UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI BIOLOGIA

Corso di Laurea in Biologia

ELABORATO DI LAUREA

INTERACTION BETWEEN ALPHA-SYNUCLEIN AND RAB PROTEINS IN HEALTH AND DISEASE

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ANNO ACCADEMICO 2024/2025

Abstract

Intracellular trafficking pathways are extremely complex and need a high degree of regulation to work correctly. In this regard, Rabs are small molecular switches that are involved in many cellular functions, such as regulating vesicle trafficking on all levels, from vesicle formation all the way to vesicle fusion.

α-synuclein is a protein, mostly present in nervous tissue, whose function is not fully understood. Nevertheless, it seems to be involved in synaptic activity, mainly neurotransmitters release, reuptake and synaptic vesicle trafficking. In addition, α-synuclein is the main constituent of Lewy bodies, intracellular proteinaceous aggregates that cause neurodegeneration and play a major role in Parkinson's Disease and related synucleinopathies.

We will be analysing the interactions between α-synuclein and Rab proteins in health and disease: in particular how α-synuclein impacts Rab activity, leading to a dysregulation in vesicle trafficking, and how Rabs play a role in α-syn clearance, seeding and spreading.

Contents

Rab proteins

Introduction

The Rab GTPases family is the largest in the Ras superfamily[1]. Rabs are very conserved proteins: the first homologous protein, known as Ypt1, was identified in *Saccharomyces cerevisiae* and since then around 70 members of the Rab superfamily have been identified in humans so far[2]. Rabs are small "molecular switches" than can be switched 'ON' and 'OFF' by alternatively binding GDP and GTP or by hydrolysing GTP to GDP: this results in a drastic change of its three-dimensional structure[1,2]. A variety of factors is constantly moving between organelles in a eukaryotic cell and this is possible thanks to vesicular transport. However, the vesicles require additional signalling to navigate between the cytoplasm and accurately deliver cargoes[2,3]. Rabs regulate all steps of vesicle traffic, including formation, transport, tethering and fusion, through different effector proteins[3].

Molecular structure

Rabs share the "GTPase fold", composed of a six-stranded β-sheet ensheathed by five α-helices in parallel configuration[2,3]. The switch I and II regions are responsible for Rabs' conformational changes: as they make contact with the γ phosphate of GTP, the disordered switch regions change to a well-ordered configuration[2,3]. α1 has a stretch which presents a GTP-binding platform and an element to bind Mg^{2+}, required for binding and hydrolysing nucleotides[2]. The C-terminus was named "hypervariable" region due to its highly variable amino acid sequence. It is followed by the CAAX box where a geranylgeranyl tail is attached to regulate Rabs membrane insertion[3].

Mechanism of action

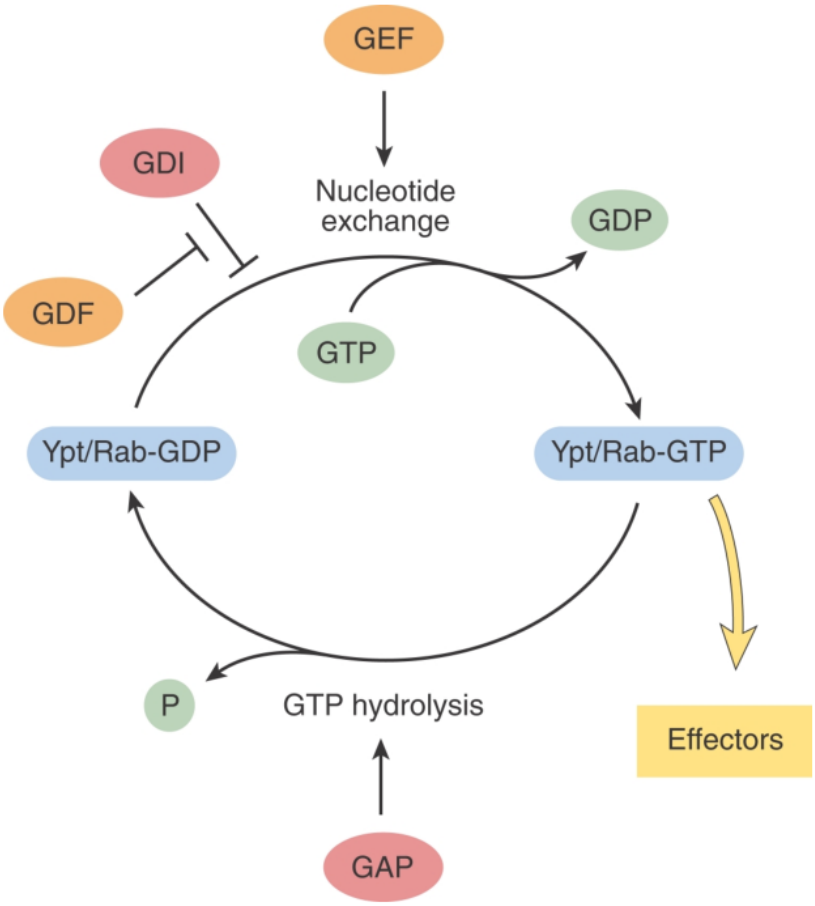

Figure 1: Rab regulation cycle[1]

Rab proteins cycle from the cytosol to membranous departments: the protein localization is influenced by its nucleotide-bound state, which also determines Rab activity (Figure 1). GTP-bound Rab is considered the 'active' form of the protein, while GDP-bound Rab is considered the 'inactive' form[2]. After translation, the new Rab-GDP associates with Rab escort protein (REP) that allows its interaction with Rab geranylgeranyl transferase (RabGGT) and a geranylgeranyl tail is attached to the carboxy-terminus[3]. REP, then, inserts Rab-GDP into its target membrane and a guanine nucleotide exchange factor (GEF) activates the Rab by exchanging GDP for GTP. The now active Rab-GTP interacts with its effectors which coordinate the cellular transport pathway[1,3]. A GTPase-activating protein (GAP) catalyses the hydrolysis of GTP and reverts Rab to its inactive state. The Rab-GDP is extracted from the membrane by GDP dissociation inhibitor (GDI) and a GDI dissociation factor (GDF) releases Rab from GDI to

insert Rab back into the membrane[3]. This cycle is essential to cellular traffic and a mutation in any of the proteins involved can lead to disease[3].

Functions

Rabs are present in almost all organelles and seem to be involved in regulating all aspects of vesicle transport, including vesicle formation, vesicle movement, membrane remodelling, vesicle docking and vesicle fusion[1–3]. The interaction between the GTP-bound form and different effector proteins regulates the Rabs pathway[3].

Role in neurodegenerative diseases

Alzheimer's disease

Alzheimer's disease (AD) is the main cause of dementia in the world. It is caused by progressive loss of neurons, particularly in the hippocampus and cerebral neocortex, due to the hallmark deposition of β-amyloid plaques [4]. Symptoms mainly include the progressive memory loss and the inability to carry out simple tasks.

Some Rab GTPases (Rab4, Rab5, Rab7 and Rab27) might play a role in AD pathogenesis through overactivation of the endocytic pathway, which would lead to imbalanced endosomal signalling and protein turnover. This would cause the accumulation of β-amyloid plaques by disrupting normal lysosomal activity. Nevertheless, it is still unknown if altered levels of Rabs are a cause or a correlate of AD pathology[4].

Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder characterized by the expansion of a trinucleotide repeat cytosine-adenine-guanine. It is caused by CAG

repeated expansions in the *huntingtin* (*htt*) gene, causing a Poly-Glutamine stretch in the protein[4]. Htt protein interacts with Rab5, Rab8 and Rab11 to control different levels of vesicle trafficking. Additionally, increased activity of Rab5 and Rab11 respectively seems to rescue the mutated phenotype and decrease the mutant Htt toxicity[4].

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the whole nervous system. It is associated with many cellular defects and membrane trafficking is fundamental to all of these processes[4]. Intronic expansion of a hexanucleotide GGGGCC repeat in the *c9orf72* gene is the main cause of both familial ALS and frontotemporal dementia. C9ORF72 functions as a RabGEF for Rab8a and Rab39b and as a Rab1a effector. Reduction of *C9orf72* expression leads to accumulation of p62 aggregates associated with ALS, indicating autophagic defects. According to this data, the mutated C9ORF72 might lead to a disruption in endosomal traffic[4]. In addition, mutations in SOD1, TDP-43 or FUS, which are involved in ALS pathogenesis, cause mislocalization of Rab1, but the impact this has on pathology is still unknown[4].

Charcot-Marie-Tooth Disease

Charcot-Marie-Tooth disease (CMT) is a motor and sensory disorder characterized by progressive muscle atrophy. The disease's hallmarks include abnormalities in myelin formation and maintenance or axon degeneration of motor and sensory neurons[4]. CMT type 2B is a direct consequence of Rab7 dysfunction, but it's still not clear whether the pathology involves a Rab7 gain-of-function or loss-of-function mutation [4]. Mutations in Rab28 and Rab11 effectors might lead to CMT type 4 via altered GTPase function[4].

α-Synuclein

Introduction

α-synuclein (α-syn) is a member of the synuclein family, alongside β-synuclein and γ-synuclein, which are neuronal proteins that mostly localize to presynaptic terminals [5]. Its function is not fully understood; what seems to be clear however is the implication of abnormal aggregates of α-syn, namely Lewy bodies, in many neurodegenerative diseases called α-synucleinopathies, including Parkinson's disease (PD) [6].

Molecular structure

α-syn is a small 140 amino acid protein encoded by the SNCA gene [5]. α-syn presents three main regions: a N- and a C-Terminus separated by a Non-amyloid Component (NAC) domain which is the most aggregation-prone part[6] (Figure 2).

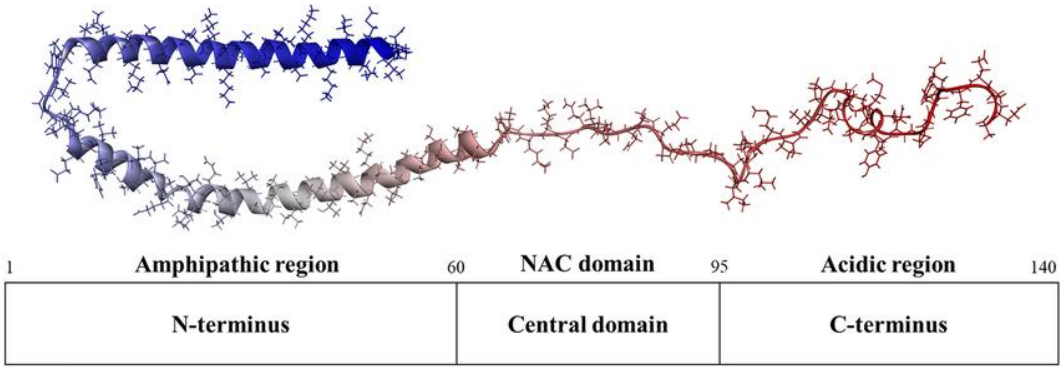

Figure 1: α-syn molecular structure[7]

Two different forms coexist: a "natively unfolded" monomer and a folded tetramer with little aggregation potential. An imbalance in the tetramer:monomer ratio may lead to a high number of pro-aggregating forms[6]. Amyloidogenic aggregation requires partially folded intermediates, an unstable form which is stabilized by dimerization. This means that the protein aggregation process is highly dependent on the protein concentration[8]. [7]

Physiological Functions

α-syn function is only partially understood. It is mostly expressed in the nervous system and in particular at the presynaptic terminals where it plays a role in release and reuptake of neurotransmitters. It is also involved in stabilizing curved membranes and regulating the vesicle trafficking; this last function may be particularly relevant in the substantia nigra pars compacta, the region which is most affected in PD[6].

Regulation, modifications and propagation

There are many factors affecting α-syn expression. Toxins that are known to cause PD also increase α-syn expression, along with some drugs like cocaine and alcohol. The biggest risk factor however is aging, as protein level increases over time[8]. Other systems that appear to be involved in α-syn accumulation are impairment in the ubiquitin–proteasome system and the lysosomal autophagy system. Despite the fact that the protein physiological degradation mechanism remains unclear[8], it appears that chemical inhibitors of proteasomal enzyme activity block the degradation of α-syn, while autophagy activity decreases with aging[8]. In addition, α-syn itself might impact protein degradation systems, leading to a vicious cycle involving α-syn accumulation[5].

Post-translational modifications play a central role in α-syn physiopathology. Indeed, α-syn α-synuclein may be modified by phosphorylation, oxidation, nitrosylation, glycation, or glycosylation[5]. It seems that phosphorylation in particular plays a major role in α-syn aggregation, as 90% of the protein in Lewy bodies is phosphorylated[6].

Intracellular α-syn can be secreted through vesicle transport. However, it appears that only damaged proteins are discarded through exocytosis, possibly leading to accumulation and aggregation of misfolded α-syn in specific vesicle populations[8]. Subsequent α-syn endocytosis can lead to propagation. The other way α-syn can spread from cell to cell is through a “prion-like” mechanism, where α-syn fibrils enter inside cells and induce endogenous α-syn aggregation[9]. Oligomeric-

aggregated α-syn seems to be prone to uptake and has the potential to promote fibrilization of endogenous α-syn[5].

Role in Parkinson's Disease

PD is the second-most common neurodegenerative disorder in the world, characterized by motor symptoms, like bradykinesia, resting tremor and rigidity, but also cognitive disorders that add to overall burden. The pathological hallmark for PD is the loss of dopaminergic neurons in the substantia nigra and two abnormal protein deposits, Lewy bodies and Lewy neurites[8,10]. α-syn is the main component of those aggregates and its deposition is the driving force in PD pathogenesis[5].

The cause of sporadic PD is still unknown; familial PD, on the other hand, is most commonly caused by mutations in various genes, among which the leucine-rich repeat kinase 2 (*LRRK2*), a protein involved in different roles such as vesicular trafficking and autophagy, is the most frequent[11].

Pathogenic effects

Among familial cases of PD, mutations or duplication/triplication of the α-syn gene have been reported. Indeed, overexpression of α-syn leads to different synaptic effects including loss of presynaptic proteins, decrease of neurotransmitter release, redistribution of SNARE proteins, enlargement of synaptic vesicles, and inhibition of synaptic vesicle recycling[5]. The toxicity of α-syn assemblies is partially caused by their binding to cell membranes. This leads to membrane curvature impingement, affecting cell survival[12]. Pathological α-syn also negatively affects mitochondrial activity, potentially inducing reactive oxygen species production. Oxidative stress may impair the ubiquitin-proteasome system, causing abnormal protein aggregation which in turn leads to neuronal death[5,6]. In addition, α-syn may also influences actin polymerization, affecting cellular trafficking and neuronal survival[5].

One of the main pathological aspects of α-syn pathology is its aggregation and

spreading mechanism. Theoretically, in vitro, aggregation process happens in three phases (Fig. 3): lag phase, elongation phase, where α-syn can associate with other α-syn monomers to elongate indefinitely[12], and stationary phase, where most of α-syn monomers form amyloid fibrils. During lag phase a process called "primary nucleation" takes place, where an aggregation competent nucleus is formed that converts into fibrils[9].

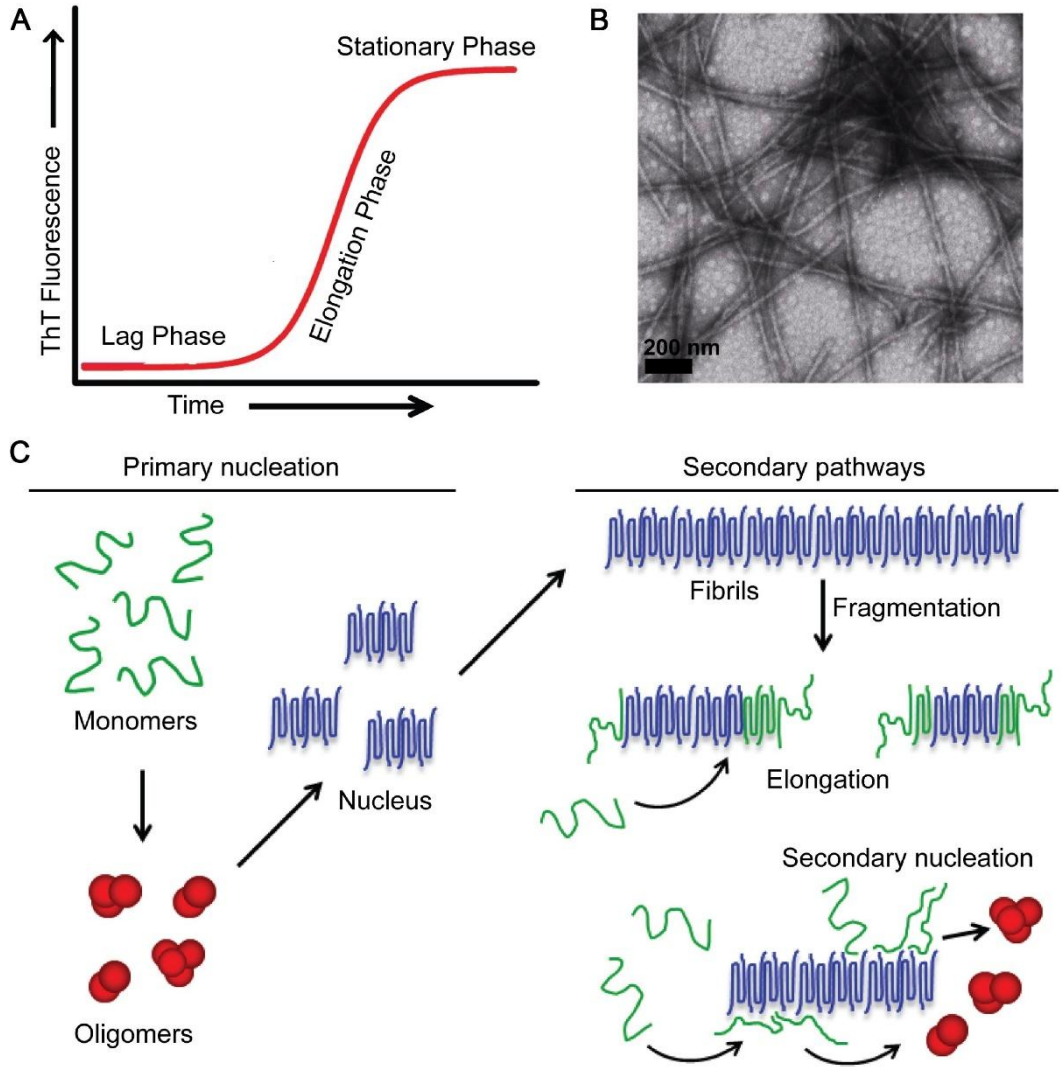

Figure 3: In vitro simulated α-syn aggregation process[9]

Interactions between Rab and α-Syn

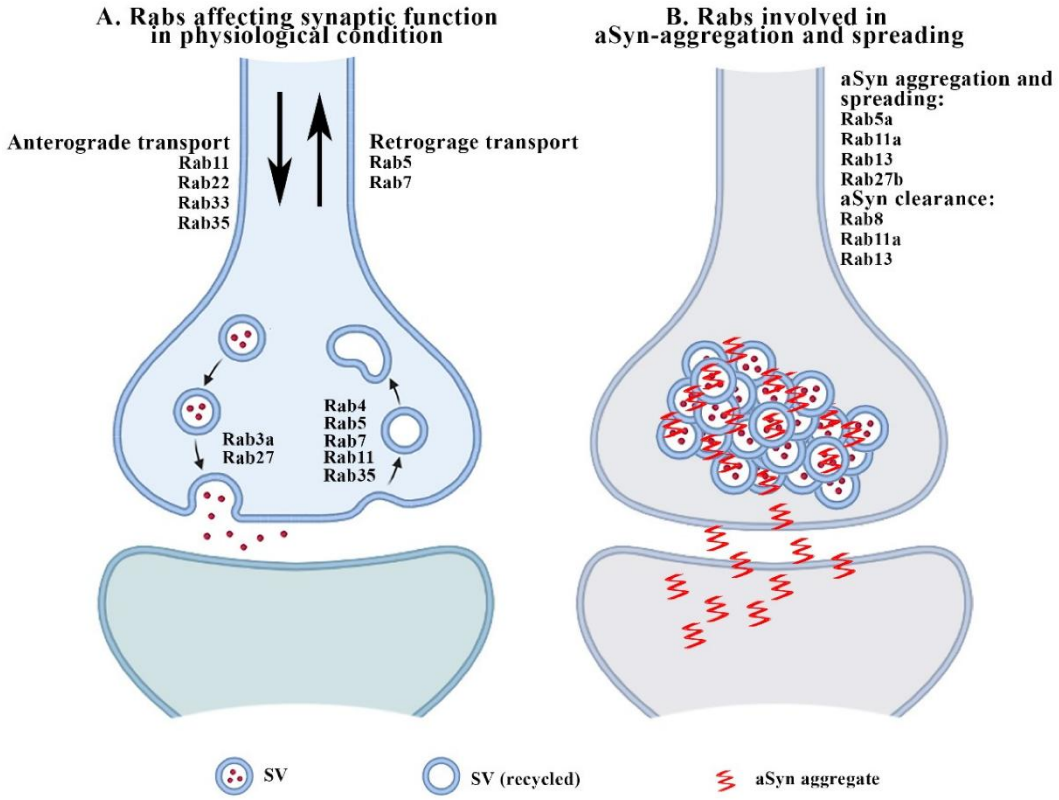

Figure 2: Rabs involved in the modulation of synaptic function in physiological conditions and in α-syn aggregation and spreading[13].

Rab1

α-syn accumulation leads to its interaction with GM130, which in turn negatively impacts ER-Golgi traffic by affecting Rab1. On the other hand, overexpression of Ypt1/Rab1 can protect against α-syn toxicity and restore Golgi fragmentation in damaged dopamine cells[11]. In a similar fashion, α-syn/Rab1 interaction leads to the inhibition of autophagy through the mislocalization of Atg9 and a lower omegasome number. Overexpression of Rab1 enhances autophagy and reduces the number of α-syn aggregates[11]. Research is currently underway to find new

therapeutic agents that interact (directly) with Rab1 or (indirectly) with its effectors[11].

Rab3a

Rab3a plays a major role in the regulation of synaptic vesicle release by interacting with rabphilin[13]. Rab3a also regulates WT α-syn attachment to the membrane of presynaptic vesicles[14]. Interactions between Rab3a, rabphilin and aberrant α-syn may lead to impaired neurotransmitter exocytosis. This hypothesis is supported by the positive correlation between Rab3a and α-syn blood levels and the loss of rabphilin/Rab3a coupling in PD patients[13].

Rab7

Overexpression of a HOPS complex protein, necessary for lysosomal fusion, rescues neurotoxicity through Rab7 by fusing endosomal material to the lysosome for degradation. Overexpression of Rab7 reduces apoptosis and rescues motor deficits by clearing α-syn. Consequently, Rab7 has been proposed as a possible new therapeutic target[15].

Rab8b

Rab8b seems to be an α-syn regulator since its knockdown leads to a higher α-syn aggregation, while its overexpression seems to increase endocytic recycling of α-syn[14].

Rab11a

Rab11 is involved in α-syn secretion via exosomes, a process which might favour α-syn propagation from cell to cell. Overexpression of Rab11 however has been shown to reduce α-syn intracellular aggregation through the same mechanism[14].

Rab13

Rab13 promotes α-syn clearance and rescues α-syn-induced toxicity, while also improving α-syn extracellular secretion[13].

Rab27b

A recent study shows how Rab27b knockdown disrupts lysosomal function, leading to diminished α-syn clearance, and reduces lysosomal exocytosis, a process which has been implicated in synucleinopathies[16].

Rab35

Phosphorylated Rab35 seems to disrupt α-syn clearance by promoting its release trough exocytosis, thus enhancing α-syn extracellular diffusion[15].

Rab39b

Rab39b plays a role in α-syn homeostasis regulation and loss-of-function mutations in Rab39b are causally linked to an X-linked form of early-onset PD[14].

LRRK2

LRRK2 is a negative regulator of autophagic activity and interacts with many different Rabs (Rab1, Rab3, Rab8, Rab10, Rab12, Rab29, Rab35 and Rab43). The G2019S mutation increases the kinase activity, disrupting autophagic activity, thus in turn impacting α-syn[11].

Conclusions

From the data we can conclude that Rabs and α-syn accumulation play an antagonistic role in the brain's vesicle trafficking pathway, with relevant implications for PD and other neurodegenerative diseases.
α-syn aggregates directly interact with a handful of different Rabs and interfere with vesicle transport. This leads to the disruption of fundamental systems such as ER-Golgi transport and lysosomal fusion, contributing to overall cellular damage but also to a vicious cycle of α-syn accumulation.
Overexpression of Rab proteins, on the other hand, promotes α-syn clearance by restoring autophagy and lysosomal pathways, potentially reducing its neurotoxic effects. For instance, Rab8b, Rab11a, Rab13 and Rab39b have been identified as α-syn regulators as they regulate α-syn aggregation, toxic potential and levels[13]. Rabs that are involved in extracellular secretion (Rab11, Rab 13, Rab35), however, might exacerbate α-syn propagation.

As of now, the only effective therapeutic tools for PD are symptomatic treatments that often carry undesirable side effects [12,14]. By targeting Rabs, new therapeutic strategies that promote α-syn clearance and inhibit neurodegeneration could be developed, thus directly counteracting the pathogenesis of PD itself [13]. There are, however, a few challenges to this approach: the degenerative process starts years before the first symptoms appear, so once the therapy starts most of the damage has already been done; another issue is that α-syn aggregation is not the only mechanism involved in PD pathogenesis, so a combination of different approaches might be necessary [6].

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Abbreviations

Rab escort protein	REP
Rab geranylgeranyl transferase	RabGGT
Guanine nucleotide exchange factor	GEF
GTPase-activating protein	GAP
GDP dissociation inhibitor	GDI
GDI dissociation factor	GDF
Alzheimer’s disease	AD
Huntington’s disease	HD
Huntingtin	Htt
Amyotrophic lateral sclerosis	ALS
Charcot-Marie-Tooth disease	CMT
α-synuclein	α-syn
Non-amyloid Component	NAC
Parkinson’s disease	PD
Leucine-rich repeat kinase 2	LRRK2
Endoplasmic reticulum	ER
Wild-type	WT