Lipids play a more significant role in Parkinson’s disease and its related brain disorders than is currently recognized, supporting a “lipid cascade”. The 14 kDa protein α-synuclein (αS) is strongly associated with Parkinson’s disease (PD), dementia with Lewy bodies (DLB), other synucleinopathies such as multiple system atrophy, and even certain forms of Alzheimer’s disease. Rigorously deciphering the biochemistry of αS in native systems is the key to developing treatments. αS is highly expressed in the brain, the second most lipid-rich organ, and has been proposed to be a lipid-binding protein that physiologically interacts with phospholipids and fatty acids (FAs). αS-rich cytoplasmic inclusions called Lewy bodies and Lewy neurites are the hallmark lesions of synucleinopathies. Excess αS–membrane interactions may trigger proteinaceous αS aggregation by stimulating its primary nucleation. However, αS may also exert its toxicity prior to or independent of its self-aggregation, e.g., via excessive membrane interactions, which may be promoted by certain lipids and FAs. A complex αS-lipid landscape exists, which comprises both physiological and pathological states of αS. As novel insights about the composition of Lewy lesions occur, new lipid-related PD drug candidates emerge, and genome-wide association studies (GWAS) increasingly validate new hits in lipid-associated pathways, it seems timely to review our current knowledge of lipids in PD and consider the roles for these pathways in synucleinopathies.

**Introduction**

The 14 kDa protein α-synuclein (αS) is strongly associated with Parkinson’s disease (PD), dementia with Lewy bodies (DLB), other synucleinopathies such as multiple system atrophy, and even certain forms of Alzheimer’s disease. Rigorously deciphering the biochemistry of αS in native systems is the key to developing treatments. αS is highly expressed in the brain, the second most lipid-rich organ, and has been proposed to be a lipid-binding protein that physiologically interacts with phospholipids and fatty acids (FAs). αS-rich cytoplasmic inclusions called Lewy bodies (LBs) and Lewy neurites are the hallmark lesions of synucleinopathies. Excess αS–membrane interactions may trigger proteinaceous αS aggregation by stimulating its primary nucleation. However, αS may also exert its toxicity prior to or independent of its self-aggregation, e.g., via excessive membrane interactions, which may be promoted by certain lipids and FAs. A complex αS-lipid landscape exists that comprises both physiological and pathological states of αS (Fig. 1). As novel insights about the composition of Lewy lesions occur, new lipid-related PD drug candidates emerge and genome-wide association studies (GWAS) increasingly validate new hits in lipid-associated pathways, it seems timely to review our current knowledge of lipids in PD and consider the roles for these pathways in synucleinopathies.

**αS transiently binds to lipid membranes physiologically**

Early biochemical characterization identified αS as soluble and brain extract fractionation showed only a weak association with synaptic vesicles, confirming immunogold-EM that had detected αS throughout cytoplasmic matrices in axon terminals. Photobleaching microscopy also indicated that αS interacts only weakly with membranes of the nerve terminal and switches rapidly between the cytosol and membrane. The earliest characterization of αS already suggested that binding of αS to membranes may occur via the formation of amphipathic helices mediated by an 11-amino acid repeat motif having the core consensus sequence KTKEGV. This motif appears imperfectly six to nine times in the first two-thirds of the protein and resembles lipid-binding domains often observed in apolipoproteins. “Cis” and “trans” factors mediate the transient αS–membrane interaction when the N-terminal two-thirds of αS form an 11/3 helix (11 amino acids/three turns) interacting with the lipid “tails” via van der Waals forces. Lysine residues (+1) in the KTKEGV motifs interact with membrane lipid head groups (−1). On the “trans” side, vesicle membrane composition and vesicle size affect αS binding; negatively charged head groups promote the interaction with lysine residues (see above) and small vesicles that exhibit “lipid packing defects” promote αS “insertion” into the membrane. The helix formation is only transient, not stable, because some nonpolar residues are found in the hydrophilic half of the helix and some polar residues interact with lipid bilayer, most importantly threonine residues (see Fig. 2d). A recent, elegant in vitro study suggests αS that comes off the membrane does not immediately lose its fold, but may actually retain it, and this may go hand-in-hand with native αS–αS assembly. The authors studied αS binding to small unilamellar vesicles composed of phosphatidylycholine (PC) with 13:0 fatty acyl side chains. Modulating αS binding via phase transitions of the vesicle lipids allowed the reconstitution of soluble helical αS species that behaved as multimers. These observations are in agreement with previous descriptions of soluble αS multimers (and trimers might be the most abundant species among them). Membrane-associated αS multimers, however, have also been described, e.g., in the context of mediating SNARE complex assembly and vesicle

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**References**

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Fig. 1. αS → lipid interplay: aspects of cellular αS homeostasis (blue oval), aspects of lipid homeostasis (green oval), and overlapping aspects. Pathological states are labeled in red. Simplified schematic of both select αS and select lipid species. Several existing publications suggest αS effects on lipids and vice versa, as indicated by arrows. DG diglyceride, ER endoplasmic reticulum, FA fatty acid, LD, lipid droplet, TG triglyceride.

αS CYTOTOXICITY: EXCESS MEMBRANE BINDING VS. FIBRILLAR AGGREGATION

The structure of αS suggests that transient αS–membrane interactions could be stabilized biochemically by either amplifying the electrostatic interaction between positive lysines in αS and negative lipid head groups (e.g., via the engineered "3K" αS mutant) or by increasing the hydrophobicity in the lower, membrane-inserted half of the αS amphipathic helix (e.g., via the engineered "KLK" αS mutant) (Fig. 2e). We have found that such membrane-enriched mutants of αS decrease multimers and lead to acute toxicity and inclusion formation when expressed in cultured cells. The resulting inclusions, however, were shown by electron microscopy (EM) not to be overtly fibrillar but rather rich in αS-decorated vesicles.15 This experimental finding was seemingly at odds with the original isolation of filamentous aggregates from LBs that were αS-positive by immunogold electron microscopy.65,66 However, it may be consistent with an experimental study of random αS point mutants that assessed their fibrillation in vitro (test tubes) and in living yeast.11 Here, in vitro fibrillation rate and in vivo yeast toxicity did not correlate, suggesting that fibrillation is not necessary for αS-induced yeast toxicity. A second screen in a library of several thousand αS-mutant yeast clones identified 25 non-cytotoxic αS sequence variants.11 Most of these sequence variants contained an αS mutation to either proline (P) or glutamic acid (E), which abnormally decreased αS membrane binding relative to wild-type (wt) αS. The authors hypothesized that αS cytotoxicity in yeast is caused by the protein binding to membranes at levels sufficient to nonspecifically disrupt membrane homeostasis. Subsequent yeast studies helped further characterize this membrane-associated toxicity: wt human αS expression in yeast (which lack an αS gene) led to vesicle clustering/aggregation and vesicle-trafficking defects.59 "Amyloid" was typically not obvious (see also review by Jarosz and Khurana70), even though at least one study also reported fibrillar aggregates upon αS expression in yeast.71 The relevance of the vesicle-related observations beyond yeast was supported when similar trafficking defects were described in αS A53T and αS triplication iPSC-derived human neurons.72 Nonetheless, the putative lack of amyloid-type αS aggregates in yeast was viewed critically by some investigators, because filaments of αS had long been considered the hallmark of human Lewy cytopathology, calling into question the relevance of the αS yeast model. A debate thus arose between those that only accepted amyloid-type αS fibrillar aggregation as disease relevant and others who were open to other forms of αS misfolding, including membrane-associated aggregation (Fig. 3). Although the former group could generally rely on the support of neuropathologists, the literature on human LBs has actually provided some evidence of membrane-associated αS aggregation.

MEMBRANE-ASSOCIATED αS AGGREGATION IN LEWY PATHOLOGY

Early descriptions of human Lewy cytopathology in the 1960s and 70s reported filament-rich regions in LBs but also some vesicle/membrane components73,74 and occasional reports in the 1980s and 90s confirmed this observation.75–77 Nonetheless, the acceptance of this insight and its impact on conceptualizing PD pathogenesis and developing PD biomarkers and drugs has been limited so far. This situation may change after a striking recent publication: a detailed analysis13 of PD brain tissue by correlative light and electron microscopy, a technique that allows immunohistochemical and EM ultrastructural analysis of the same lesions. The authors found that the majority of human LBs consisted of αS intermingled with clusters of various membranous structures or components (Fig. 4, right). Of special significance, the authors identified in the core of LBs various vesicle clusters that were coated with high local concentrations of non-fibrillar αS. Surprisingly, only about 20% of all LBs/LNs had large amyloid fibrils (at
least 5 nm diameter, at least 25 nm in length; smaller structures were likely not detectable with the method) associated with them, indicating that amyloid-type $\alpha_S$ aggregation is not as integral or required a part of PD cytopathology as formerly believed. Raman scattering and infrared spectroscopy showed that the LB core comprised large amounts of lipids, most importantly sphingomyelin and PC, as determined by mass spectrometry. These striking new insights raise the question of why these features of human LBs were apparently overlooked in the past. As the authors point out, the short postmortem intervals of their cases and the special tissue preservation methods they applied may be responsible. Traditional LB analyses (e.g., see ref. 79) have largely relied on immunohistochemical staining at the light microscopic level, providing limited resolution. Thus, a bias might have been created in the past for areas that showed the expected rod-like or fibrous structures (see a commentary by Bartels79). The important new findings of Shahmoradian et al.13 are based on state-of-the-art imaging techniques and, if confirmed by others, have the potential to change the ways in which we conceptualize and model PD pathogenesis and design therapeutics.

MEMBRANE-ASSOCIATED AGGREGATION IN CELLULAR MODELS OF $\alpha_S$ DYSHOMEOSTASIS

The proposed lipid vesicle-rich clusters within human LBs are reminiscent of the effects of expressing human $\alpha_S$ at relatively high concentrations in Saccharomyces cerevisiae (see above). The $\alpha_S$ inclusions in yeast had initially been interpreted in light micrographs as proteinaceous aggregates. Gitler et al.,68 however, provided ultrastructural evidence that $\alpha_S$ accumulations in yeast were not comprised of fibrils but rather were clusters of many vesicles (Fig. 4, left), and this was confirmed by another publication.67 Similarly, it was observed in yeast that accumulation of undocked vesicles coalesce into massive vesicle clusters in an $\alpha_S$ dose-dependent manner.68 By immuno-fluorescence microscopy and immuno-EM, these non-filamentous $\alpha_S$ inclusions in yeast were associated with vesicle markers of diverse subcellular origin (endosomes, Golgi, lysosomes). In contrast to yeast, mammalian neural cells expressing very high levels of wt, or even familial PD (fPD) single-mutant $\alpha_S$ (e.g., E46K), may not readily show discrete $\alpha_S$ inclusion formation (e.g., see ref. 59). In an HEK293 model, aggregation propensity of $\alpha_S$ was shown to be exacerbated by fPD mutants A30P, A53T, and E46K.80 fPD mutants A53T, A30P, E46K, H50Q, and G51D were found to have the same oligomerization propensity but differing inclusion formation in a similar HEK293 model.81 The exact nature of these aggregates/inclusions...
as well as a possible cell-type dependence of the observed inclusions will require further analysis. It is important to mention that the known fPD-linked aS point mutations have diverging effects on membrane binding: A30P binds membranes less, E46K binds more strongly, and this may indicate that the initial pathways leading to toxicity may differ between mutants (see review for further details). Indeed, the degree to which the protein binds membranes in mammalian models seems to be recapitulated in yeast models of aS toxicity, e.g., aS A53T was shown to localize to (plasma) membranes, whereas A30P remained cytoplasmic, recapitulating the poor membrane binding nature of A30P reported in many models. To further elucidate the relationship between aS membrane binding and aggregation/ toxicity, engineered aS mutations may prove to be useful tools. Certain engineered mutations in the aS KTKEGV motifs that decrease the cellular multimer:monomer ratio increase the interactions of the excess aS monomers with membranes. Such mutations include aS "3K" (E35K + E46K + E61K), an amplification of the familial PD-causing E46K (Fig. 2e) or "KLK" (T12L + T23L + T34L + T45L + T60L + T71L + T82L; Fig. 2e). These exaggerated, membrane-enriched mutations have provided evidence that the vesicle- and tubule-rich membranous inclusions: are they amphiphilic helices, the species that was shown to form at aS nature of the familial PD-causing E46K (Fig. 2e) or engineered (3K) KTKEGV mutants, which accumulate on membranes; the resultant cytoplasmic vesicle aggregates are often in the vicinity of LDs (see Figs 2 and 3 in ref. ). Moreover, it was recently reported that aS expression in yeast and aS excess in rodent neurons or induced pluripotent stem cell (iPSC)-derived human neurons lead to marked alterations in lipid profiles, including increases in neutral lipids. Among FAs, oleic acid (OA) was found to be specifically elevated in response to excess aS monomers. Strikingly, reciprocal effects were also seen in this and a related study: lowering the enzymatic formation of monounsaturated FAs (MUFAs; e.g., OA) appeared to benefit aS biochemistry; it increased physiological aS solubility, decreased aS aggregation in the membrane-rich inclusions, and decreased serine 129 phosphorylation. In contrast, conditioning cells with MUFAs had the opposite effects. These observations align in part with earlier work, demonstrating pathological aS oligomer accumulation upon conditioning cultured neural cells with polyunsaturated FAs (PUFAs). The proposed aS-OA interplay is consistent with a scenario in which excess aS, in particular membrane-associated aS monomers, leads to an increase in MUDA levels, which in turn render aS more neurotoxic (Fig. 5). Such a model is reminiscent of a “bidirectional pathogenic loop” that had been proposed for another cellular lipid, glucocerebrosidase (GCase), and aS (see below). A novel therapeutic strategy emerging from the work just summarized as follows: inhibiting the rate-limiting enzyme in the biosynthesis of MUFAs, stearoyl-CoA desaturase (SCD). This approach, which emerged simultaneously from another group based on unbiased compound screens in aS-expressing yeast, could potentially (a) neutralize the upregulation of MUFAs by excess aS and (b) prevent detrimental structural changes in aS that are the consequence.

HUMAN GENETICS, PATIENT SAMPLES, AND EXPERIMENTAL MODELS LINK PD TO LIPID PATHWAYS

The theory we emphasize here that lipid metabolism is central to aS homeostasis is particularly well supported by human genetic evidence that strongly suggest a key aS-lipid interplay (Fig. 1) and a major role for certain lipids in modulating aS physiology and

![Fig. 4 aS membrane-associated aggregation in LBs and models thereof.](image-url)

**A** Vesicle-rich membranous aS aggregation in the aS::GFP expression model in yeast. Insert: immunogold staining for aS. Reprinted by permission from the National Academy of Sciences, USA. **B** Vesicle- and tubule-rich membranous aS aggregation in the aS 3K neuroblastoma cell model. Reprinted from Dettmer et al. (CC-BY license). **C** Vesicle/membrane/lipid-rich aS aggregation in human Lewy bodies. Reprinted by permission from the Springer Publishing Group.
consequent toxicity in the brain.\textsuperscript{11,88} This concept is in line with a systematic analysis of GWAS data and genetic networks that revealed lipid homeostasis as a common link between several processes involved in PD pathogenesis.\textsuperscript{89} GWAS have identified—and postmortem brain analyses have confirmed—several proteins that help regulate lipid metabolism, including LD biology, to be associated with PD. First and foremost, mutations in GBA (GCase), a key gene in glycolipid metabolism, significantly increase PD risk\textsuperscript{32,34}—and certain other genes in related pathways have also been implicated\textsuperscript{24,27} (see below). In addition, a diacylglycerol kinase, DGKQ, which controls diglyceride and phosphatidic acid content, emerged from several GWAS as a PD risk factor.\textsuperscript{17,22–25} FA elongase 7, a determinant of fatty acyl side-chain length, and hence membrane composition and fluidity, was recently designated as another significant PD risk gene.\textsuperscript{28,90} A phospholipase, PLAT2G6, has been proposed to affect risk for PD and other brain diseases with “high brain iron”.\textsuperscript{91} Furthermore, seipin, an integral membrane protein localized at endoplasmic reticulum (ER)/LD contact sites and involved in LD biogenesis and maintenance,\textsuperscript{92,93} may be differentially expressed in the brains of PD vs. control subjects.\textsuperscript{94,95} These findings suggest phospholipid group and side-chain nature (dictated by FA type) play a critical role in PD, likely through αS interaction alterations. As far as non-cell-autonomous lipid homeostasis is concerned, variants in LRP10, a low-density lipoprotein receptor protein, were also reported to be linked to PD dementia, DLB, and Lewy pathology,\textsuperscript{96,97} but this remains controversial.\textsuperscript{98} LRP10 mutations have also been associated with increased Alzheimer’s disease pathogenesis.\textsuperscript{99} Moreover, SREBF-1, a transcription factor that binds sterol regulatory element-1 and controls lipid homeostasis through sterol biosynthesis, has been identified in GWAS as a PD risk factor.\textsuperscript{99} This finding, in addition to certain studies on statin use, suggests that sterol pathways in PD pathogenesis should not be ignored either.

We hypothesize that the downstream effects of PD-relevant lipid alterations may involve changes in vesicle trafficking and vesicle function (lysosomes and synaptic vesicles in particular). Several known and emerging PD risk genes such as LRRK2 (PARK8), RAB7 (PARK16), VPS35 (PARK17), SYNJ1 (PARK20), VPS13C (PARK23), SYT11, and LIMP2 (all reviewed in ref.\textsuperscript{90}) underline the relevance of vesicle trafficking and function in PD pathogenesis, and both are affected by lipid and/or αS alterations (further details are beyond the scope of this review). Focusing on putative upstream events and in the context of a possible “bidirectional interplay” between lipids and αS, the following paragraphs will summarize in some detail what genetics, patient samples, and model systems have taught us about how certain lipid species may alter αS biology—and how they might be altered by αS.

PHOSPHATIDYCHOLINE

PC is the most abundant phospholipid in cellular membranes.\textsuperscript{104} Decreased levels of PC containing the polyunsaturated fatty acyl side chains denoted 34:5, 36:5, and 38:5 were observed in the frontal cortex of PD brains.\textsuperscript{105} Similarly, PD visual cortex has been reported to have reductions in some PC species with polyunsaturated 34 and 36 carbon species, as well as decreases in 16:0, 18:0, 18:1, and 18:2 lyso-phosphatidylcholines (LPCs).\textsuperscript{106} In a study of PD patient plasma, PC 44:6 and 44:5 were increased and PC 35:6 was decreased.\textsuperscript{107} Such changes in PUFAs could be the consequence of αS accumulation on membranes, because trends for lower PC species were observed in yeast and rat cortical neuron models of αS excess.\textsuperscript{108} Treating rats with the dopaminergic neurotoxin 6-hydroxydopamine\textsuperscript{108} led to early (defined by the authors as preceding “full blown primary symptoms”) lipid changes in the substantia nigra (SN), with most PC species decreased. Exceptions were LPC 16:0 and LPC 18:1, which were increased. Interestingly, sex differences in lipid changes in PD patients have been observed: in one study, PC was significantly decreased in male patients only.\textsuperscript{109} An in vitro study, highlighting the importance of using native αS forms for studies, reported that PC affects conformation and aggregation of the N-acetylated form of αS.
of αS, specifically that N-acetylation enhances binding to PC micelles and small unilamellar vesicles with high curvature.110

PHOSPHATIDYLETHANOLAMINE
Phosphatidylethanolamine (PE) is the next most abundant phospholipid after PC, comprising ~25% of total mammalian cellular phospholipids.111 In the brain ~45% of phospholipids are PE.112 Using magnetic resonance spectroscopic imaging, decreased PE (and PC) were observed in brains of early (Hoehn and Yahr stages I and II) PD patients but not in advanced (Hoehn and Yahr stages III and IV) cases.113 Decreases in multiple PE species (PE 34:2, 34:1, 36:4, 36:3, 36:1, 38:7, 38:6, 38:4, 40:6, 40:5, 40:4, 38p:7, and 40p:7) and Lysophosphatidylethanolamine species (16:0, 18:0), and 20:0p), particularly in the visual cortex, of PD patients relative to controls were reported.106 PE 36:3 was reduced in the amygdala and species 34:1, 36:3, and 36:2 were decreased in the anterior cortex cingulate of PD patients.106 PE 34:1 was found to be decreased in PD patient plasma.112,113 A sex difference, as observed for PC above, was also noted for PE, with significantly lower PE levels in male PD patients (vs. controls) but not in females.109 The consequences of reduced PE for αS phenotypes have been analyzed by genetically reducing cellular PE content. Deletion of a phosphatidylinositol (PS) decarboxylase (PSD1), which synthesizes PE from PS, increased cytoplasmic αS inclusion formation and enhanced αS toxicity in a yeast model. Importantly, dopaminergic neuron degeneration from expressing wt human αS was enhanced by PSD1 RNAi silencing in a Caenorhabditis elegans model of synucleinopathy, whereas supplementation with ethanolamine, a building block for PE synthesis, led to partial rescue.114 By way of follow-up, low levels of PE in the mitochondria resulted in ER stress and induced αS to form cytoplasmic foci in this model. Feeding with ethanolamine rescued this phenotype.115 Trends for lower levels of shorter chain PE species were observed in yeast expressing wt human αS, whereas longer-chained PE increased. Alterations in PE species appeared αS dose- and time-dependent in a rat cortical neuron model of αS accumulation.14

PHOSPHATIDYLINOSITOL
Phosphatidylinositol (PI) is the third or fourth most abundant phospholipid in cells (after PC, PE, and potentially PS).104 A study in rat brain identified PI 18:0/20:4 as the major PI species and stated that all major PI species contain at least one PUFA.116 PI decreases with age in both male and female mice, whereas lyso-PI decreases in females only.117 The PI total lipid class was shown to be significantly reduced in the SN of male PD patients relative to controls.109 Decreased PI, particularly saturated PI species, was observed in yeast expressing human αS, whereas rat and human cortical neuron models overexpressing αS tended to decreased PI also.14 Although not the most prominent phospholipid or lyso-phospholipid, changes in the visual cortex and amygdala included decreases in PI 38:5 and increases in PI 36:1, 38:3, and 40:4 in PD vs. control brain.106

PHOSPHOTIDYLSERINE
Phosphatidylserine (PS) is a quantitatively minor membrane phospholipid that makes up 2–10% of total phospholipids in mammalian cells.112 PS is an important precursor of mitochondrial PE, which is produced by the mitochondrial enzyme PS decarboxylase. As part of a study analyzing membrane phospholipid synthesis of the SN in PD vs. control brains, it was observed that such synthesis may increase during the course of PD development.118 suggesting increased PS, PE, and/or PC would be observed in PD patients. In this regard, increases in PD frontal cortex of specific PS species have been observed, namely PS with 36:1, 36:2, and 38:3 fatty acyl side chains. PS has also been suggested to play a role in regulating αS-facilitated synaptic vesicle docking by aiding SNARE complex formation.119 Lipid raft levels of PS were 36% higher in the brains of “incidental PD” patients (cases with brain stem LB pathology but devoid of motor symptoms), but not in typical PD brains, relative to controls.120

CONCLUSIONS: PROTEINOPATHY VS. LIPIDOPATHY IN PD AND RELATED BRAIN DISEASES
PD has been principally thought of heretofore as a classical “proteinopathy”—a disease that is caused by the misfolding of a protein into β-sheet-rich fibrillar aggregates. In this scenario, neurons are typically thought to suffer from an imbalance between protein synthesis or folding and protein degradation, leading gradually to neuronal dysfunction and death. Both reduced63 and excess10 αS–membrane interactions, as well as interactions with certain FAs7 have been discussed as potential triggers for toxic αS oligomerization and fibril formation, consistent with the concept of a “lipid-induced proteinopathy”. However, recent advances in LB analysis and PD modeling now provide evidence for the opposite sequence, i.e., synucleinopathies may be “protein-induced lipidopathies”. In this hypothesis, an imbalance in cellular lipid homeostasis is the actual neurotoxic process and αS dyshomeostasis (e.g., excess or reduced vesicle binding of αS associated with fibrillar aggregation) is the trigger. Perhaps it is more sensible not to propose a dichotomy: PD and related human synucleinopathies may simultaneously be protei-nopathies and lipidopathies, and a vicious cycle of dyshomeostasis in protein folding and lipid metabolism might be triggered by early and subtle changes in either lipid or protein handling (the initial alteration may differ from case to case).

Although support for all three scenarios can be found in previous and recent research, the question becomes whether it is important to put a conceptual label on the disease or more scientifically relevant to keep both protein and lipid alterations in mind when exploring new ideas for therapeutics. As an example, the inhibition of SCD, a new therapeutic target, may indeed both counteract changes in lipid metabolism that are triggered by αS accumulation and prevent any negative feedback of these lipid changes on αS structure.14 Regulation of FAs other than MUFA's may also prove valuable, given the report that arachidonic acid (20:4) promotes the formation of helical αS structure.14 Regulation of FAs other than MUFA's may also prove valuable, given the report that arachidonic acid (20:4) promotes the formation of helical αS structure.14 Regression may become promising targets.14,121,122 may have to be rethought and complemented with models of intracellular membrane-mediated αS aggregation such as the αS “3K” model that was developed in cultured cells19 and then shown in transgenic mice to cause PD-like phenotypes.123 Second, positron emission topography tracers for synucleinopathies may have to be designed differently than attempted thus far, taking into account the protein’s excess on cellular membranes in a yet undefined conformation. Third, drugs that alter lipid and FA homeostasis in addition to SCD inhibitors may become promising targets.14,14,16 Fourth, the common assumption that proteinopathy means amyloid aggregation may have to be modified in the case of αS in PD and DLB. From these and other considerations, a new model is emerging in which αS “misfolding” in the cell can occur on several levels. Just an excess amount of the wt αS monomer that normally interacts with vesicles, i.e., which forms physiological amphipathic helices, may already have to be considered a type of “misfolding”. Thus, excess membrane-associated αS may be cytotoxic and disease relevant in the absence of actual β-sheet-rich fibrillar aggregation. A progression to β-sheet-rich fibrillar aggregates may confer further
detrimend, but it might also be temporarily beneficial by sequestering αS monomers away from membrane vesicles, thereby mitigating negative effects on vesicle trafficking. The recently published αS “3K” mouse model that exhibits a pronounced neurodegenerative and movement disorder phenotype indeed developed vesicle-rich aS+ aggregation in young animals, whereas older animals seemed to display occasional “classical” filament-rich amyloid aggregates.12 This finding could indicate that vesicle clustering is potentially an early event in LB formation, whereas fibrillar aggregates are characteristic of more mature inclusions. Related to these considerations may be the question of the nature of the so-called “pale bodies” of αS aggregation that can be observed in PD patient brains and that have been discussed to potentially be a stage in the formation of LBs (e.g., see ref.124).

It should also be noted that the genetics of αS mutations per se suggest that two ways of LB formation may exist (recently reviewed in ref. 50): one via excess membrane binding of monomers (E46K, A53T) and one via excess accumulation of soluble monomers in the cytosol (A30P, G51D). Both pathways seem to have in common the accumulation of monomers at the expense of putative physiological multimers.59

Our final figure (Fig. 6) summarizes the potential pathways leading to membrane-associated and fibrillar αS aggregation we have emphasized in this review; the bidirectional aspects of the interplay are highlighted. In the final analysis, lipid homeostasis appears to loom ever larger in the fundamental mechanisms of human synucleinopathies.
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