Parallel mechanisms for direct and indirect membrane protein trafficking by synucleins

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More than two decades of work have yet to conclusively determine the physiological role of the synuclein proteins, even though these abundant brain constituents are participants in a broad array of cellular processes. Among proposed physiological roles is a functional interaction between the synuclein proteins and monoamine transporters contributing to transporter trafficking through direct protein–protein interactions. Recent work shows that an antagonistic effect of the synuclein proteins on the secretory functions of the endoplasmic reticulum and the Golgi apparatus appears to simultaneously influence trafficking of the dopamine transporter and other membrane proteins. Here, we highlight these new findings in view of the broader literature identifying the role of synucleins in protein trafficking and suggest emerging themes for ongoing and future work in the field of synuclein biology.

After more than 2 decades of intense effort, the synuclein (Syn) proteins, particularly α-Syn (α-Syn), remain a focal point for research because of their strong link to the genetics and pathology of Parkinson disease (PD). Despite their abundance both in the brain and in the literature, the Syn proteins continue to elude a concise description of their normal and pathological functions. Among proposed functions is a role for Syn proteins as regulators of membrane protein trafficking. In our most recent work, we have continued our examination of the involvement of the 3 family members α-Syn, β-Syn (β-Syn), and gamma-Syn (γ-Syn) in regulated trafficking of the dopamine transporter (DAT) in both cellular and animal models of Syn protein overexpression.[1,2] Trafficking of DAT and other monoamine transporters of norepinephrine (NET) and serotonin (SERT) has been linked to the Syn proteins for over a decade.[3] Nonetheless, knowledge gaps exist concerning the details of the trafficking mechanism as well as the broader effects of the Syn proteins on DAT and the other transporters.

Syn protein modulation of DAT: an alternative mechanism

Previous evidence supported a functional relationship between Syn proteins and the monoamine transporters that was dependent on direct protein–protein interactions.[3] In

several earlier studies, modulation of transporter trafficking was mediated by cytoskeletal tethering, with the Syn proteins acting as a link between DAT, NET, or SERT and the actin- or microtubule-based cytoskeleton (**Fig. 1C-1D**). Our new findings, however, suggest that Syn protein modulation of DAT can be accomplished through this direct mechanism as well as the indirect effects of the Syn proteins on endoplasmic reticulum (ER) and Golgi function (**Fig. 1A-1B**) that lead to accumulation of DAT within the biosynthetic compartment.[1] The existence of these parallel mechanisms may be critical to understanding the relationship between Syn proteins and DAT in the brain. While modulation of NET in the brain by α-Syn and γ-Syn has been modeled successfully,[4] the in vivo effects of α-Syn on DAT have been more difficult to identify. Though there is some evidence of DAT trafficking deficits in α-Syn KO mice,[5] many contradictory findings have emerged from this model.[6-8] More broadly, work in double KO[9,10] and triple KO[11] mice has thus far failed to provide evidence that Syn proteins influence DAT trafficking. Age-dependent deficits in the TKO mice, however, do demonstrate that Syn proteins are critical for normal brain function,[12] and suggest that significant functional overlap exists between α-Syn, β-Syn, and γ-Syn. Nonetheless, as the Syn proteins appear to have broad but subtle effects on many components of the synapse and cell soma, it is very likely that the absence of effects in these KO models is due to compensatory or competing mechanisms that merit further exploration.

DAT as a link between Syn proteins and ER pathology

Even if the evidence for a direct interaction in vivo is limited, it is clear that a relationship exists between the Syn proteins and the cellular processes that produce and distribute DAT. Dopaminergic lesions result in the loss of DAT terminals through degeneration, but there are additional losses in DAT function that result from a simultaneous reduction of DAT export, leading to its accumulation in the ER–Golgi compartment.[13] Also, the ratio of glycosylated to non-glycosylated DAT is elevated in the dopaminergic neuronal populations most affected in PD,[14] suggesting that trafficking of DAT in the neurons comprising the nigrostriatal pathway is especially dependent on efficient ER and Golgi function. Furthermore, pathological excess of α-Syn can trigger ER stress in rats,[15] and similar effects have also been reported recently in a mouse model of synucleinopathy.[16,17] These findings show that the Syn proteins are involved in both the normal function of the dopaminergic ER and the ER-based pathology that develops during dopaminergic neurodegeneration.[18]
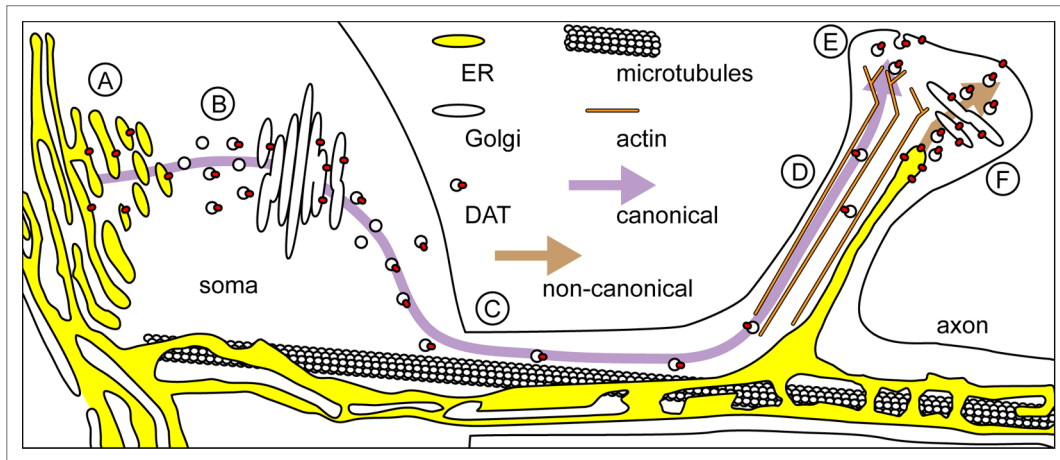
Figure 1. Evidence supports many distinct possible mechanisms that may operate simultaneously and in parallel to modulate trafficking of DAT and similarly secreted proteins in neuronal cells. The earliest steps of biosynthesis, including chaperone-mediated folding events (**A**) inside the ER, may be subject to interference from the Syn proteins, as several recent studies show binding with ER chaperone Grp78 and identify α-Syn inside the ER lumen.[15-17] Syn proteins also antagonize many elements that contribute to (**B**) export of newly synthesized membrane proteins out of the ER and to the Golgi apparatus.[1] The consequences of this function, which have varied widely across different model systems, range from a general slowing of the ER–Golgi transition to significant cytotoxicity.[24-27] Syn proteins can bind tubulin and have been shown to (**C**) act as a bridge between DAT or NET and the microtubule cytoskeleton.[4,33,34] This tethering function serves to increase the cytosolic fraction of the transporters and reduce their distribution to the cell surface. A similar relationship has been described between α-Syn, NET, and (**D**) the actin cytoskeleton, which penetrates even further into the axonal synapse.[35] The final steps required to insert DAT and related cargos into the plasma membrane at or near axonal synapses are (**E**) mediated by SNARE proteins.[36,37] Assembly of SNARE proteins into functional complexes is in part dependent on appropriate levels of the Syn proteins.[12] These findings together suggest a role for the Syn proteins in regulating the insertion of trafficked proteins into the pre-synaptic plasma membrane. Syn mRNA has been identified in the axonal transcriptome, suggesting that local translation could contribute to accumulation of the Syn proteins in the pre-synaptic area.[32] This accumulation puts the Syn proteins in position to have a potentially large contribution to regulating (**F**) non-canonical secretory functions performed by axonal ER and Golgi outposts that to date remain poorly described.

Although it remains controversial, evidence is also accumulating for a role of the Syn proteins inside the ER lumen. Several studies have shown that α-Syn interacts functionally with the lumenal ER chaperone Grp78/Bip and further suggest that intra-ER accumulations of α-Syn are directly linked with the processes of synucleinopathy.[16,19] Grp78 is a critical determinant of the folding capacity of the ER lumen, with Grp78 assisting in the functional expression of complex transmembrane proteins such as SERT, a close homolog of DAT.[20] Like the IgG heavy chain (C_H1), a classical Grp78 substrate,[21] the Syn proteins are recognized as intrinsically disordered proteins.[22] Structural studies have shown that by binding to and stimulating the ATPase activity of Grp78, intrinsically disordered proteins are constantly engaged in sequestering the "open" population of the chaperone, thus reducing availability of Grp78 for enabling the folding reactions of other ER-resident proteins.[23] The site of interaction between Grp78 and the Syn proteins is unclear, but whether they occupy the substrate binding pocket or merely associate with the substrate binding domain, physical interactions with this portion of Grp78 are likely to modify its activity. Further analysis of the interaction of Syn proteins with the components of the ER lumen, in particular description of a mechanism of ER entry, is an effort that should continue to generate exciting results.

Syn proteins in the context of neuronal secretion

Many studies now implicate the Syn family generally, and α-Syn in particular, in both regulatory and pathological roles with regard to ER and Golgi function. While some of the relevant work has been conducted in neuronal culture systems or in vivo models, much of the data supporting involvement of the Syn proteins in the process of transmembrane protein trafficking comes from more generic mammalian cell culture models (eg NRK or COS-7 cells)[24,25] or even yeast and other organisms that lack endogenous Syn protein expression.[26,27] As these results continue to accumulate, it is important to relate the findings in these model systems to the admittedly more complex and less understood reality of transmembrane protein trafficking in actual neurons. It has been established that neuronal soma contain the familiar ER and Golgi configuration, with a well-described ER–Golgi interface and a post-Golgi pathway that appears similar in many respects to the trafficking systems described in other cell types.[28] Most of the proposed models describing the involvement of Syn proteins in regulating ER – and Golgi-dependent trafficking rely on this canonical understanding of the function and arrangement of these secretory organelles. There is a growing consensus, however, that neurons possess alternative modes of secretory trafficking that are topologically and functionally dissimilar to the generic model often presented.[29] Non-canonical ER–Golgi trafficking is best described in the dendritic compartment, but similarly specialized secretory machinery has also been identified in neuronal axons.[30] Though the Syn proteins can be expressed throughout the various neuronal cell compartments, it is generally agreed that these proteins accumulate pre-synaptically, and recent work shows that the Syn proteins are enriched in pre-synaptic membranous

structures that remain poorly described.[31] This places the Syn proteins in an optimal position to directly influence non-canonical neuronal secretion by interaction with various protein and lipid components of putative axonal ER and Golgi outposts (**Fig. 1F**). Furthermore, Syn mRNA has been identified in the axonal transcriptome,[32] strengthening the evidence that the Syn proteins are involved in regulation of this specialized biosynthetic compartment.

Conclusions

The Syn proteins have the potential to be simultaneously involved in regulating the core biosynthetic processes as well as modulating specialized secretory and trafficking events at or near the pre-synaptic membrane. These facts indicate the potentially broad impact of the Syn proteins on neuronal function, but also highlight the difficulties involved in dissecting these diverse activities. This difficulty is exacerbated for DAT and other pre-synaptic transmembrane proteins that still lack comprehensively described neuronal trafficking mechanisms. Ongoing and future efforts should be directed at elucidating the details of Syn protein biology as well as answering more fundamental questions regarding the unique biosynthetic pathways present in neurons.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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