Pathology, Prevention and Therapeutics of Neurodegenerative Disease

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Preface

Neuroscience is a large field founded on the premise that all of behavior and mental abilities have their origin in the structure and function of the nervous system. This book attempts to provide an overview of major neurodegenerative diseases with a special focus on diseases related to the central nervous system (CNS). Neurodegenerative diseases add up to tremendous medical and financial burden due to their non-partisan share for individuals of all ages, with elderly population contributing the largest share. Due to the enigmatic and complex nature of neurodegenerative diseases, therapeutic intervention to address the same is of immense challenge for the researchers. To date, research has suggested the involvement of diverse factors and complex mechanisms in disease etiology, with a bolting approach still lacking to thwart neurodegeneration. Such impuissance of researchers is mainly due to delayed appearance of behavioral symptoms: the only diagnostic marker for most of the neurodegenerative diseases presently. In fact, the visible symptoms manifest at later and peak stage of disease act as barrier for timely intervention.

Brain has postmitotic neurons thereby lacking restoration of damaged neurons. Previous studies have implicated neurogenesis mainly in the hippocampal area of the brain, while the disease pathology may encompass any brain region. Further, restoration of damaged neurons by stem cell therapy failed to achieve the desired effect due to the lack of versatile utilization for treatment and its financial impact. The prime focus of this book is to introduce students to the major CNS-related neurodegenerative diseases. The chapters aim to introduce the readers about disease pathologies, related mechanisms involved, and available therapeutics. As the disease diagnosis is a huge challenge for physicians and researchers alike, specific chapters focusing on the same have been included to assist the reader in getting a comprehensive view of the disease. Further, the book focuses on neurodegenerative diseases involving mental abilities and motor responses, specifically Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Collectively, research to date strongly supports the view that prevention might be a better approach to fight the disease. In line with disease etiology and diagnosis, we have also endeavored to expose the readers to the existing alternative preventive therapeutic approaches. Alternative therapies derived from natural products may outweigh the side effects of the conventional approaches, thereby a potential option for long-term treatment.

We express our gratitude to all the authors for their efforts in bringing out this compilation in the field of neurosciences. We are also thankful to Eti Dinesh at Springer for her constant support throughout the project. N. S. Pandian (Senior Production Manager) and Kumar Athiappan (Project Coordinator) are also acknowledged for their contribution.

Lucknow, India — Sarika Singh
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About the Editors

Sarika Singh completed her postgraduate degree in biochemistry at Lucknow University in 2001 and subsequently received her doctoral degree from the same university for a dissertation on the role of nitric oxide in the pathology of Parkinson's disease. In 2006, she assumed her current position as a Senior Scientist at CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India. She is a life member of the Indian Academy of Neurosciences and a member of the Indian Academy of Sciences. She is a recipient of international Indo-US and CSIR-Raman research fellowships and has worked toward the identification of diagnostic markers for autism and Parkinson's disease. Having published in several international peer-reviewed journals, she also serves as an editorial board member and reviewer for various international and national journals. Her chief research focus is on investigating the neurodegenerative and neuroprotective mechanisms involved in various brain diseases.

Neeraj Joshi received his master's (M.Sc.) degree in biochemistry from Lucknow University, India, in 2001, after which he was recruited to Bhabha Atomic Research Center (BARC), Mumbai, India. After completing the Orientation Course in Nuclear Science and Engineering at BARC, he worked at its Radiation Biology and Health Sciences Division as a Scientific Officer from 2002 to 2006. His research at BARC focused on investigating DNA damage repair and radiation hormesis in the context of cancer biology and neurodegeneration. To further understand the mechanisms of genomic integrity, Neeraj chose to pursue his doctorate at Cleveland State University, USA, where he explored the mechanistic aspects of meiotic chromosome segregation. This resulted in (1) unraveling the role of genome architecture in DNA damage repair (DDR), (2) the discovery of a new, ultrasensitive DNA damage responsive checkpoint system, and (3) the development of a novel molecular assay: "Homolog Pairing Capture."

Collectively, his doctoral studies provided a new perspective on cellular DDR mechanisms and the indirect involvement of proteasome in the DDR process. From 2015 to 2017, his postdoctoral work at the University of California-San Francisco (UCSF), USA, centered on investigating both the selective and comprehensive repertoire of Cullin-RING-like (CRL) ubiquitin ligases under defined stress conditions.

Alpha Synuclein and Parkinson's Disease

1

Arti Parihar, Priyanka Parihar, Isha Solanki, and Mordhwaj S. Parihar

1.1 Introduction

Parkinson's disease (PD) is the age-related neurodegenerative disorder diagnosed by tremor at rest, rigidity, and bradykinesia symptoms. The prevalence of PD increases with the increase in age and about 2–3% population worldwide suffer from the disease ≥65 years [1]. The major neuropathology of PD patients is the deficit of dopaminergic neurons the substantia nigra pars compacta (SNpc) region of the midbrain. The lesions caused in these brain regions cause severe depletion of striatal dopamine. Non-motor symptoms like dementia, depression, anxiety, insomnia, excessive daytime sleepiness, rapid eye movement sleep disorder, constipation, difficulty in swallowing, and dyspepsia may also be involved in PD symptoms and pathology. Histological characteristic of PD includes the occurrence of Lewy bodies (LBs) in existing neurons [2]. However, little is known about the formation of LBs. The rising evidence revealed that LB biogenesis may involve neuroprotective reactions [3]. Numerous studies have been executed to elucidate the role of α-synuclein in the pathogenesis of PD.

Reports have shown the expression of α-synuclein in neurons which abundantly distributed in presynaptic neuronal terminals of synapses [4]. The distribution of α-synuclein in the synaptic terminals indicates that this protein may take an important role in synaptic plasticity, kinetics of vesicle, and in the dopamine synthesis and its release. The role of α-synuclein in the pathogenesis of PD has been extensively analyzed. The observation of fibrillar α-synuclein in LBs and the occurrence of mutations in the α-synuclein gene in familial forms of PD have led to the belief that this protein has a critical role in PD pathology. The relationship of α-synuclein and PD has been identified by a genetic finding of A53T mutation of α-synuclein gene (*SNCA*) in a family with autosomal-dominant familial PD [5]. Furthermore the implication of α-synuclein in PD has been corroborated by the discovery of the other mutations of *SNCA*, involving A30P and E46K in other families with inherited PD [6, 7]. The function of α-synuclein in PD was further strengthened by the investigation in which presence of this protein was found as the primary structural constituent of LBs [8]. Here, we present an overview of existing knowledge on the physiological functions, oligomerization, and aggregation of α-synuclein and its pathological

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role in PD. Considering the nature of the various α-synuclein structures and its mechanism of toxicity may be important in developing attractive treatment options against the pathologic hallmarks of PD and α-synucleinopathies.

1.2 Localization and the Structure of α-Synuclein

The varied forms of synuclein protein, α, β, and γ are expressed at numerous locations in the nervous system [9]. Synuclein α- and β-forms are chiefly present in nerve terminals, near synaptic vesicles in the central nervous system [10], whereas γ-synuclein is present in neuronal cells of the peripheral nervous system [10]. α-Synuclein is mainly located in the cytoplasm but extracellular α-synuclein has also been studied [11]. In PD, the levels of α-synuclein are higher in cerebrospinal fluid (CSF) than age matched controls [12], indicating that α-synuclein is also present in extracellular brain fluids. Most significantly, α-synuclein oligomers have abundantly distributed in the extracellular space in PD. The presence of α-synuclein both at intra- and extracellular spaces could explain that the extracellular α-synuclein oligomers may disperse from one neuron to another, and this movement might channelize the succession of the disease from one brain region to other regions.

α-Synuclein is a 14 kDa protein (140 amino acids; pKa of 4.7) expressed by the *SNCA* gene on human chromosome 4 [13]. It is the cytoplasmic and/or membrane-bound protein found in presynaptic terminals of neurons [14] categorized by an amphipathic lysine-rich amino terminus (Fig. 1.1a–d). α-Synuclein is intrinsically located in the cytoplasm (Fig. 1.1b) but exhibits alpha helical confirmation when bound to cellular membranes [15]. In addition, α-synuclein is also located in other subcellular compartments such as mitochondria (Fig. 1.1c, d) [16] and it can also be secreted and transferred to nearby cells [17, 18]. The normal cellular state of alpha synuclein is the α-helically folded 58 KDa tetrameric complex that primarily exists as an unfolded monomer in the central nervous system [19]. By structure α-synuclein protein consists of three domains like an amino terminus (residues 1–60), a central hydrophobic region (61–95), so-called NAC (non-Aβ component), and a carboxyl terminus which is extremely negatively charged (Fig. 1.2) and is prone to be unstructured [20]. The N-terminal domain is particularly significant for the pathological dysfunction of *α*-synuclein as the rare point mutations like Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu are present in this region [21]. However, NAC domain is accountable for the aggregation attributes of *α*-synuclein via inhibition of its degradation and promotion of its fibrillation [22]. Although the normal physiological role of *α*-synuclein is not known, still it appears to be involved in compartmentalization, storage, and recycling of neurotransmitters [23]. α-Synuclein has been shown to interrelate directly with the membrane phospholipids, especially vesicles and have a role in the vesicle trafficking during the neurotransmission release. It also appears to be associated with directive of various enzymes and tends to augment the integer of dopamine transporter molecules [24]. In addition, recombinantly α- and β-synucleins inhibit mammalian phosphatidylcholine (PC)-specific phospholipases D2 activity in vitro [25], suggests that inhibition of PLD2 may be a function of synucleins.

In aqueous solution, α-synuclein normally has natively unfolded protein structure but may assume oligomeric and/or fibrillar conformations in definite pathological conditions like mutations in the SNCA gene, overexpression, oxidative stress, and posttranslational amendment (Fig. 1.3a–d). Studies indicate that the pathogenic species of α-synuclein involve the posttranslationally modified, mutant, oligomeric, or aggregated forms that could induce adverse effects by disturbing the physiological function of α-synuclein in release of neurotransmitters [26, 27]. Pathological form of α-synuclein may impair mitochondrial functions and mitophagy [28, 29]. It may also result in endoplasmic reticulum (ER) stress by disrupting ER-Golgi vesicular transport [30, 31] and vitiating the effectiveness of some protein degradation pathways [32]. Thus α-synuclein adversely affects the cellular physiology which consequently causes cellular injury and death.

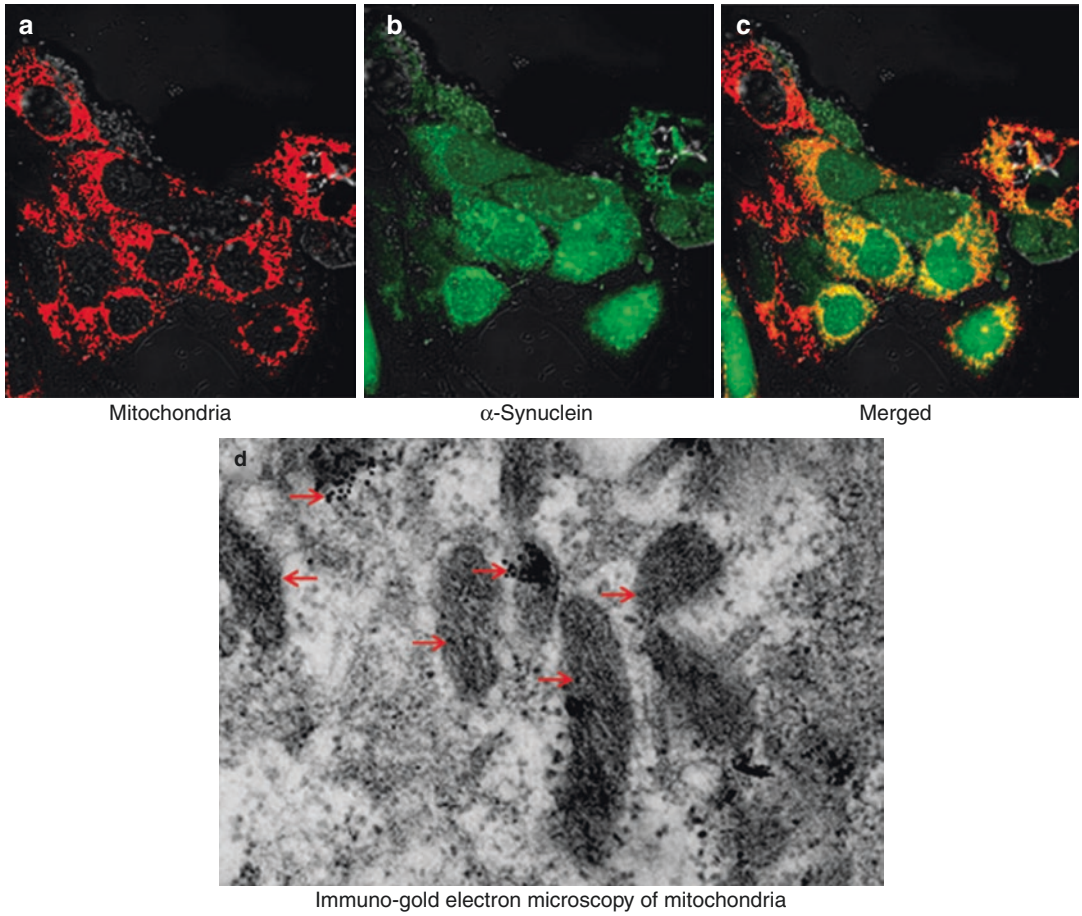

Fig. 1.1 Localization of α-synuclein in the cytoplasm and mitochondria of neurons. (**a**) Human neuroblastoma cells were loaded with mitotracker red (Mitochondria) and (**b**) immunostained for α-synuclein using monoclonal α-synuclein antibody (α-Synuclein). Fluorescence was detected by confocal microscopy. The α-synuclein immunoreactivity is shown in green, mitochondria staining in red, and the merge image (merge) is yellow for overlapping red and green signals (**c**). (**d**) Immuno-gold electron microscopic localization of α-synuclein in the mitochondria of human neuroblastoma cells. Immuno-gold-labeled particles are shown by arrows

1.3 The Transmission and Release of α-Synuclein in Brain Cells

α-Synuclein has self-propagating property, therefore it extends gradually among interconnected brain regions. Different brain regions have the presence of pathological α-synuclein aggregates involving both the peripheral nervous system (PNS) and central nervous system (CNS) [33]. Several observations in human samples revealed the transmission and secretion of α-synuclein in the brain cells. Together monomeric and oligomeric forms of α-synuclein species have been observed in samples of human plasma and cerebrospinal fluid [11, 34], which suggests that α-synuclein can be secreted in brain cells. The exact machinery of α-synuclein release is not entirely understood; however, it is well identified that α-synuclein can be secreted into the culture medium by varied types of neuronal cells [35, 36]. Internalization of α-synuclein has also been demonstrated [37–39], possibly through passive diffusion by enacting with membranes and lipids [40]. Majority of experiments verified that α-synuclein may be spread from one cell to

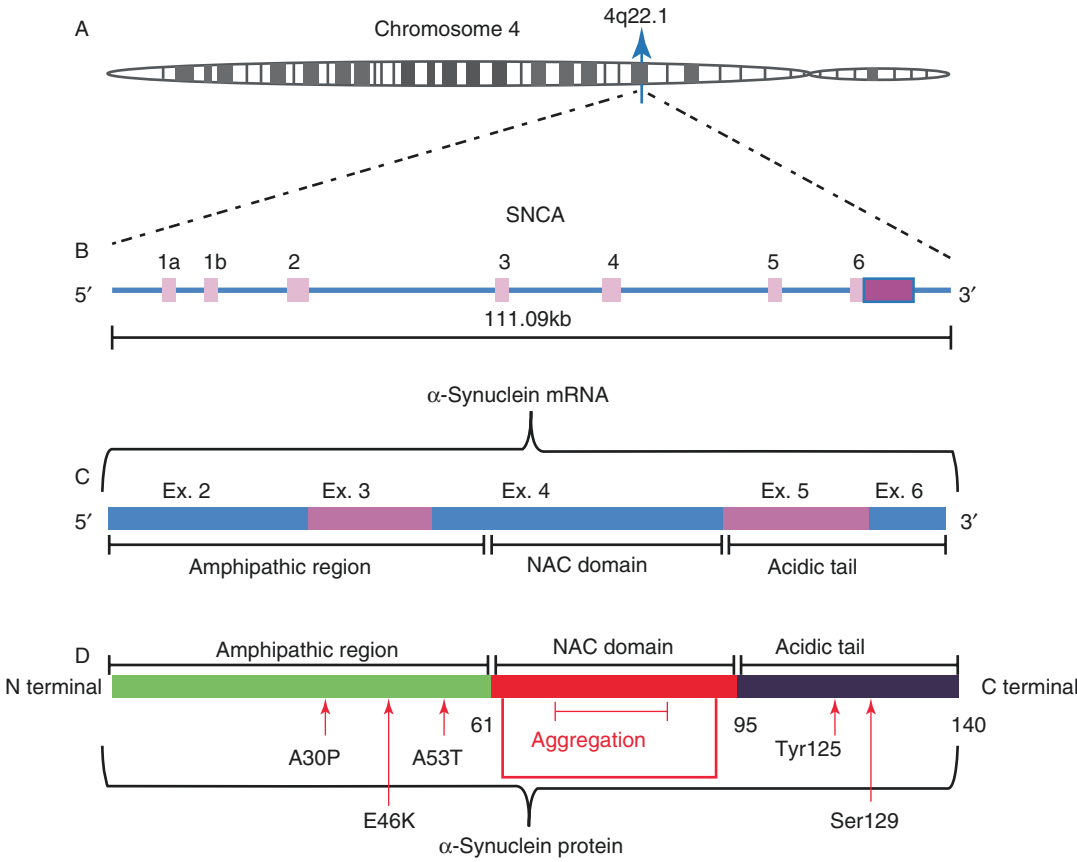

Fig. 1.2 Schematic representation of α-synuclein regions: (**a**) α-Synuclein (SNCA) genomic region on chromosome 4q22.1, (**b**) SNCA gene structure, (**c**) mRNA, and (**d**) protein domains. The amino-terminal from amino acids 1–60 is an amphipathic region. This region is responsible for α-synuclein–membrane interactions. Localized in this region of α-synuclein are three point mutations (A30P, E46K, and A53T). The amino acids 61–95 is termed as central region (NAC), NAC is required for the aggregation process. The C-terminal region from amino acids 96–140 possesses acidic residues and several negative charges. The residue serine 129 in this region is phosphorylated in Lewy bodies. The three missense mutations known to cause familial PD (A30P, E46K, and A53T) lie in the amphipathic region. The non-amyloid-β component or the NAC domain of α-synuclein is associated with an increased tendency of the protein to form fibrils

another by a cell-to-cell transmission machinery [41]. The study confirmed that diverse forms of human α-synuclein, involving monomers, oligomers, and fibrils, might be absorbed by neurons in vivo by endocytosis [42]. In addition, host-to-graft transmission of human α-synuclein has also been demonstrated in rats [43].

1.4 α-Synuclein Physiological Functions

The physiological functions of α-synuclein are the subject most debated in the neuroscience field. However, several researches in the field suggest that α-synuclein enacts at the presynaptic terminal and controls the synaptic transmission. The subcellular localization of α-synuclein at the synapse supports this idea [44, 45]. Evidences suggest that α-synuclein perform many functions at the synapse, i.e., in the rhythm of synaptic vesicles, regulating the vesicle pool size, militarization, and endocytosis [4, 46]. C-terminus region of α-synuclein has been observed to interact with the synaptobrevin-2 (VAMP2) [47], a central player in synaptic exocytosis [48]. Burre et al. [47] reported that the N-terminus of the protein might bind to phospholipids and endorse soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor

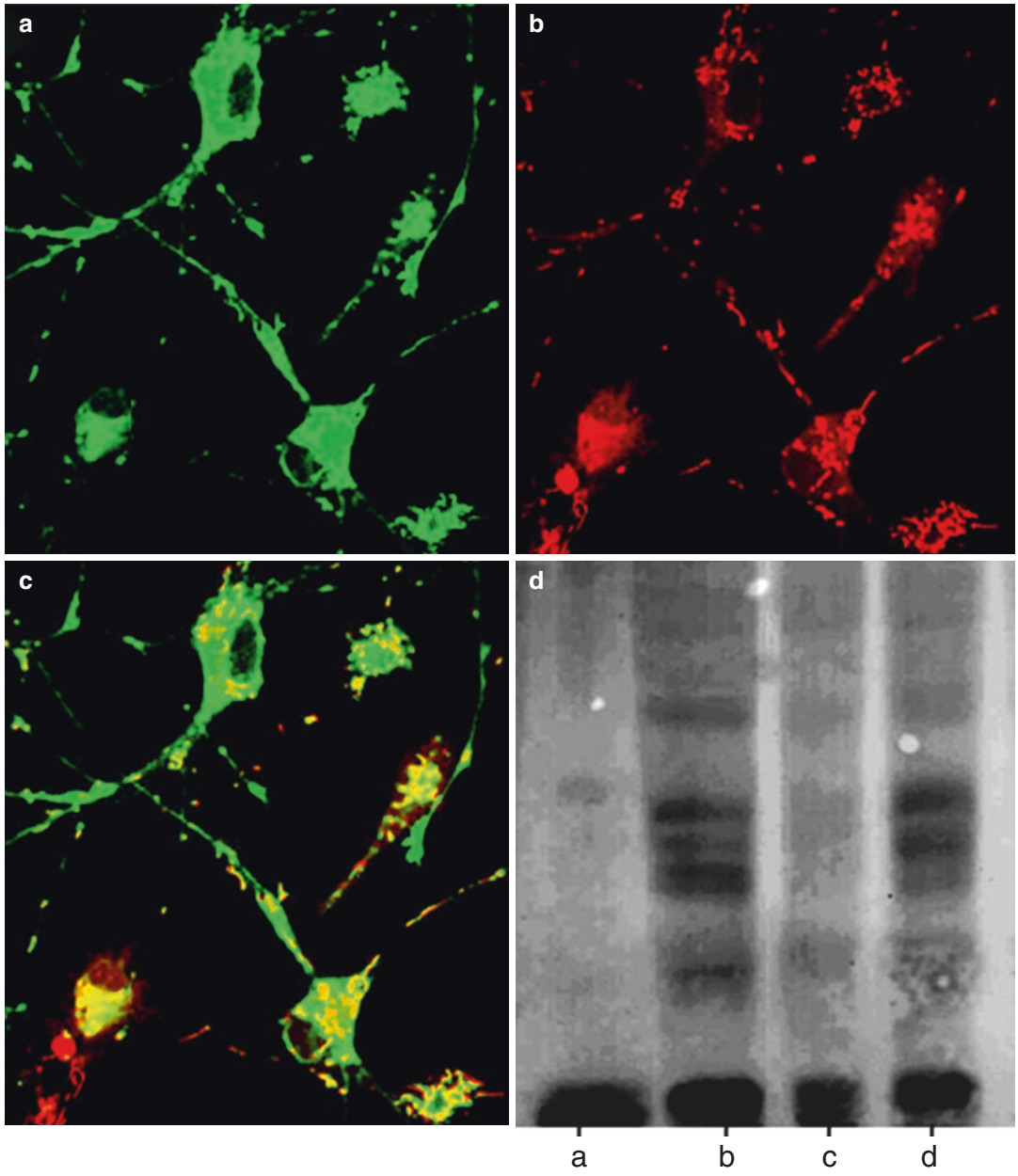

Fig. 1.3 Aggregation of α-synuclein in human neuroblastoma cells. (**a**) Human neuroblastoma cells were overexpressed with wild-type α-synuclein and immunostained for α-synuclein using monoclonal antibody. (**b**) Mitochondria were labeled with mitotracker red. (**c**) Merge shows mitochondria and α-synuclein images overlaid. Aggregates are shown by arrows. (**d**) Silver-stained SDS-PAGE of cell homogenates [lane (a) unaggregated (control) α-synuclein, lane (b) aggregated α-synuclein (mutant A53T), lane (c) aggregated α-synuclein (A30P), and lane (d) aggregated α-synuclein (wild type). Unaggregated α-synuclein migrated at about 19 kDa, consistent with monomeric size. Aggregated α-synuclein showed both low and high molecular mass]

(SNARE) complexes assembly. SNARE proteins encounter important roles in synaptic vesicle exocytosis [49]. Study by Diao et al. [50] revealed that α-synuclein was involved in synaptic transmission by increasing vesicle clustering. These studies suggest that the α-synuclein may delay vesicle trafficking by enhancing vesicle clustering. These studies support the complex multimerization dependent function of α-synuclein, which is vastly reliant on its lipid-binding domains. α-Synuclein can continuously transport between cytosolic monomeric and membrane-bound multimers. α-Synuclein also has an important role in the nucleus. The N- and C-termini of α-synuclein have a signal-like role for its nuclear translocation. Familial mutations and oxidative stress has been found to increase its nuclear localization [51–53]. However, the mechanism of nuclear import of α-synuclein is still not understood. Once α-synuclein enters the nucleus, it may participate in the regulation of transcription. It has been observed that α-synuclein binds to the GC1α promoter, a vital mitochondrial transcription factor, eventually having a negative effect on mitochondria homeostasis [54, 55]. Although several questions are still unclear, currently there is strong evidence for the role of α-synuclein in intracellular trafficking, with particular focus on synaptic vesicle trafficking.

α-Synuclein has been shown to defend dopaminergic cells against apoptosis by signaling pathways involving protein kinase C (PKC). PKC is a serine-threonine kinase involved in phosphorylation of different target proteins and therefore controls many cellular mechanisms, such as apoptosis. PKC is very sensitive to oxidative stress and triggers an apoptotic cascade in dopaminergic cells. α-Synuclein has been shown to be a PKC downregulator that can protect dopaminergic cells against apoptosis. α-Synuclein has been shown to switch off the proteolytic cascade by downregulation of PKCδ expression. Thus in dopaminergic cells, α-synuclein may be considered to be a neuroprotective protein [56]. α-Synuclein regulates different cellular functions via activation of Ras. The activated Ras can activate other signaling molecules including the ERK/MAPK pathway which is involved in sending a signal of growth factor from the cell receptor to transcription factors in the nucleus [57].

α-Synuclein expression has also been recorded in many other cell types, involving cells pertained to secretory processes. α-Synuclein interacts with insulin-containing secretory granules K_{ATP} channels that leads to the inhibition of insulin secretion triggered by glucose stimulation. These observations suggest a function of α-synuclein in diabetes. Moreover, it has been shown that in type 2 diabetes, there is a deposition of amyloidogenic protein in pancreatic β-cells and these patients are most likely to develop PD. However, when α-synuclein combines to amyloid fibrils, an amyloidogenic protein deposits in pancreatic β-cells and forms irreversible damaging complexes in dopaminergic cells [58]. Another important function of α-synuclein has been suggested for modulation of calmodulin (CaM) activity. Calmodulin (CaM) is a messenger protein that can be activated through binding to Ca^{2+} ions and triggers various mechanisms such as those involved in short- and long-term memory. Studies have revealed that both wild-type and mutant α-synuclein can interrelate with CaM both in vitro and in vivo. This interaction of CAM with wild-type and mutant α-synuclein causes α-synuclein fibrillization. α-Synuclein interacts with many cellular proteins and acts as a molecular chaperone, because it comprises regions that are homologous with 14-3-3 proteins which interact with many cellular proteins. Chaperone activity of α-Synuclein is dependent on both its N- and C-terminal regions. The N-terminus is accountable for interfacing of α-synuclein with substrate proteins, leading to the arrangement of a large complex while the C-terminus is responsible for the solubilization of that complex [59].

α-Synuclein may act as an antioxidant in precluding oxidation of unsaturated lipids in synaptic vesicles. Dopaminergic neurons are very sensitive to oxidative damage including the oxidants produced by the metabolism of dopamine. The α-synuclein in its monomeric form can protect lipids from oxidation by interaction with lipid membranes. Fibrillar form of α-synuclein does not have this capability of protecting lipids from oxidation. Thus monomeric form of