 doi:10.20944/preprints202104.0606.v2

Title

Role of α-Synuclein in Cell Biology: A Hypothesis and its Implications in Neurodegenerative Diseases

Author

Renu Minda

Abstract

I wish to suggest a physiological function for α-synuclein (a-syn) that has the potential to explain its role in pathology. Intraneuronal proteinaceous Lewy Bodies (LBs), the pathological hallmark of Parkinson's disease and other synucleinopathies, consist majorly of a-syn. Ample evidence suggests that LBs are not the result of simple amyloidosis of cytosolic a-syn. Benign soluble unstructured a-syn gets converted into toxic species which preferentially accumulates in LBs. But how these aberrant a-syn molecules are produced in the cytosol, is still not clear. The present hypothesis is an effort to relate a metabolic reaction specific to neuronal function, that is, phase transition, with the pathobiology of a-syn. During high frequency stimulation, which entails rapid phase transition reactions at the presynaptic compartment, aberrant interaction of a-syn with the membrane occasionally generates toxic a-syn molecules. My conjecture is that the physiological function of a-syn is to modulate membrane fluidity by a process wherein it goes through a conformation cycle driven by a flux of energy from mitochondria. It is the range of toxic a-syn produced during aberrant phase transition reaction that is responsible for pathology, not the normal a-syn that reenters the conformation cycle, thereby, resolving the paradox of the Janus-face of a-syn.

Keywords

Parkinson's disease, α-synuclein, Lewy bodies, phase transition, thermodynamics, and mitochondria

Contact details

E-mail: mindarenu@gmail.com

Affiliation

Ex. Research Fellow, Dept. of Biosciences,

Sri Sathya Sai Institute of Higher Learning,

Puttaparthi, Andhra Pradesh, India.

ORCID id: 0000-0002-2359-9146

 doi:10.20944/preprints202104.0606.v2

Main Text

Thermodynamic View

α-Synuclein, a small abundant pre-synaptic protein, has been the focus of intense research as it is involved in both genetics and pathology of Parkinson's disease (PD). Missense mutations and locus multiplications of the *SNCA* gene, which encodes α-synuclein (a-syn) can lead to rare, familial, autosomal dominant forms of PD (Polymeropoulos MH et al Science 1997; Singleton AB et al Science 2003). In the more common sporadic form of PD, which constitutes 90-95% of cases, common genetic variants at the *SNCA* locus have been associated with an increased lifetime risk of PD (Nalls MA et al Nat Genet 2014). Lewy bodies (LBs), intraneuronal proteinaceous inclusions, pathological hallmark of PD and other synucleinopathies, consist majorly of a-syn protein (Spillantini MG et al Nature 1997).

Despite decades of intense research both pathophysiology and physiology of a-syn remains poorly understood (Burré J et al Cold Spring Harb Perspect Med 2018; Ray B et al Front Cell Dev Biol 2021). The elusive characteristic of a-syn mainly arises from the fact that it is an intrinsically disordered protein (Weinreb PH et al Biochem 1996); lacking a well-defined three-dimensional structure dictating its function. a-Syn protein has access to multiple local energy minima and can thus easily shift between multiple folded or unfolded states in response to environmental conditions (Lucas HR et al Neural Regen Res 2020). These include beta-sheet rich fibrils which is the major form in LBs (Spillantini MG et al Nature 1997), a large number of higher order oligomers that are often linked to toxicity (Chen SW et al PNAS 2015), physiologically relevant membrane bound alpha-helical monomers and multimers (Burré J et al PNAS 2014), and free-cytosolic alpha-helical tetramers and unfolded monomers (Bartels T et al Nature 2011; Theillet FX et al Nature 2016). Due to conformational plasticity it exhibits a kind of functional promiscuity, interacting with multiple partners (Longhena F et al Int J Mol Sci 2019). Accordingly, a multitude of functions have been attributed to a-syn (Bellucci A et Brain Res 2012). Though, not many take into account the varied conformations that it can acquire under physiological conditions, a recent study underscores the importance of a-syn to populate diverse conformational ensembles for modulating synaptic activity (Fonseca-Ornelas L et al Cell Rep 2021). The present hypothesis assumes a conformation cycle of a-syn induced by a flux of energy from mitochondria which results in a-syn function as a "refrigerant" of Carnot cycle

 doi:10.20944/preprints202104.0606.v2

in a sub-cellular milieu (Minda R Preprint 2021). The proposed physiological function of a-syn provides a plausible solution to the origin of prodromal phase of PD and other synucleinopathies as discussed later.

Although, at a gross level, cells of living organisms are isothermal systems, at the sub-cellular level heterogeneity in temperature exists (Tanimoto R et al Sci Rep 2016). This is largely attributed to the presence of specific organelles as the source of heat associated with inhomogeneous dissipation. Nucleus, mitochondria, and the endoplasmic reticulum generate heat (Okabe K et al Nat Commun 2012; Rajagopal MC et al Commun Biol 2019; Bal NC et al Nat Med 2012). In neuronal cells, mitochondrial uncoupling mediated by uncoupling proteins leads to local thermogenesis, thereby providing a thermal basis for modulation of presynaptic activity (Andrews Z et al Nat Rev Neurosci 2005). Concepts of classical thermodynamics have been developed for systems operating close to equilibrium, that is, for reversible processes. This is not the case for biological cells which are maintained far from equilibrium, requiring a continuous flux of matter and energy (Ornes S PNAS 2017; Fang X and Wang J Annu Rev Biophys 2020). However, principles of equilibrium thermodynamics are applied in biological processes as an approximation and continue to be used for gaining insights that has been assumed in the present hypothesis (Harold F 1986).

A gradient in temperature, pressure, or chemical concentration sets up the possibility for work cycles. Gradients not only run heat engines – gradients also run living organisms. Chemotrophs derive their energy from chemical redox gradients; photosynthetic organisms derive their energy from solar energy gradient. Consistent with the nonequilibrium thermodynamic version of the second law – dissipation of gradients of energy through non-living matter on Earth, organised it, and evolved life as we know it (Prigogine I and Stengers I 1990). My conjecture is that a-syn protein evolved to dissipate thermal gradient between the hot mitochondria and the cool membrane.

The proposed a-syn conformation cycle can be considered analogous to a thermodynamic cycle operating in a refrigerator. In refrigerators, a working fluid operating on the principles of Carnot cycle uses external energy to cool the inner compartment and to dump heat outside in the environment. Analogously, a-syn uses mitochondrial energy to cool the membrane and dissipates heat in the surrounding cytosol. Mitochondria by converting a-syn multimers to monomers, poise it for the next conformation cycle thereby reusing a-syn from a buffered pool at the synapse.

 doi:10.20944/preprints202104.0606.v2

Background

a-Syn, exists *in vivo* in equilibrium between free-cytosolic and membrane-bound states with membrane partitioning being tightly regulated (Lee HJ et al J Biol Chem 2002). This dynamic equilibrium is probably maintained by stress-induced chaperone Hsp70 which can interact with monomeric cytosolic a-syn by an *ATP-independent* mechanism (Tao J et al J Biol Chem 2021). On contact with lipid-water interface a-syn folds into an imperfect amphipathic α-helix extending parallel to the membrane surface, involving ~100 amino acid residues from the N-terminus while the C-terminal forty residues remain unstructured (Jao CC et al PNAS 2008). Interaction of a-syn with membrane shows characteristics of cooperativity reminiscent of enzyme-ligand interaction (Nuscher B et al J Biol Chem 2004). Notably, homomeric helical multimers of a-syn whose formation requires the presence of membranes have been observed in mouse brain tissue (Burré J et al PNAS 2014). In addition, higher order a-syn conformers that are sensitive to SDS, heat, and final protein purification step have been identified in the human brain (Gould N et al J Biol Chem 2014; Luth ES et al Biochem 2015), and these native metastable conformers differ from the pathologically relevant oligomers which are both SDS- and heat-stable (Tsika E et al J Neurosci 2010). Depending upon lipid composition and charge density of membrane, a-syn shifts conformation from α-helix to β-sheet rich amyloid fibrils (Galvagnion C et al Nat Chem Biol 2015; Galvagnion C et al PNAS 2016). Membrane induced fibrillation is promoted by the central aggregation prone region of a-syn, that is, the Aβ component of amyloid (NAC domain from residues 61 – 95) (Fusco G et al Nat Commun 2014). How do transient interaction of a-syn with membrane help in synaptic function?

Synaptic vesicle cycle involves fusion for neurotransmitter release and endocytosis for vesicle recycling. In addition, it is crucial to maintain integrity of synaptic vesicle membrane to prevent leakage of filled-in neurotransmitters. Fission, fusion, and maintaining integrity of biological membrane are executed by specific enzymes. However, these processes can also be controlled by phase separation if the resting state of the membrane is close to a phase transition (Roux A et al EMBO J 2005; Papahadjopoulos D & Poste G Biophys J 1975; Döbereiner HG et al Biophys J 1993; van Meer G et al Nat Rev Mol Cell Biol 2008). In other words, a change in membrane fluidity might cause variations in membrane integrity, permeability, fusion, and capacitance (Li Z et al Cell Metabolism 2006; Lim L and Wenk MR 2009; Sornette D et al Biochimie 1981; Heo P and Pincet F Nature 2020). Indeed,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 January 2022 doi:10.20944/preprints202104.0606.v2

in vitro studies show that a-syn modulates membrane fluidity (Galvagnion C et al PNAS 2016; Nuscher B et al J Biol Chem 2004).

A model of *in vivo* phase transition catalysed by a-syn

To begin with, native unfolded a-syn in the cytoplasm adopts α-helical conformation upon interaction with anionic phospholipid membrane. Motional restriction of the lipid acyl chains induces lipids in membrane to undergo a phase transition from liquid-disordered to solid gel state. Interaction of a-syn with membrane is transient and at the same time cooperative. After the binding of the first molecule of a-syn subsequent molecules bind more readily. Exothermic heat released due to lipid ordering induces a conformation change from α-helix to β-sheet in the NAC domain of a-syn, which assists a-syn multimerization on the surface of the membrane. This reduces the binding affinity of a-syn with membrane, and a-syn multimers dissociate. Heat generated by mitochondria helps to regenerate free unfolded a-syn monomers from multimers in the cytosol, thereby poising it for the next conformation cycle (Figure 1).

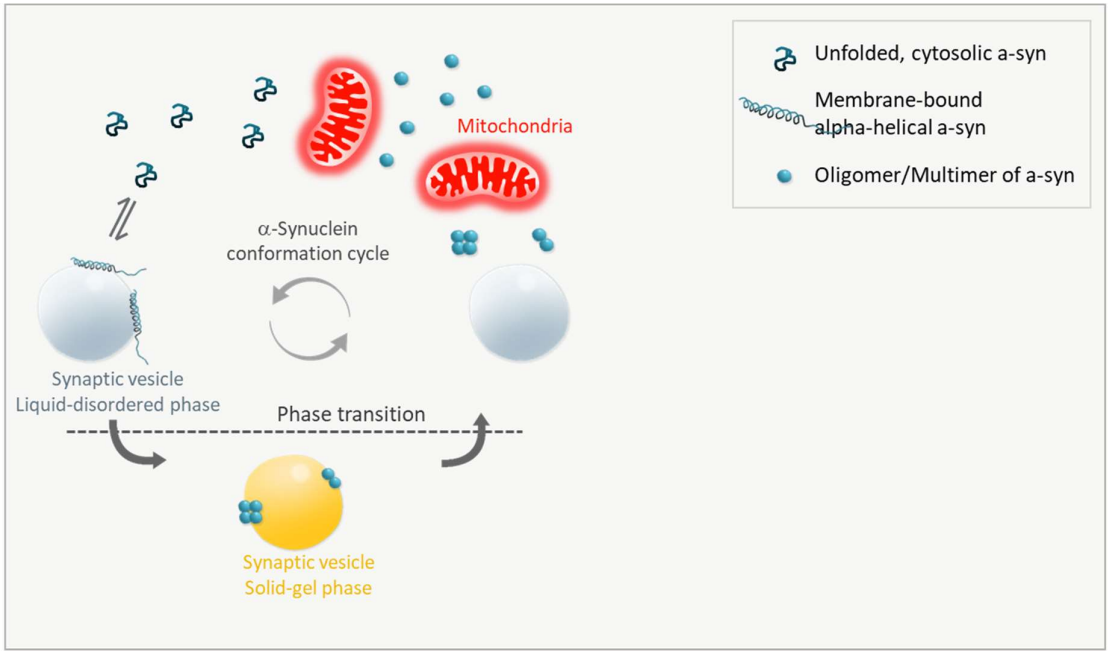

Figure 1. A schematic diagram to illustrate the essential function of α-synuclein (a-syn) assumed in the hypothesis. Driven by mitochondrial energy, transient interaction with membrane induces a-syn to go through a conformation cycle in the process the membrane undergoes a phase transition.

The energy required to disaggregate a-syn multimers could be derived not only from heat generated by uncoupling in mitochondria but may also be supplemented by other

 doi:10.20944/preprints202104.0606.v2

possible sources of energy within mitochondria. It has been observed in artificial lipid membranes that a surface pH gradient generates proton current (Teissie J et al PNAS 1985). Such a current could produce heat due to electrical resistance of the surface. Recently it has also been demonstrated that such a proton current in pulses could generate sonic waves (Fichtl B et al Sci Rep 2016). Further, synchronous fluctuations in transmembrane potential in groups of mitochondria (Diaz G et al FEBS Lett 2000) may raise the temperature of the surrounding in pulses.

Multimers invoked upon in the present study are metastable structures assembled on the surface of the membrane and disaggregating in the cytosol. These native multimers of a-syn are different from toxic oligomers by the fact that they have reduced affinity for membranes. Indeed, in case of the amyloidogenic bacterial protein HypF-N, it has been shown that the major difference between toxic and non-toxic oligomers is exposure of hydrophobic residues (Campioni S et al Nat Chem Biol 2010). Non-toxic oligomers have a well-packed hydrophobic core, associate loosely with membranes, and do not permeabilize them.

In the following section, I will try to interpret several *in vivo* studies which seem to be consistent with the proposed membrane phase transition catalysed by a-syn.

Implications of the hypothesis in resolving the paradox of Janus-face of a-syn

Role of a-syn in physiology

Neuronal plasticity requires a pliable membrane which in turn may depend on membrane fluidity, explaining changes in a-syn mRNA level during song acquisition in male zebra finch (George JM et al Neuron 1995). According to the present hypothesis absence of a-syn will cause an increase in membrane fluidity which in turn will correlate with increased membrane permeability (Li Z et al Cell Metabolism 2006) explaining storage defect of synaptic vesicle associated reduction in the total striatal dopamine level in a-syn null mice relative to wild type controls (Abeliovich A et al Neuron 2000). In addition, increase in membrane fluidity correlates with a decrease in membrane capacitance (Heo P and Pincet F Commun Biol 2020), explaining reduced nerve conduction velocity in aged synuclein triple knockout mice compared to age-matched wild type controls (Greten-Harrison B et al PNAS 2010).

 doi:10.20944/preprints202104.0606.v2

On the contrary, increased a-syn level will correspondingly increase a-syn interaction with the membrane causing a decrease in membrane fluidity, which in turn correlates with enhanced membrane fusion (Sornette D et al Biochimie 1981), explaining profound deficit in neurotransmitter release concomitant with enlargement of synaptic vesicles due to chronic, elevated human a-syn in transgenic mice boutons (Scott DA et al J Neurosci 2010). Similarly, a drop in membrane fluidity in synaptosomes from transgenic mice expressing the amplified form of E46K familial PD mutation ("3K") in a-syn which enhance membrane interaction can explain the observed alterations in synaptic vesicle ultrastructure (Fonseca-Ornelas L et al Cell Rep 2021).

Taken together, these *in vivo* studies, in congruence with the present hypothesis, indicate that a functional role of a-syn at the synapse involves *phase transition of membrane, its absence causing an increase in fluidity while an excess causes rigidity*. Is the physiological phase transition function of a-syn related to its pathological effects, or are they independent?

Role of a-syn in pathology

Although a large number of proteins possess a potential to form amyloid-like fibrils, not all aggregate in pathological states (David DC et al PLoS Biol 2010; Chapman E et al PNAS 2006). Besides the intrinsic propensity of a protein to aggregate, cellular concentration is also a key factor that determines the ability of a protein to form amyloid fibrils. Thus, supersaturation leads to a metastable subproteome which makes the protein vulnerable to aggregation (Ciryam P et al Cell Rep 2013). In agreement with this hypothesis, a slight increase in *SNCA* expression due to single nucleotide polymorphism increases a life time risk of PD, and familial PD cases that have extra copies of *SNCA* exhibit dose-dependent pathology (Soldner F et al Nature 2016; Singleton AB et al Science 2003). Accordingly, increase in a-syn levels have been associated with misfolding and LB pathology (Fares MB et al Nat Rev Neurosci 2021), giving an impression that the pathological effect of a-syn is unrelated to its physiological function. But this hypothesis fails to explain how point mutations in *SNCA*, namely A53T, A30P, E46K, H50Q, G51D, and A53E cause pathology. Even though these mutations influence the rates of the various steps involved in amyloid formation, they show no correlation between these rates and the onset of disease (Ghosh D et al Biochem 2014; Flagmeier P et al PNAS 2016; Nussbaum RL Cold Spring Harb Perspect

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 January 2022 doi:10.20944/preprints202104.0606.v2

Med 2017). The fact that LBs are prevalent in the brains of familial Alzheimer's disease patients (Lippa CF et al Am J Pathol 1998), lent further support to the speculation that LB formation is independent of mutations in the a-syn gene.

Further, the composition of a-syn in LBs is different from that in the cytosol. Phosphorylation at ser-129 (P-S129) is the most abundant post-translational modification of a-syn found in LBs, however, this form of a-syn constitute only a small fraction of the total soluble a-syn in the brain (Anderson JP et al J Biol Chem 2006), suggesting that LB formation is not the result of a simple amyloidosis of cytosolic a-syn. The authors further suggest that the soluble post-translational modified a-syn is the product of normal synuclein metabolism which preferentially accumulates in LBs (Anderson JP et al J Biol Chem 2006). But, why these altered a-syn molecules are produced in the cytosol, is still unclear. In addition, it is now widely believed that different toxic conformers of a-syn whose origin is still uncertain may give rise to different synucleinopathies (Hoppe SO et al Biomolecules 2021). The present hypothesis implies that most likely these altered a-syn conformers are produced during high frequency stimulation, which entails rapid phase transition reactions at the presynaptic compartment (Figure 2).

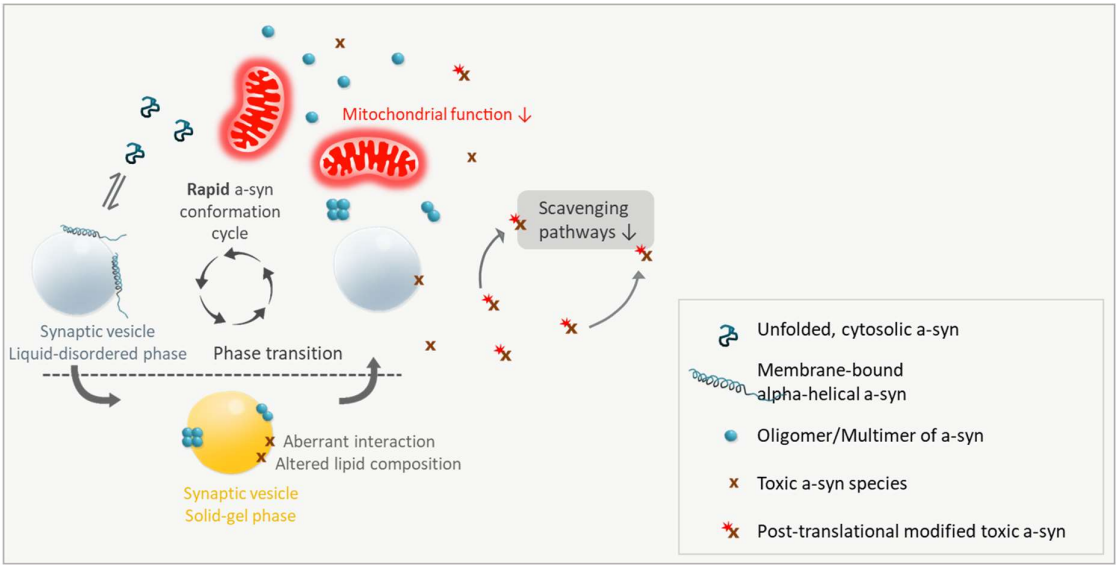

Figure 2. A schematic diagram to illustrate the *de novo* formation of toxic α-synuclein (a-syn) – the seeds of Lewy bodies (LBs). During high frequency stimulation, rapid phase transition reactions may lead to aberrant interaction of α-synuclein with membrane giving rise to alien a-syn species which after post-translational modifications are cleared by scavenging pathways. Ageing and genetic predisposition that results in altered lipid composition and decreased efficiency of scavenging pathways and mitochondria, may enhance production and accumulation of aberrant a-syn molecules which are eventually channeled into LB formation pathway. The leaked out toxic a-syn may initiate the prodromal phase of pathology.

 doi:10.20944/preprints202104.0606.v2

Continuous operation of Carnot cycle in a gasoline engine generates exhaust, analogously toxic a-syn molecules which cannot be recycled are generated occasionally during the phase transition reaction in the proposed a-syn conformation cycle at the nerve terminus. A biological corollary is the formation of toxic metabolic intermediates in cellular biochemical pathways. Under normal circumstances, these toxic a-syn molecules undergo post-translational modifications, including P-S129 (Waxman EA and Giasson BI J Neuropathol Exp Neurol 2008), and are subsequently channeled to scavenging pathways, including chaperones, proteasomes, and lysosomes (Schneider MM et al Nat Commun 2021; Xilouri M et al Mol Neurobiol 2013). However, during high frequency stimulations, rapid phase transition reactions may increase the rate of production of toxic a-syn. In addition, leakiness of the channels (Pareek V et al Mol Cell 2021) associated with scavenging pathways may result in the buildup of aberrant a-syn molecules. These toxic a-syn species may undergo further chemical modifications, including nitration, and are eventually drawn into a pathway to form cytoplasmic inclusions (Giasson BI et al Science 2000).

With age, composition of lipids in membrane changes which could accentuate formation of aberrant a-syn molecules (Kiechle M et al Front Cell Dev Biol 2020). Moreover, efficiency of scavenging pathways and mitochondria decreases on ageing which leads to accumulation of altered a-syn species within the cell (Chiti F and Dobson CM Annu Rev Biochem 2017). Genetic predisposition may further compound this problem. Slowly, the cytoplasmic inclusions grow in size and matures into LBs. The path to LB formation is a defense mechanism specific to the brain which being insulated from the circulating blood has evolved to retain its toxic waste. LBs not only sequester toxic a-syn but also defective mitochondria and lysosomes (Shahmoradian SH et al Nat Neurosci 2019). In case of PLA2G6 associated mutations, which cause neurodegeneration commonly involving a-syn related Lewy pathology, it has been suggested that the initial event that triggers formation of cytoplasmic inclusions and subsequent LB formation is prominent association of P-S129 a-syn with damaged mitochondria (Beck G et al PLoS One 2015; Sumi-Akamaru H et al Acta Neuropathol Commun 2016).

Besides sequestering another mechanism that neurons employ to get rid of toxic a-syn is sharing. The overwhelmed neuron may discharge excess toxic a-syn molecules in the extra-cellular space which are taken up and scavenged by adjacent healthy neurons, glial cells, and oligodendrocytes (Mavroeidi P and Xilouri M Int J Mol Sci 2021). That cell-to-cell

 doi:10.20944/preprints202104.0606.v2

transmission of toxic a-syn is *not* to spread pathology but rather a mechanism for sharing the burden is evident from the fact that when stressed human astrocytes transfer presumably aggregated a-syn to nearby healthy astrocytes via tunneling nanotubes, the healthy astrocyte reciprocates by delivering healthy mitochondria indicating a rescue mechanism (Rostami J et al J Neurosci 2017).

During the process of sharing and spreading, vulnerable cells may die giving rise to symptoms specific for the neuronal subtype being affected. This sets up the stage for the prodromal events of PD and other synucleinopathies initiated by toxic a-syn (Chiti F and Dobson CM Annu Rev Biochem 2017) which having escaped scavenging mechanisms and sequestering pathways were free to adversely interact with vital parts of the cell (Pareek V et al Mol Cell 2021). In fact, certain post-translational modified species of a-syn, but not monomeric a-syn, have been shown to interact with high affinity to TOM20 (translocase of the outer mitochondrial membrane 20) eventually leading to mitochondrial dysfunction (Di Maio R et al Sci Trasl Med 2016). Non-motor symptoms, which include difficulties to smell, sleep, swallow, pass stools and anxiety are the major characteristics of prodromal phase of PD; may appear decades before the actual diagnosis of PD (Pont-Sunyer C et al Mov Disord 2015).

Mechanism of neurodegeneration in PD involves impaired protein turnover, mitochondrial dysfunction, and disturbances in synaptic function (Lill CM and Klien C 2017). Since protein degradation pathways are well conserved among eukaryotes, simple PD model organisms, including, yeast, *C. elegans*, and *Drosophila*, even though lacking an endogenous homolog of a-syn, display some common cellular pathological aspects underlying PD (Auluck PK et al Annu Rev Cell Dev Biol 2010). This is because when human wild type a-syn is expressed in these model organisms it is sensed as a foreign molecule eliciting scavenging pathways, in the same way as toxic a-syn produced during aberrant phase-transition reaction is perceived by neurons. As a consequence, studies involving these model organisms, by and large, have remained confined to the late stage of disease, obscuring identification of the initial events that lead to the prodromal stage of PD. The present hypothesis is the first effort to relate an aberrant metabolic reaction specific to neuronal function, that is, phase transition, with the prodromal stage of PD and other synucleinopathies.

 doi:10.20944/preprints202104.0606.v2

Mechanism of neurodegeneration is shared among different pathologies. Although extracellular amyloid plaques are the pathological hallmark of Alzheimer's disease (AD), it is now widely believed that amyloid-independent mechanisms may contribute to the pathology (Bali J et al PNAS 2012). Since LBs are detected in the majority of sporadic AD cases (Hamilton RL Brain Pathol 2000), it has been suggested that at the molecular level toxic a-syn could be the common player (Twohig D and Nielsen HM Mol Neurodegener 2019). The present hypothesis implies that the identification of the range of toxic a-syn generated by aberrant phase transition reaction may suggest new strategies of drug design for neurodegenerative disorders. Intervention to reinforce or assist scavenging of toxic a-syn without perturbing the steady state level of normal a-syn monomers in the conformation cycle may be a sure way to success, but that would need an understanding of the mechanism of the phase transition reaction in vivo. A more significant aspect of the hypothesis is its implication that drugs aimed to block the transport of toxic a-syn and formation of LBs will only enhance the death of the initial aberrant a-syn producing neurons even faster.

Acknowledgements

I earnestly thank Swapan Bhattacharjee for allowing me to use his personal library, for in-depth discussions, and encouragement. I thank Dr. Ravi Makde for insightful comments, Dr. Venketesh Sivaramakrishnan for inducting me into the exciting field of neurobiology, and Anushka Gupta for the illustrations.

Competing Interest Declaration

I declare no competing interest.

References

Abeliovich A, et al. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron. 2000 Jan; 25(1):239-52.

Anderson JP, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem. 2006 Oct 6; 281(40):29739-52.

Andrews ZB, Diano S, Horvath TL. Mitochondrial uncoupling proteins in the CNS: in support of function and survival. Nat Rev Neurosci. 2005 Nov; 6(11):829-40.

Auluck PK, Caraveo G, Lindquist S. α-Synuclein: membrane interactions and toxicity in Parkinson's disease. Annu Rev Cell Dev Biol. 2010; 26:211-33.

Bal NC, et al. Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. Nat Med. 2012 Oct; 18(10):1575-9.

Bali J, Gheinani AH, Zurbriggen S, Rajendran L. Role of genes linked to sporadic Alzheimer's disease risk in the production of β-amyloid peptides. Proc Natl Acad Sci U S A. 2012 Sep 18; 109(38):15307-11.

Bartels T, Choi JG, Selkoe DJ. α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature. 2011 Aug 14; 477(7362):107-10.

Beck G, et al. Deficiency of Calcium-Independent Phospholipase A2 Beta Induces Brain Iron Accumulation through Upregulation of Divalent Metal Transporter 1. PLoS One. 2015 Oct 27; 10(10):e0141629.

Bellucci A, et al. From α-synuclein to synaptic dysfunctions: new insights into the pathophysiology of Parkinson's disease. Brain Res. 2012 Oct 2; 1476:183-202.

Burré J, Sharma M, Südhof TC. Cell Biology and Pathophysiology of α-Synuclein. Cold Spring Harb Perspect Med. 2018 Mar 1; 8(3):a024091.

Burré J, Sharma M, Südhof TC. α-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proc Natl Acad Sci U S A. 2014 Oct 7; 111(40):E4274-83.

Campioni S, et al. A causative link between the structure of aberrant protein oligomers and their toxicity. Nat Chem Biol. 2010 Feb; 6(2):140-7.

Chapman E, et al. Global aggregation of newly translated proteins in an Escherichia coli strain deficient of the chaperonin GroEL. Proc Natl Acad Sci U S A. 2006 Oct 24; 103(43):15800-5.

Chen SW, et al. Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation. Proc Natl Acad Sci U S A. 2015 Apr 21; 112(16):E1994-2003.

Chiti F, Dobson CM. Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress over the Last Decade. Annu Rev Biochem. 2017 Jun 20; 86:27-68.

Ciryam P, Tartaglia GG, Morimoto RI, Dobson CM, Vendruscolo M. Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. Cell Rep. 2013 Nov 14; 5(3):781-90.

David DC, et al. Widespread protein aggregation as an inherent part of aging in C. elegans. PLoS Biol. 2010 Aug 10; 8(8):e1000450.

Di Maio R, et al. α-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. Sci Transl Med. 2016 Jun 8; 8(342):342ra78.

Diaz G, Falchi AM, Gremo F, Isola R, Diana A. Homogeneous longitudinal profiles and synchronous fluctuations of mitochondrial transmembrane potential. FEBS Lett. 2000 Jun 23; 475(3):218-24.

Döbereiner HG, Käs J, Noppl D, Sprenger I, Sackmann E. Budding and fission of vesicles. *Biophys J*. 1993; 65(4):1396-1403.

Fang X, Wang J. Nonequilibrium Thermodynamics in Cell Biology: Extending Equilibrium Formalism to Cover Living Systems. Annu Rev Biophys. 2020 May 6; 49:227-246.

Fares MB, Jagannath S, Lashuel HA. Reverse engineering Lewy bodies: how far have we come and how far can we go? Nat Rev Neurosci. 2021 Feb; 22(2):111-131.

Fichtl B, Shrivastava S, Schneider MF. Protons at the speed of sound: Predicting specific biological signalling from physics. Sci Rep. 2016 May 24; 6:22874.

Flagmeier P, et al. Mutations associated with familial Parkinson's disease alter the initiation and amplification steps of α-synuclein aggregation. Proc Natl Acad Sci U S A. 2016 Sep 13; 113(37):10328-33.

Fonseca-Ornelas L, et al. Altered conformation of α-synuclein drives dysfunction of synaptic vesicles in a synaptosomal model of Parkinson's disease. Cell Rep. 2021 Jul 6; 36(1):109333.

Fusco G, et al. Direct observation of the three regions in α-synuclein that determine its membrane-bound behaviour. Nat Commun. 2014 May 29; 5: 3827.

Galvagnion C, et al. Chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of α-synuclein. Proc Natl Acad Sci U S A. 2016 Jun 28; 113(26):7065-70.

Galvagnion C, et al. Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation. Nat Chem Biol. 2015 Mar; 11(3):229-34.

George JM, Jin H, Woods WS, Clayton DF. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron. 1995 Aug; 15(2):361-72.

Ghosh D, et al. The newly discovered Parkinson's disease associated Finnish mutation (A53E) attenuates α-synuclein aggregation and membrane binding. Biochemistry. 2014 Oct 21; 53(41):6419-21.

Giasson BI, et al. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science. 2000 Nov 3; 290(5493):985-9.

Gould N, et al. Evidence of native α-synuclein conformers in the human brain. J Biol Chem. 2014 Mar 14; 289(11):7929-34.

Greten-Harrison B, et al. αβγ-Synuclein triple knockout mice reveal age-dependent neuronal dysfunction. Proc Natl Acad Sci U S A. 2010 Nov 9; 107(45):19573-8.

Hamilton RL. Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain Pathol. 2000 Jul; 10(3):378-84.

Harold F. *The Vital Force: A Study of Bioenergetics* Ch. 1 (W. H. Freeman and Co., New York, 1986).

Heo P, Pincet F. Freezing and piercing of in vitro asymmetric plasma membrane by α-synuclein. Commun Biol. 2020 Mar 31; 3(1):148.

Hoppe SO, Uzunoğlu G, Nussbaum-Krammer C. α-Synuclein Strains: Does Amyloid Conformation Explain the Heterogeneity of Synucleinopathies? Biomolecules. 2021 Jun 23; 11(7):931.

Jao CC, Hegde BG, Chen J, Haworth IS, Langen R. Structure of membrane-bound alpha-synuclein from site-directed spin labeling and computational refinement. Proc Natl Acad Sci U S A. 2008 Dec 16; 105(50):19666-71.

Kiechle M, Grozdanov V, Danzer KM. The Role of Lipids in the Initiation of α-Synuclein Misfolding. Front Cell Dev Biol. 2020 Sep 15; 8:562241.

Lee HJ, Choi C, Lee SJ. Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. J Biol Chem. 2002 Jan 4; 277(1):671-8.

Li Z, et al. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab. 2006 May; 3(5):321-31.

Lill CM, Klein C. *The Neurogenetics of Parkinson's disease and Putative Links to Other Neurodegenerative Disorders*. In: *Parkinson's disease: Molecular Mechanisms Underlying Pathology.* (ed: Verstreken P) 1-40 (Academic Press, Cambridge, 2017).

Lim L, Wenk MR. *Neuronal Membrane Lipids – Their Role in the Synaptic Vesicle Cycle*. In: *Handbook of Neurochemistry and Molecular Neurobiology*. (ed: Lajtha A, Tettamanti G, Goracci G) 223-238 (Springer, Boston, MA, 2009).

Lippa CF, et al. Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. Am J Pathol. 1998 Nov; 153(5):1365-70.

Longhena F, Faustini G, Spillantini MG, Bellucci A. Living in Promiscuity: The Multiple Partners of Alpha-Synuclein at the Synapse in Physiology and Pathology. Int J Mol Sci. 2019 Jan 2; 20(1):141.

Lucas HR, Fernández RD. Navigating the dynamic landscape of alpha-synuclein morphology: a review of the physiologically relevant tetrameric conformation. Neural Regen Res. 2020 Mar; 15(3):407-415.

Luth ES, Bartels T, Dettmer U, Kim NC, Selkoe DJ. Purification of α-synuclein from human brain reveals an instability of endogenous multimers as the protein approaches purity. Biochemistry. 2015 Jan 20; 54(2):279-92.

Mavroeidi P, Xilouri M. Neurons and Glia Interplay in α-Synucleinopathies. Int J Mol Sci. 2021 May 8; 22(9):4994.

Minda, R. Role of alpha-Synuclein in Cell Biology: A Hypothesis. *Preprints* 2021, 2021040606 (doi: 10.20944/preprints202104.0606.v1).

Nalls MA, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet. 2014 Sep; 46(9):989-93.

Nuscher B, et al. Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study. J Biol Chem. 2004 May 21; 279(21):21966-75.

Nussbaum RL. Genetics of Synucleinopathies. Cold Spring Harb Perspect Med. 2018 Jun 1; 8(6):a024109.

Okabe K, et al. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. Nat Commun. 2012 Feb 28; 3:705.

Ornes S. Core Concept: How nonequilibrium thermodynamics speaks to the mystery of life. Proc Natl Acad Sci U S A. 2017 Jan 17; 114(3):423-424.

Papahadjopoulos D, Poste G. Calcium-induced phase separation and fusion in phospholipid membranes. Biophys J. 1975 Sep; 15(9):945-8.

Pareek V, Sha Z, He J, Wingreen NS, Benkovic SJ. Metabolic channeling: predictions, deductions, and evidence. Mol Cell. 2021 Sep 16; 81(18):3775-3785.

Polymeropoulos MH, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997 Jun 27; 276(5321):2045-7.

Pont-Sunyer C, et al. The onset of non-motor symptoms in Parkinson's disease (the ONSET PD study). Mov Disord. 2015 Feb; 30(2):229-37.

Prigogine I, Stengers I. *Order out of Chaos: Man's new dialogue with Nature* (Fontana Paperbacks, London, 1990).

Rajagopal MC, et al. Transient heat release during induced mitochondrial proton uncoupling. Commun Biol. 2019 Jul 26; 2:279.

Ray B, et al. "Janus-Faced" α-Synuclein: Role in Parkinson's disease. Front Cell Dev Biol. 2021 May 28; 9:673395.

Rostami J, et al. Human Astrocytes Transfer Aggregated Alpha-Synuclein via Tunnelling Nanotubes. J Neurosci. 2017 Dec 6; 37(49):11835-11853.

Roux A, et al. Role of curvature and phase transition in lipid sorting and fission of membrane tubules. *EMBO J*. 2005; 24(8):1537-1545.

Schneider MM, et al. The Hsc70 disaggregation machinery removes monomer units directly from α-synuclein fibril ends. Nat Commun. 2021 Oct 14; 12(1):5999.

Scott DA, et al. A pathologic cascade leading to synaptic dysfunction in alpha-synuclein-induced neurodegeneration. J Neurosci. 2010 Jun 16; 30(24):8083-95.

Shahmoradian SH, et al. Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. Nat Neurosci. 2019 Jul; 22(7):1099-1109.

Singleton AB, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003 Oct 31; 302(5646):841.

Soldner F, et al. Parkinson-associated risk variant in distal enhancer of α-synuclein modulates target gene expression. Nature. 2016 May 5; 533(7601):95-9.

Sornette D, Hesse-Bezot C, Ostrowsky N. Fusion kinetics of dimyristoyl phosphatidylcholine vesicles around the phase transition of the aliphatic chains. Biochimie. 1981 Nov-Dec; 63(11-12):955-9.

Spillantini MG, et al. Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28; 388(6645):839-40.

Sumi-Akamaru H, et al. High expression of α-synuclein in damaged mitochondria with PLA2G6 dysfunction. Acta Neuropathol Commun. 2016 Mar 30; 4:27.

Tanimoto R, et al. Detection of Temperature Difference in Neuronal Cells. Sci Rep. 2016 Mar 1; 6:22071.

Tao J, et al. Hsp70 chaperone blocks α-synuclein oligomer formation via a novel engagement mechanism. J Biol Chem. 2021 Jan-Jun; 296:100613.

Teissié J, Prats M, Soucaille P, Tocanne JF. Evidence for conduction of protons along the interface between water and a polar lipid monolayer. Proc Natl Acad Sci U S A. 1985 May; 82(10):3217-21.

Theillet FX, et al. Structural disorder of monomeric α-synuclein persists in mammalian cells. Nature. 2016 Feb 4; 530(7588):45-50.

Tsika E, et al. Distinct region-specific alpha-synuclein oligomers in A53T transgenic mice: implications for neurodegeneration. J Neurosci. 2010 Mar 3; 30(9):3409-18.

Twohig D, Nielsen HM. α-synuclein in the pathophysiology of Alzheimer's disease. Mol Neurodegener. 2019 Jun 11; 14(1):23.

van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008 Feb; 9(2):112-24.

Waxman EA, Giasson BI. Specificity and regulation of casein kinase-mediated phosphorylation of alpha-synuclein. J Neuropathol Exp Neurol. 2008 May; 67(5):402-16.

Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT Jr. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. Biochemistry. 1996 Oct 29; 35(43):13709-15.

Xilouri M, Brekk OR, Stefanis L. α-Synuclein and protein degradation systems: a reciprocal relationship. Mol Neurobiol. 2013 Apr; 47(2):537-51.