects of the focal adhesion sites. Mutations in the *DNM2* gene cause two hereditary neuromuscular disorders; dominant centronuclear myopathy and Charcot-Marie-Tooth peripheral neuropathy. Potential impairment of focal adhesions as a pathophysiological hypothesis in *DNM2*-related human diseases is discussed.

**Keywords:**

Dynamin 2, Focal adhesion, endocytosis, focal adhesion disassembly

Dynamin 2 (DNM2) is a ubiquitously expressed large GTPase, involved in various membrane trafficking events. At the plasma membrane, DNM2 is involved in the formation and release of vesicles for the clathrin-mediated [1] and clathrin-independent endocytosis [2, 3]. DNM2 also participates in the formation of transport vesicles from the endosomal system and the Golgi apparatus [4, 5]. Moreover, several studies have demonstrated that DNM2 directly interacts with the microtubule [1, 6] and actin cytoskeleton [7, 8]. DNM2 is a 98 kDa protein composed of a N-terminal catalytic GTPase domain, a middle domain involved in DNM2 self-assembly, a pleckstrin homology domain (PH) which interacts with membrane phosphatidylinositol 4,5-bisphosphate (PI4,5P2) and therefore is involved in the targeting of dynamin to membranes [9], a GTPase effector domain regulating the GTPase activity, and a C-terminal proline rich domain (PRD) containing multiple Src homology 3 (SH3) binding motifs engaged in protein-protein interactions [10]. Heterozygous mutations in the *DNM2* gene cause rare forms of the Charcot-Marie-Tooth peripheral neuropathy (CMT) [11] and autosomal dominant centronuclear myopathy (CNM) [12]. There is no treatment for *DNM2*-related diseases and the pathophysiological mechanisms are still largely unknown.

The focal adhesions (FAs) represent the major site of cell attachment to the extracellular matrix (ECM) where a structural and functional link between the ECM and the intracellular actin cytoskeleton occurs. FAs are macromolecular complexes composed of transmembrane receptors including integrins, structural proteins including vinculin, talin, and  $\alpha$ -actinin, and signalling proteins such as Focal adhesion kinase (FAK) [13]. FAs act in cell adhesion and migration and dynamics of FA assembly-disassembly is a crucial process to achieve these functions. The molecular mechanisms leading to FA assembly have been well characterized. In contrast, FA disassembly mechanisms are not fully understood. During the last decade, a role of DNM2 in FAs disassembly emerged. The purpose of this review is to summarize the

studies showing the role of DNM2 as a regulator of FAs dynamics. In addition, we discuss the hypothesis of FA dysfunction in the *DNM2*-related human diseases.

### **DNM2-mediated endocytosis at FAs**

The first link between dynamin and components of the FA was reported in 1995 in human monocyte primary cultures stimulated by the macrophage colony-stimulating factor (M-CSF) [14]. Ligand-activated M-CSF receptor recruits a protein complex including dynamin, FAK and the adaptor protein Grb2 (growth factor receptor-bound protein-2). Interestingly, dynamin is phosphorylated and co-immunoprecipitated with activated FAK in M-CSF-treated monocytes suggesting a regulation of dynamin function by FAK that may contribute to receptor internalization. FAK is a non receptor tyrosine kinase involved in the signaling pathway of several agonist-activated membrane receptors including integrins and is mainly expressed at the FA. In addition, FAK is a key element of the regulation of FA disassembly [15] and actin polymerization by phosphorylation of downstream targets [16]. This first demonstration of DNM2 association with FAK had strongly suggested a role for DNM2 at the adhesion sites.

In cultured cells, microtubule depolymerisation by nocodazole results in FA disassembly. After washing the drug, microtubules grow, contact the FAs and lead to FA disassembly [17]. Using this microtubule-induced FA disassembly assay, Ezratty and collaborators showed that disassembly is independent of Rho- and Rac-GTPase activities but dependent on FAK-induced recruitment of DNM2 at the FA via its PRD domain [18]. Before FA disassembly, DNM2 colocalizes with FA and co-immunoprecipitation studies suggest that DNM2 interacts with the phosphorylated, *i.e.* activated, form of FAK (p-FAK). By the development of a phospho-antibody against DNM2's tyrosine 231, Wang and collaborators further defined the

DNM2 regulation occurring during FA turnover [19]. The authors showed that activation of Src is responsible for the phosphorylation of DNM2 at the FA where the phosphorylated DNM2 (pDNM2) interacts directly with FAK. Consequently, the formation of a pDNM2-FAK-Src complex promotes  $\beta$ 1-integrin endocytosis and FA disassembly [19]. Such Src-mediated phosphorylation of DNM2 was already shown to induce endocytosis [20]. Inhibition of the DNM2 activity, using dominant negative DNM2-K44E mutant and DNM2-siRNA, inhibits FA disassembly [18] and impairs cell migration [18, 21-23] confirming the central role of DNM2-dependent processes in FA disassembly.

Additional data strongly suggested that clathrin-mediated endocytosis is the main route involved in the DNM2-dependent endocytosis of FA components leading to FA disassembly. A rapid accumulation of clathrin occurs in a microtubule-dependent manner as FAs disappear [21] and clathrin depletion impairs microtubule-induced FA disassembly [21, 22] and cell migration [18, 21-23] as shown for DNM2. Clathrin- and DNM2-mediated endocytosis targets specifically  $\beta$ 1-integrin engaged in extracellular matrix interaction towards the endosomal recycling pathway [21-23]. Among the clathrin adaptors responsible for clathrin recruitment to endocytic pits, endocytosis of  $\beta$ 1-integrin at the FA involves either Dab2 (disabled 2) and ARH (autosomal recessive hypercholesterolemia protein) in NIH-3T3 cells [21] or AP2 (adaptor-related protein complex 2) and Dab2 in the HT1080 cell line [22]. These apparent differences suggest a cell-specific combination of clathrin adaptors for integrin endocytosis and FA disassembly. Even if clathrin-mediated endocytosis seems to be the major route for integrin endocytosis leading to FA disassembly, involvement of DNM2- and caveolin-dependent endocytosis was reported [24, 25] suggesting that caveolin-mediated endocytosis may also participate in the FA disassembly. Further studies will be necessary to better define if clathrin- or caveolae-dependent endocytosis pathways could be exploited in a cell-specific manner or if they both coexist in a single cell.

## **DNM2 targeting to FAs**

In addition to its interaction with FAK, DNM2 may be targeted to the FA by interacting with syndecan 4 mediated by the PH domain of DNM2 [26]. Syndecan 4 is a heparan sulphate proteoglycan involved in the formation of FAs and actin stress fibres [27, 28]. It regulates FAK activity by increasing FAK phosphorylation in a Rho-dependent manner [29], and participates in integrin internalization [25]. In NIH-3T3 cells treated with lipophosphatidic acid to induce FA formation, DNM2 and syndecan 4 are both recruited and interact at FAs [26]. This study shows that DNM2 is recruited at the FA as soon as FAs are formed and engaged via ECM interaction. Even if syndecan 4 do target DNM2 to FAs, syndecan 4 endocytosis itself occurs via a clathrin- and DNM2-independent process [30] showing a tightly regulated sorting of FA components targeted to the clathrin- and DNM2-associated endocytic vesicles.

DNM2 may also be targeted at FAs by interaction via its PH domain with the PI4,5P2 enriched at the FA. In fact, local production of this phosphoinositide which also interacts with syndecan 4 [31], may increase recruitment and activation of DNM2 and the endocytic machinery leading to vesicle formation [32]. Interestingly, it was shown that FA disassembly is dependent on the type 1 phosphatidylinositol phosphate kinase beta (PIP2K $\beta$ ) located at the FA before disassembly which locally produces the PI4,5P2 allowing recruitment of clathrin, clathrin adaptors (Dab2 and AP2) and DNM2 and formation of the FAK-DNM2 complex [23]. Concerning clathrin and adaptors, the cytoplasmic domain of  $\beta$ -integrins contains NPXY motif [33] necessary for their recruitment. However, an interesting possibility is that clathrin could be targeted to the FA by interacting with other components of FAs. Indeed,  $\alpha$ -actinin

and vinculin, two components of the integrin-based adhesion sites [13], have been shown to interact directly with clathrin heavy chain [34, 35].

### **DNM2-dependent mechanisms in FA regulation**

Processes involved in the turnover of FA components include the microtubule-mediated regulation of FA dynamics [17, 36] leading to DNM2-dependent integrins endocytosis as discussed earlier. FA turnover also includes proteolysis by calpains [37, 38] and protein dephosphorylation by phosphatases [39]. The importance of calpain 2, a calcium-dependant protease, was shown for the proteolysis of talin and the subsequent disassembly of the macromolecular complexes also including paxilin and vinculin [37]. More recently, calpain 2-mediated proteolysis of FAK was also shown to contribute to the regulation of FA turnover [38]. FAK is composed of a N-terminal FERM domain (F for 4.1 protein, E for ezrin, R for radixin and M for moesin), which interacts with DNM2 [19], the catalytic kinase domain containing the binding site for Src and a FAT (focal adhesion targeting) domain involved in the recruitment of FAK to the FA [40]. The cleavage site of FAK by calpain 2 was mapped at the serine residue in position 745 located between the kinase and FAT domains [38]. The consequences of calpain-mediated proteolysis on the FAK-Src-DNM2 complex and on the DNM2-dependent FA disassembly were not investigated until now. It is tempting to speculate that the proteolysis contributes to the activation of the clathrin and DNM2-mediated endocytosis maybe by allowing an increased polymer formation and GTPase activity of DNM2.

Another mechanism of DNM2-dependent regulation of FA emerged from studies performed on podosomes, a transient adhesion site specific to motile cells [41]. In osteoclasts, DNM2 is recruited at podosomes by the activated phosphorylated form of Pyk2; *i.e.* a focal



adhesion kinase highly homologous to FAK, which in turn induces a negative feedback loop leading to the dephosphorylation and inactivation of Pyk2 [42]. The DNM2-dependent dephosphorylation of FAK was also reported [42]. Recently, the mechanism of the DNM2-dependent Pyk2 dephosphorylation was demonstrated to be mediated by the tyrosine phosphatase PTP-PEST leading to the arrest of Pyk2 downstream signaling [43]. PTP-PEST is known to dephosphorylate several components of FAs [44, 45]. Eleniste and collaborators showed that dynamin's GTPase activity is required for the formation of the DNM-Pyk2-PTP complex [43]. The recruitment of the PTP-PEST phosphatase by DNM2 when its GTPase activity increases may be a more general mechanism at the FAs resulting to the dephosphorylation of FA components, inactivation of signaling cascades, disengagement of integrins and finally FA disassembly. Further studies will be necessary to better define the temporal regulation and coordination of these different processes. For example, it was already demonstrated that calpain is required for microtubule-mediated FA disassembly [46] showing that FA disassembly is an intricately regulated process.

In addition to its role in endocytosis, DNM2 is a well known regulator of the actin network dynamics [47-49]. One can hypothesize a wider function of DNM2 at the FA besides its role in endocytosis of FA components. In particular, interactions of DNM2 with several other proteins need to be further characterized at the FA. DNM2 interacts with Cbl (Casitas B-lineage Lymphoma) in podosomes of osteoclasts [50] and with CAP (Cbl-associated protein) [51] involved in remodeling of the actin cytoskeleton at adhesion sites [52, 53] suggesting that DNM2 may also participate in actin network dynamics at the FA before disassembly. It was also shown that the CAP-DNM2 complex negatively regulates receptor-mediated endocytosis [51]. A similar complex may occur at FAs and inhibit DNM2 activity before disassembly signaling occurs.

Figure 1 summarizes the known interactions of DNM2 with FA components including FAK, PTP-PEST phosphatase and syndecan 4. The available data demonstrate that DNM2 is *per se* a component of FA and that the endocytic machinery including clathrin adaptors, clathrin, and DNM2 probably start being recruited at the FA during their formation and maturation by interaction with specific FA components. The subsequent clathrin- and DNM2-mediated endocytosis of FA components, which recycle back to the plasma membrane [54], allows turnover of FA.

### **Relevance in *DNM2*-related human diseases**

Mutations in the *DNM2* gene cause rare forms of Charcot-Marie-Tooth peripheral neuropathy (CMT) [11]. Among the hypotheses proposed to explain the CMT pathogenesis, impaired intracellular transport is of particular interest due to the number of CMT genes involved in intracellular trafficking [55]. Given that CMT-related *DNM2* mutations impaired clathrin-mediated or clathrin-independent endocytosis [6, 11, 56, 57], a defect in integrin internalization and FA turnover may participate in the pathomechanisms of the disease. In agreement with this hypothesis, CMT-related *DNM2* mutants inhibit clathrin-mediated endocytosis in motor neuronal cells and Schwann cells and strongly impaired myelination [58]. In this study, over-expression of the K562E CMT-mutant in rat primary Schwann cells increases the plasma membrane content of  $\beta$ 1-integrin probably by endocytosis impairment. On the other hand, integrin-based adhesion sites play important roles in the regulation of cell surface receptor, neurotransmitter receptors, and calcium signaling in neurons [59]; several processes potentially impaired by *DNM2* mutation.

Mutations in the *DNM2* gene also cause autosomal dominant centronuclear myopathy (CNM) [12], a slowly progressive congenital myopathy. The mature skeletal muscle fibres

have two distinct FA types: the integrin- and the  $\beta$ -dystroglycan ( $\beta$ -DG)-mediated adhesions [60]. The  $\beta$ -DG is an integral membrane protein included in the dystrophin-associated glycoprotein complex (DGC) which links extracellular laminin and ECM proteins to the intracellular actin cytoskeleton. The two types of FA are concentrated at two specialized adhesives structures of muscle fibres; i.e. the muscle-tendon attachment at myotendinous junctions and the muscle-ECM attachment at costameres along the fibres. Due to muscle intrinsic functional properties, these two adhesives structures have a crucial protective role against contractile damages during contraction and relaxation cycles and are essential for maintaining integrity of sarcomeres; i.e. the contractile unit of the muscle fibres. Several muscular dystrophies are linked to mutations in components of the two FA types [61]. In muscle, integrin-mediated adhesion sites are dynamic structures, crucial for structural integrity [62], which require clathrin-mediated endocytosis for turnover [63]. The first link between CNM and adhesion sites was recently demonstrated in drosophila [64]. MTM1, a phosphoinositide phosphatase mutated in the X-linked recessive form of CNM [65] participates in maintenance of muscle attachment sites through a role in integrin trafficking at myotendinous junctions and FAs [64]. Given that CNM-related DNM2 mutations may impair clathrin-mediated and clathrin-independent endocytosis [56, 57, 66], future studies will be necessary in order to determine if similar impact may be due to *DNM2* mutations.

One important question is to understand the tissue-specific impact of *DNM2* mutations on skeletal muscle or peripheral nervous system. In cultured neurons, DNM2 interacts with ArgBP2 (Arg and Abl Binding Protein 2) and its brain-specific isoform nArgBP2; two proteins involved in the regulation of actin cytoskeleton at the cell adhesion sites [67]. Interaction of DNM2 with cell type-specific FA components may participate to the tissue-specific impact of *DNM2* mutations. Alternatively, one may hypothesize a cell-type specific effect of distinct *DNM2* mutants. Indeed, CMT-mutants but not CNM-mutants impair

endocytosis in peripheral nerve model [58]. Similar comparative studies on FA disassembly using skeletal muscle and peripheral nerve models will be necessary for deciphering the tissue-specific impact of *DNM2* mutations.

## Conclusions

Consistent data now exist to show the importance of *DNM2* at the cell-matrix adhesion sites. By interacting with syndecan 4 and FAK, *DNM2* is *per se* a component of the focal adhesion sites. *DNM2* is probably recruited early during the FA formation process and may regulate actin dynamics. In addition, *DNM2* plays a central role in FA disassembly through the link with four disassembly signaling: i) *DNM2* is a regulator of the microtubule cytoskeleton, ii) Disassembly is dependent on the activity of the  $\text{PIP}\text{K}\beta$  which locally produces  $\text{PI4,5P2}$  which binds *DNM2*, iii) Calpain-dependent proteolysis acts on FAK which interacts with *DNM2*, and iv) *DNM2* interacts with PTP-PEST, a phosphatase involved in FA disassembly. These data highlight a new promising pathophysiological hypothesis that should be further studied in *DNM2*-related human diseases.

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**Disclosure of potential conflict of interest**

There is no conflict of interest to disclose.

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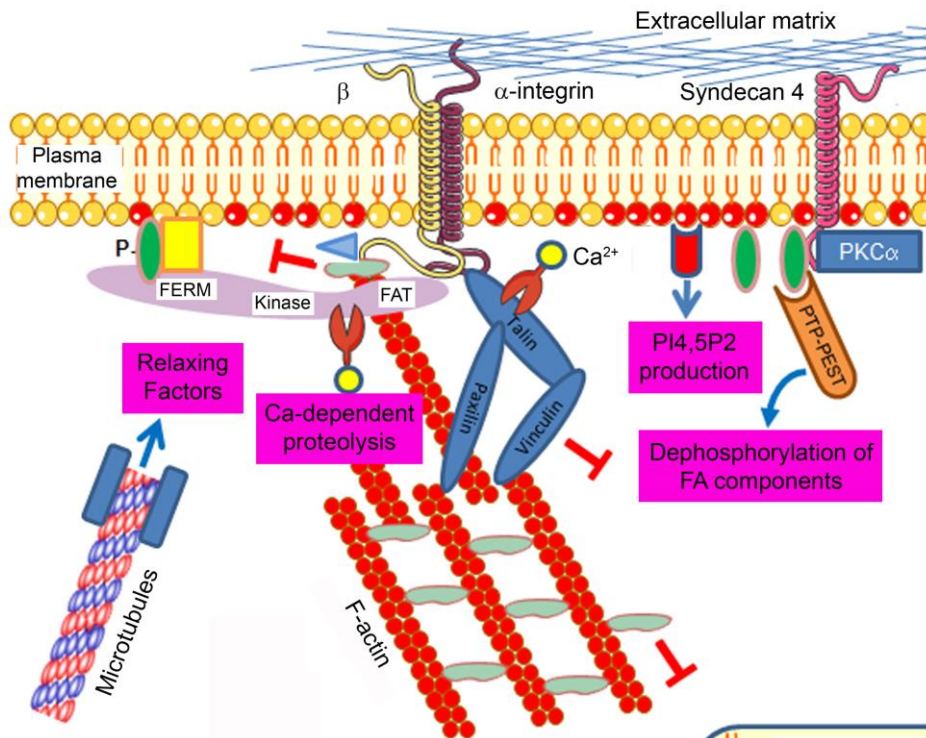
## Legends

### **Figure 1. Summary of the DNM2 interactions with FA components.**

**A.** Several processes may participate to the recruitment of DNM2 to FA: i) interaction with FAK in a DNM2-FAK-Src complex, ii) interaction with Syndecan 4, iii) interaction with the PTP-PEST phosphatase, and iv) interaction with PI4,5P2 enriched at the FA. Clathrin adaptors and clathrin heavy chain may be also targeted to the mature FA by interacting with integrin heterodimers, vinculin and  $\alpha$ -actinin. Under disassembly signaling, the calcium-dependent proteolysis by calpain cleaves FAK and other FA components. In addition, the production of PI4,5P2 by PIPK $\beta$  may increase DNM2 membrane recruitment. On the other hand, the dephosphorylation of FA components by phosphatases involves the PTP-PEST phosphatase which interacts with DNM2. **B.** The combination of the four disassembly stimuli (pink boxes in A) activates the clathrin- and DNM2-mediated endocytosis and recycling of FA components. The figure is focused on DNM2-related processes and consequently, additional interactions between FA components have been omitted.

A

### Focal Adhesion



B

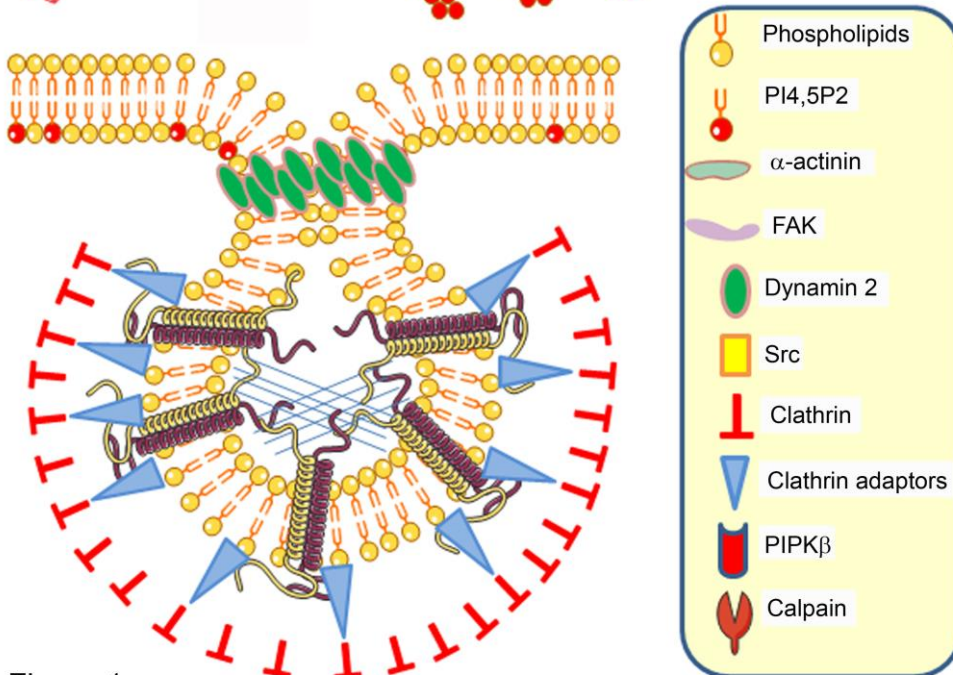


Figure 1