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### Commentary

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# Dynamin 2 the rescue for centronuclear myopathy

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**Centronuclear myopathy is a lethal muscle disease. The most severe form of the disease, X-linked centronuclear myopathy, is due to mutations in the gene encoding myotubularin (MTM1), while mutations in dynamin 2 (DNM2) and amphiphysin 2/BIN1 (AMPH2) cause milder forms of myopathy. MTM1 is a lipid phosphatase, and mutations that disrupt this activity cause severe muscle wasting. In this issue of the JCI, Cowling and colleagues report on their finding of increased DNM2 levels in human and mouse muscle with MTM1 mutations. Partial reduction of *Dnm2* in mice harboring *Mtm1* mutations remarkably rescued muscle wasting and lethality, and this effect was muscle specific. DNM2 regulates membrane trafficking through vesicular scission, and it is presumed that reducing this activity accounts for improved outcome in X-linked centronuclear myopathy.**

## Defective membranes in centronuclear myopathies

Centronuclear myopathy (CNM) is an inherited muscle-wasting disease with onset in infancy or childhood. Muscle biopsies from CNM patients characteristically show enlarged and centrally placed nuclei throughout the muscle, and clinically, CNM-associated mutations may result in weakened respiratory musculature, leading to early death without supportive care. Three main forms of CNM are attributable to mutations in myotubularin (*MTM1*), amphiphysin 2 (*AMPH2*), and dynamin 2 (*DNM2*), which all encode membrane-associated proteins that potentially act in muscle at the transverse tubules (T-tubules), membrane invaginations specialized for calcium handling (Figure 1 and ref. 1). The most common and severe form of CNM is X-linked CNM (XLCNM), referred to as myotubular myopathy. XLCNM is clinically evident at birth, with patients presenting with hypotonia and respiratory compromise. Skeletal muscles biopsies from XLCNM patients show abnormal T-tubules, including defective triads, struc-

tures critical for excitation-contraction coupling. XLCNM is the result of mutations in *MTM1*, which encodes a ubiquitously expressed phosphoinositide 3-phosphatase that functions to dephosphorylate PI3P and phosphatidylinositol-3,4-bisphosphate, two phospholipids essential for membrane sorting and signaling (2). *MTM1* is required for normal endocytosis, receptor degradation, and early endosome maturation (1).

Mice harboring a deletion of *Mtm1* (*Mtm1*<sup>-/-</sup> mice) recapitulate human disease and display many characteristic XLCNM-associated features, including muscle weakness, centralized nuclei, and muscle atrophy (3). *Mtm1*<sup>-/-</sup> mice exhibit muscle atrophy, become weak at 3 to 4 weeks of age, and have a greatly reduced life expectancy of only 6 to 12 weeks (3). The role of *MTM1* in human muscle has also been effectively modeled in zebrafish using morpholinos to reduce *mtm1* expression (MTM MO) (4). MTM MO fish, like XLCNM patients and *Mtm1*<sup>-/-</sup> mice, develop weakness and muscle atrophy. MTM MO fish display accumulation of PI3P, especially surrounding muscle nuclei, and T-tubule defects. The phenotypes observed in MTM MO fish provide further support that *MTM1* is critical for the normal biogenesis and maintenance of membrane structures within muscle (4). Because of its broad role as a lipid phosphatase, the

indispensable targets of *MTM1*'s enzymatic action are not fully known.

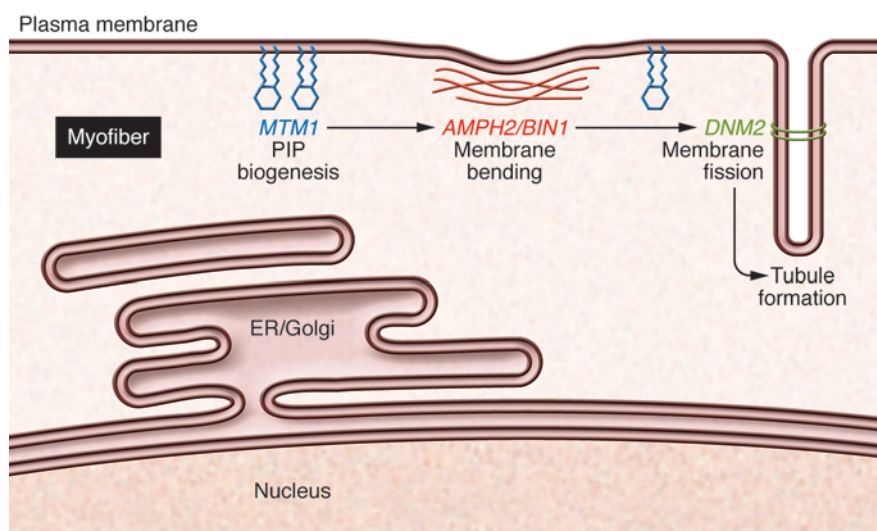
*DNM2* is a ubiquitously expressed GTPase that has been implicated in multiple cellular functions, including endocytosis, membrane scission, and cytoskeletal remodeling (5). Dynamins assemble as rings around membrane tubules where they are thought to actively “pinch off” membranes (5). Complete loss of *Dnm2* results in embryonic lethality in mice (6). In humans, CNM is caused by dominant *DNM2* mutations (7), and overexpression of a CNM-linked *DNM2* mutation (R465W) in mouse muscle results in myopathic features, including centralized nuclei, muscle atrophy, and deformed T-tubules (8). Interestingly, overexpression of normal *DNM2* in mouse models also produces some of these same features, consistent with the model that increased *DNM2* activity contributes to CNM (8). Increased *DNM2* activity may lead to excessive membrane scission and pruning, giving the appearance of excessive membrane accumulation around nuclei or at T-tubules.

## Genetic reduction of DNM2 in a CNM model

In this issue of the *JCI*, Cowling and colleagues described elevated protein levels of *DNM2* in *Mtm1*<sup>-/-</sup> animals and in muscle biopsies from human XLCNM patients (9). To target the increased levels of *DNM2* found in *Mtm1*<sup>-/-</sup> animals, mice heterozygous for *Dnm2* were crossed with *Mtm1*<sup>-/-</sup> mice. The in vivo reduction of *Dnm2* corrected some histological abnormalities in muscle, but dramatically extended life expectancy from 6 to 12 weeks to beyond one year. To demonstrate that this effect was muscle intrinsic, Cowling and colleagues generated *Mtm1*<sup>-/-</sup> mice in which *Dnm2* was specifically reduced in skeletal muscle. Reduction of *Dnm2* in skeletal muscle alone after disease onset was sufficient to reduce pathology and

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**Figure 1**

Genes implicated in CNM. *MTM1* encodes a membrane-bound lipid phosphatase (blue). Loss of function *MTM1* mutations lead to severe, early onset myopathy characterized by myofibers with centrally placed nuclei. Dominant mutations in *DNM2*, encoding dynamin 2 (green), also lead to CNM. In this issue, Cowling and colleagues demonstrate that reducing *DNM2* dramatically improves longevity in a mouse model of *MTM1*-mediated myopathy. Mutations in *AMPH2/BIN1* (red) also cause CNM. These proteins are involved in phosphoinositide (PIP) biogenesis, membrane bending, and membrane fission. Mutations in these genes affect membranes throughout the muscle, including those that surround nuclei and those in the T-tubule.

extend the life span in *Mtm1*<sup>-/-</sup> mice; however, skeletal muscle-specific *Dnm2* deletion did not extend life span in *Mtm1*<sup>-/-</sup> animals to the same extent as heterozygous whole-body *Dnm2* reduction, suggesting that aspects of the phenotype arise from muscle extrinsic effects or the need to reduce *Dnm2* earlier in muscle development.

Based on their data, Cowling et al. propose that *DNM2* and *MTM1* function in an overlapping pathway, but there are no reports of a direct interaction between these two proteins. *DNM2* is enriched at the Z band of muscle, adjacent to but not directly overlapping with T-tubules, and *DNM2* is also seen in the membranes surrounding myonuclei (8, 10). Many *DNM2* mutations fall near the pleckstrin homology domain, which may insert directly into membranes to participate in membrane fission. These observations suggest a broader complex may be critical for the restricted action of *MTM1* and *DNM2* on membrane biogenesis and function. The large size of *DNM2* may require alternative approaches to provide adequate resolution to fully define its associated membranes in muscle.

*AMPH2*, also known as *BIN1* (bridging integrator protein), is a Bin-Amphiphysin-Rvs (BAR) domain-containing protein, known to sense membrane curvature and to bend membranes (1). Membrane bending is a key aspect of forming intracellular tubules in all cell types and also in muscle. Recent work has shown that *BIN1* binds phosphatidylinositol-4,5-bisphosphate at the T-tubule and is capable of binding both *DNM2* and *MTM1* (11, 12). The binding of *MTM1* to *BIN1* enhances tubule formation in vitro, and mutations in *MTM1* that

cause CNM result in reduced tubule formation in vitro. *MTM1*-deficient muscle lacks normal *BIN1* localization at the T-tubule (12); therefore, *MTM1* loss may result in defective membrane biogenesis through upregulation and altered localization of phosphoinositides. The increase in *DNM2* observed in XLCNM patients may arise as a consequence of altered lipid signaling.

Currently, there are no drug therapies for CNM patients, and care relies on respiratory support and management. To date, most therapeutic approaches have focused on *MTM1* replacement. For example, local restoration of *MTM1* with viral delivery improved both the structural and functional deficits of *Mtm1* deficiency in mice (13). Alternatively, direct enzyme replacement therapy of *MTM1* in mice markedly improved muscle strength after only two weeks of treatment (14). These preclinical approaches for *MTM1* replacement are promising, but have yet to be tested in a clinical setting.

The improvement in strength and pathology following *Dnm2* reduction in *Mtm1*<sup>-/-</sup> mice suggests that targeting *DNM2* is an alternative and potentially complementary strategy for XLCNM therapy. Importantly, the decrease in XLCNM-associated phenotypes observed following *DNM2* reduction after disease onset is essential for any XLCNM treatment, given that most patients present with disease at birth. The in vivo overexpression and loss-of-function studies in mice suggest a wide therapeutic window in which to manipulate *DNM2* levels safely in clinic for XLCNM treatment. Molecular strategies to inhibit *DNM2* could also be applied to other forms of CNM. For example, the cell-permeable molecule dynasore and the

serotonin reuptake inhibitor sertraline both act as reversible noncompetitive inhibitors that block the GTPase activity of *DNM2*, resulting in diminished endocytosis (5, 15). Unfortunately, these molecules lack specificity and would interact with other dynamins, including dynamin 1 and the mitochondrial dynamin *DRP1* (5, 15). Whether other dynamins are increased and/or contribute to pathology in XLCNM is not known. Down-regulating multiple dynamins within and outside of muscle could increase the risk of unwanted off-target effects. Mice with deletions of microRNA 133a-1 (*Mir133a-1*) and *Mir133a-2* develop a CNM that is mediated by upregulation of *Dnm2* (16). Because *Dnm2* is a direct target of *Mir133a*, it is possible that this microRNA could be used to reduce *DNM2* levels in CNM patients.

### Can reduction of *DNM2* be extended as a therapy in other myopathies?

The rescue of XLCNM through the genetic reduction of *DNM2* is a viable approach for this rare form of muscle disease; however, many genetically diverse forms of inherited myopathies share aspects of the phenotypes seen in CNM. For example, mutations in dysferlin (*DYSF*) and caveolin 3 (*CAV3*) also result in some histological abnormalities shared with CNM, including T-tubule defects and muscle weakness (17, 18). Similarly to *MTM1*, *DYSF* and *CAV3* also regulate endocytosis and colocalize with *BIN1* at the T-tubule (19–21). Herein, Cowling and colleagues showed abnormal internalization of *CAV3* in *Mtm1*<sup>-/-</sup> muscle, which was rescued by *Dnm2* reduction. Interestingly, both overexpression of normal *DNM2* in mice and *DNM2* loss-of-



function mutations in mice and humans results in abnormal DYSF internalization (10, 16). Whether mislocalization of CAV3 and DYSF within the myofiber contributes to CNM disease progression remains to be determined. These data suggest that a common pathway links MTM1, DNM2, BIN1, DYSF, and CAV3 in the biogenesis and maintenance of muscle, specifically at the T-tubule. If DNM2 levels are found to be upregulated in these other forms of myopathy, then targeting DNM2 becomes a common therapeutic strategy for a wider range of muscle disease.

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## PPAR<sub>γ</sub> in emphysema: blunts the damage and triggers repair?

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**Cigarette smoke is the most common cause of pulmonary emphysema, which results in an irreversible loss of lung structure and function. Th1 and Th17 immune responses have been implicated in emphysema pathogenesis; however, the drivers of emphysema-associated immune dysfunction are not fully understood. In this issue of the JCI, Shan and colleagues found that peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) is downregulated in APCs isolated from the lungs of emphysematous chronic smokers and mice exposed to cigarette smoke. Furthermore, treatment with a PPAR $\gamma$  agonist prevented emphysema development and appeared to reduce emphysema-associated lung volume expansion in mice exposed to cigarette smoke. Further work will need to be done to evaluate the potential of PPAR $\gamma$  agonists to restore lung capacity in emphysematous patients.**

Pulmonary emphysema is a major component of chronic obstructive pulmonary dis-

ease (COPD) and involves the loss of alveolar units distal to the terminal bronchioles. Even though COPD holds an unenviable position as the world's fourth-leading cause of death, current medical interventions have little to offer beyond symptomatic relief. Meanwhile, the prevalence of

COPD is expected to continue to rise as low- and middle-income countries join in the developed world's tobacco addiction. If we are to avoid this grim projection, we must expand our knowledge of the basic mechanisms behind lung injury and repair before translating these findings into novel therapeutic treatments.

### Pulmonary emphysema results from an imbalance between elastases and anti-elastases

It has been more than 50 years since Laurell and Eriksson first identified a deficiency in  $\alpha$ -1 antitrypsin, the major inhibitor of neutrophil elastase (ELANE), as the culprit behind hereditary pulmonary emphysema (1). Since the involvement of ELANE in pulmonary emphysema was first reported, our understanding of the disease's

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