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A novel role for the cytoskeletal linker protein dystonin in the maintenance of microtubule stability and the regulation of ER-Golgi transport

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Abbreviations: *dt*, *dystonia musculorum*; ER, endoplasmic reticulum; MAP1B, microtubule-associated protein-1B; MTOC, microtubule organizing complex; NE, nuclear envelope, UPR, unfolded protein response

Crosslinking proteins maintain organelle structure and facilitate their function through the crosslinking of cytoskeletal elements. We recently found an interaction between the giant crosslinking protein dystonin-a2 and the microtubule-associated protein-1B (MAP1B), occurring in the centrosomal region of the cell. In addition, we showed that this interaction is necessary to maintain microtubule acetylation. Loss of dystonin-a2 disrupts MT stability, Golgi organization, and flux through the secretory pathway. This, coupled to our recent finding that dystonin-a2 is critical in maintaining endoplasmic reticulum (ER) structure and function, provides novel insight into the importance of dystonin in maintenance of organelle structure and in facilitating intracellular transport. These results highlight the importance of cytoskeletal dynamics in communicating signals between organelle membranes and the cytoskeleton. Importantly, they demonstrate how defects in cytoskeletal dynamics can translate into a failure of vesicular trafficking associated with neurodegenerative disease.

Introduction

Cytoskeletal cross-linker proteins have been the focus of much attention in disorders of the sensory-motor system. While much investigation has focused on understanding the role of the giant cytoskeletal linker protein dystonin, the daunting size of the protein coupled to the numerous protein isoforms generated from the dystonin (*Dst*) gene have made elucidation of divergent isoform functions arduous. In the recent article *Microtubule stability, Golgi organization, and transport flux require dystonin-a2/MAP1B interaction*,[1] we highlight a novel function of the dystonin-a2 isoform in mediating Golgi organization and flux through the secretory pathway. We have previously determined that the dystonin-a2 isoform is anchored at the nuclear envelope, and is involved in organization of membranous structures of the ER.[2-5] We have now performed expression profiling of pre-phenotype stage dystonin null dorsal root ganglia and isoform-specific loss of function analysis to evaluate the role of dystonin in flux through the secretory pathway. We find defects in anterograde transport and secretion coincident with ultrastructural dilation of the Golgi complex and loss of MT acetylation resulting from depletion of the dystonin-a2 isoform. Through interaction with MAP1B, dystonin-a2 maintains perinuclear acetylation of α-tubulin necessary for discrete organization of the Golgi complex. Maintenance of MT acetylation status through chemical inhibition of deacetylation or MAP1B overexpression, maintains Golgi structure following dystonin-a2 loss, normalizing flux through the secretory pathway. Moreover, dystonin-a2 was found to be critical for MAP1B localization at the centrosome. Collectively, our recent work identifies dystonin-a2 as a novel regulator of ER-Golgi transport through structural organization of the endomembrane system.

Dystonin as a regulator of ER-Golgi transport. The endomembrane system (consisting of the ER, Golgi, nuclear envelope (NE) and lysosomes) traffics proteins and lipids among organelles and the plasma membrane along microtubules and actin filaments. Aberrations in components of the endomembrane system have been associated with numerous disorders of the nervous system resulting in both cognitive and sensory-motor deficiencies.[6,7] Our recent findings offer an improved understanding of the bridge connecting newly formed transport vesicles and the cytoskeleton that may offer important insight into the underlying

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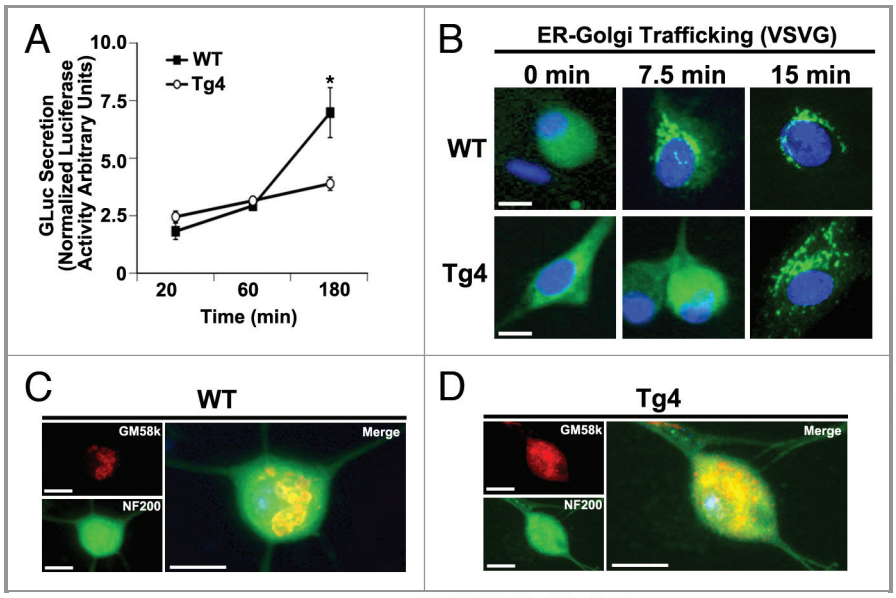

Figure 1. Transport flux in dt^{Tg4} sensory neurons is impeded. (A) Flux through the secretory pathway was assessed in primary sensory neurons. Secreted gaussia luciferase (GLuc) was delivered to WT and dt^{Tg4} sensory neurons, and its secretion was monitored by measuring luciferase activity in the medium over time. Data were normalized to non-secreted firefly luciferase. dt^{Tg4} neurons show a decrease in flux through the secretory pathway relative to WT sensory neurons, (anova post hoc Tukey, *$p < 0.05$, $n = 3$). (B) Defective trafficking of $VSVG^{ts0-45}$ (VSVG) from the ER to the Golgi was observed in dt^{Tg4} sensory neurons relative to WT. (C) WT sensory neurons labeled with the Golgi marker GM58K show a discrete perinuclear organization of the Golgi. dt^{Tg4} neurons show fragmented Golgi through the cytoplasm. Scale bars are 10 μm.

cause of sensory-motor pathologies. We find dystonin-a2 critical to maintenance of transport flux by maintaining the structural organization of both the ER and Golgi.[1,2] We further find this effect to be conserved across multiple *dystonia musculorum* (*dt*) mutant alleles.

dt is an inherited homozygous recessive sensory neuropathy, which results in a severe form of ataxia in mice. Mutations in the *Dst* gene underlie the *dt* disorder. There exist several *dt* alleles, including those induced through spontaneous mutations (e.g., dt^{27J}) or through transgene insertion (e.g., dt^{Tg4}).[8] As both dt^{Tg4} and dt^{27J} alleles are allelic and do not complement, the nature of their mutations impacts the expression of dystonin isoforms differently.[8,9] The dt^{Tg4} mutation prevents the expression of two dystonin isoforms, dystonin-a1 and -a2; while the dt^{27J} mutation disrupts the expression of all three dystonin isoforms, dystonin-a1, -a2 and -a3.[8,9]

We report here, consistent with our findings in dt^{27J} spontaneous mutant mice, that a second *dt* allele, the transgenic dt^{Tg4} mouse, displays similar deficits in secretion and Golgi organization (**Fig. 1**). A failure in transport flux results from defective ER-Golgi transport emanating from aberrant organization of the endomembrane system. Dystonin-a2 is a giant cytoskeletal linker protein flanked by an N-terminal transmembrane domain and a C-terminal microtubule binding domain. The transmembrane domain of dystonin-a2 positions the protein in the perinuclear membranes of the ER, Golgi and NE. The microtubule binding domain of dystonin anchors these membranes to the microtubular cytoskeleton. This maintains the organization of the Golgi around the microtubule organizing complex (MTOC) and centrosome (**Fig. 2**). Ultrastructural and macrostructural evaluation of the Golgi revealed both the dispersal of Golgi ribbons in addition to the dilation of Golgi vesicles in the absence of dystonin-a2. We also observed ultrastructural defects in the ER of dt^{27J} sensory neurons,[2] which preceded phenotype onset. We believe these structural abnormalities arise through impaired cytoskeletal dynamics. Support for this notion comes from previous studies wherein ectopic expression of dystonin-a2 localizes with both ER and Golgi protein markers, and reorganizes the ER via cytoskeletal filaments.[3] At the NE, the dystonin-a2 isoform associates with the NE protein nesprin 3α. However, ultrastructural analysis of the NE has not revealed any gross changes in NE structure (data not shown). As such, the cellular role of dystonin-a2 at the NE does not appear to involve structural maintenance. Rather, dystonin-a2 recruits various cytoskeletal elements to the NE.[4,5] Perturbation of this connection between the NE and cytoskeleton may underlie the eccentric nuclei observed in dystonin deficient sensory neurons[10,11] and may yet prove to be essential to intracellular communication via the endomembrane system. We propose a novel function for dystonin-a2 in the regulation of ER-Golgi transport though binding and sequestration, or in some instances activation, of multiple proteins required for normal transport flux. We find dystonin-a2 to functionally interact with multiple proteins critical to ER-Golgi transport.[12] Through interaction with MAP1B (centrosome), clathrin (Golgi), nesprin 3α (NE), neurobeachin (lysosomes) and CLIP-170 (polymerizing microtubules),[12] or direct anchoring of membranes via the N-terminal transmembrane domain, dystonin modulates activity of proteins critical to proper vesicular transport between every organelle of the endomembrane system. The full extent of this protein

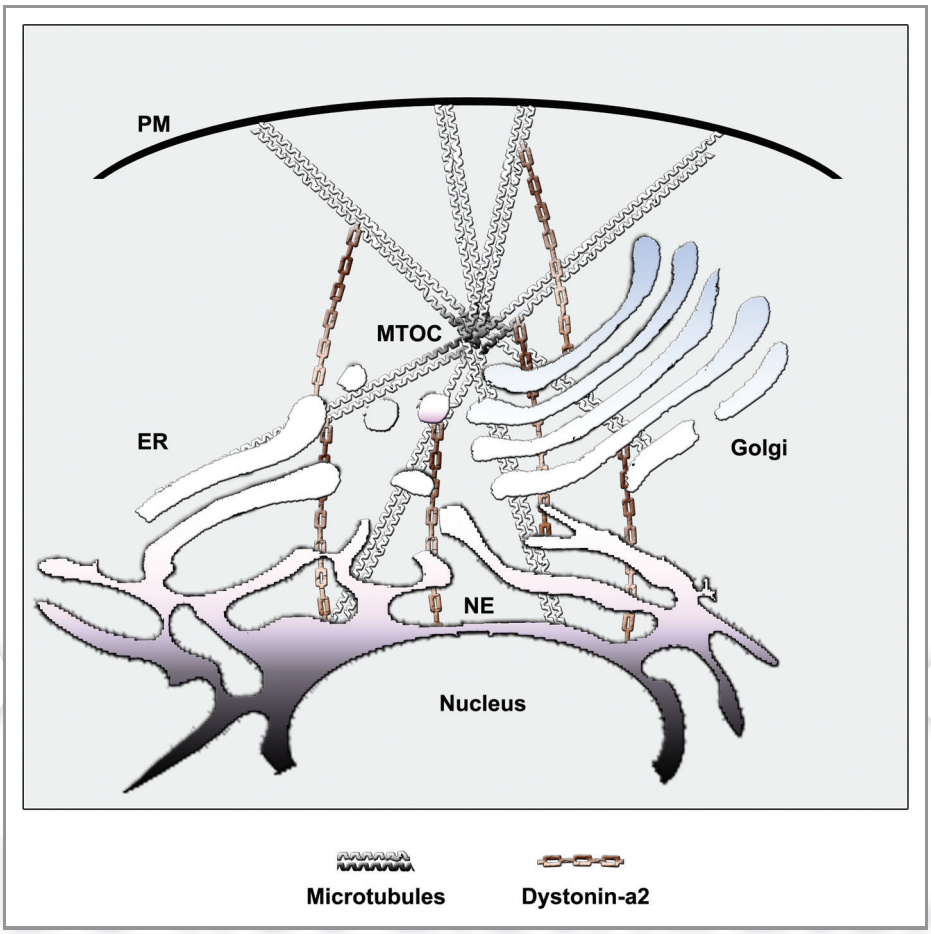

Figure 2. The role of dystonin-a2 in organization of ER-Golgi membranes. Schematic illustration of dystonin-a2 as an anchor of the endomembrane system. Dystonin-a2 links the perinuclear membrane of the ER, Golgi and NE to the microtubular cytoskeleton. This maintains the organization of the Golgi around the microtubule organizing complex (MTOC) and facilitates transport flux from the ER to the Golgi.

interaction network and its importance to cellular communication in both neuronal and non-neuronal systems remains to be elucidated.

Dystonin-a2: A transducer of second messenger signaling? Our analyses of dystonin-a2 focused on the neuronal cell soma, where we find dystonin-a2 critically associates with the ER, Golgi and NE. Within the soma, rough ER sheets and cis-Golgi elements are most prominent, and are involved in protein synthesis, trafficking, quality control, and post-translational modification.[13,14] Through modulation of organelle structure, we find dystonin-a2 to be critically linked to second messenger signal transduction. Ultrastructural aberrations in ER morphology following dystonin loss were evaluated for their impact on ER function. We found that primary sensory neurons derived from *dt*^{27J} mice show perturbations in both Ca^{2+} homeostasis (i.e., defective efflux of Ca^{2+} from the ER) and protein quality-control (i.e., activation of the unfolded protein response).[2] While this study provided important insight into the cellular functions of dystonin-a2, it suggests dystonin-a2 may act as a link between endomembrane system function and apoptosis. Maintenance of transport flux between the ER and Golgi is critical to neuronal function. As transport defects are associated with numerous neurodegenerative diseases, these processes must be tightly regulated and mechanisms must exist to translate defects in orthograde transport into apoptotic messages. Maintenance of ER Ca^{2+} homeostasis may represent one avenue through which these signals are transduced. It remains to be determined, however, whether aberrant ER morphology is causal of Ca^{2+} dyshomeostasis or whether dystonin-a2 interacts with other ER-proteins capable of regulating calcium mobilization.

Conclusion

Dystonin proteins have been implicated in a variety of cellular processes, including processing of proteins through secretory pathways, transport of organelles along neuronal processes, and maintaining cytoskeletal integrity. Their broad involvement in cellular mechanisms can be explained by the importance of cytoskeletal dynamics and the role these proteins have in communicating signals between membranes and the cytoskeleton. While the role we highlight herein for dystonin-a2 in ER-Golgi transport is novel, it is also intuitive given the integral role the cytoskeleton plays in maintaining the structure and function of membrane organelles. Dystonin-a2 is primarily expressed in

sensory neurons of the peripheral nervous system. A majority of our work has focused on the role of dystonin isoforms in post-mitotic neurons. The diverse expression of dystonin isoforms in dividing cells of the skeletal muscle and epithelial cell lineages prompts intriguing questions on the role of dystonin in endomembrane organelles at multiple stages of the cell cycle. It would be interesting to assess the impact loss of dystonin has on the integrity of organelles (i.e., ER, Golgi and NE) in dividing cells. Understanding the common functions of dystonin proteins in multiple cell systems will provide critical insight into the role of ER/Golgi/NE cytoskeletal interactions in maintaining cell integrity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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