Braak staging concept and the role of alpha-synuclein in Parkinson’s disease

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Abstract

Parkinson’s disease is the most common movement disorder and one of the most frequent neurodegenerative diseases. The disease is characterized by postural imbalance, rest tremors, rigidity and bradykinesia. These symptoms are caused by selective neurodegeneration in the brain, particularly of the dopaminergic neurons located in the substantia nigra. The most prominent cytopathological hallmark is the presence of proteineous inclusions called Lewy Bodies with as main constituent alpha-synuclein aggregates. Although the cause of this selective neurodegeneration is still quite unclear, alpha-synuclein appears to play a role in that. Braak and colleagues suggest that alpha-synuclein acts in a prion-like manner and is capable of transferring between cells and is responsible for the spread of the pathology of Parkinson’s disease. According to them, the Lewy pathology spreads in a non-random manner, which starts in the brain stem and gradually spreads throughout the brain. In this review, the staging (according to Braak) of Parkinson pathology will be described and the role of alpha-synuclein in this pathology will be discussed in detail.

Introduction

Parkinson’s disease is the second most prevalent neurodegenerative disease, clinically characterized by tremor, rigidity and bradykinesia. Despite the fact that this disease is so common, very little is known about the underlying cellular mechanisms. For several decades, Parkinson’s disease was thought to involve a relatively simple neuropathological process. With as major focus, the neurodegeneration of dopaminergic neurons in the substantia nigra and the presence of insoluble protein inclusions termed Lewy bodies and Lewy neuritis (Forno, 1996). Due to this loss of dopaminergic neurons, the motor symptoms including bradykinesia and rigidity arise. Fiedrich Lewy described the Lewy bodies for the first time in 1912 they are typically spherical globules, consisting of a dense core surrounded by a pale-stained halo of radiating filaments. Lewy neurites display a thread-like structure (Forno, 1996). The major component of the Lewy bodies and Lewy neurites is a misfolded protein called alpha-synuclein. This classical view of a relative simple neuropathology is universal accepted. However, this view is beginning to change, due to a more complex symptomology and a more widespread neuropathology for Parkinson’s disease coming to light in recent years. Braak and colleagues were the first who suggested a different theory. According to them, the neuropathology follows a non-random manner, which starts in the brain stem and then gradually spread throughout the brain to involve eventually large parts of the central nervous system (Braak and Del Tredici, 2008). The Braak model hinges on the notion that Lewy pathology is not random: vulnerable sites in the brain are affected in a predictable order. They also suggest that the pathology begins when a neurotrophic pathogen enters the body via the nasal or gastric pathway. This pathogen enters the central nervous system and spreads trans-synaptically from one vulnerable brain region to the next. In this review, first the classical pathogenesis of Parkinson’s disease will be described. Also the major component of the Lewy bodies and Lewy neurites, aggregation of alpha-synuclein will be
discussed in detail. Finally, the Braak model will be described and how this model is divided in six stages. In addition, the prion-like behavior of alpha-synuclein and how this protein can be transferred between cells will be reviewed. The goal of this review is to investigate the role of alpha-synuclein in the pathogenesis of Parkinson’s disease and how this protein can act as a prion in Braaks staging of Parkinson’s disease.

**Classical pathogenesis of Parkinson’s disease**

The major focus of the classical pathogenesis is the neurodegeneration of the substantia nigra and the presence of Lewy bodies with as main constituent aggregation of alpha-synuclein.

**Alpha-synuclein**

Alpha-synuclein is a very abundant protein in the brain. This 140-amino acid protein belongs to a small group of natively unfolded proteins. Alpha-synuclein is part of the synuclein family, which includes β- and γ-synucleins. The synuclein family shares a similar organization domain. The amino-terminal sequence is identical for each synuclein, containing imperfect repeats with the consensus sequence KT-KEGV (fig 1). This repeat region contains a conserved apolipoprotein-like class-A2 helix. All of the synucleins have a hydrophobic middle region, but they differ in the carboxy-terminal part (Tofarisi and Spillantini, 2005).

![Fig 1: Alpha-synuclein (Tofarisi 2005)](image)

Although alpha-synuclein is found in many tissues, it is mostly found in neural tissue and therefore very abundant in the brain as mentioned earlier. Parts of the brain where alpha-synuclein is predominantly expressed are the presynaptic terminals of the hippocampus and the neocortex (Jakes et al., 1994). Alpha-synuclein is a natively unfolded protein, since recombinant alpha-synuclein does not assume a uniform or consistent secondary structure in aqueous solution (Weinreb et al., 1996). However it may be capable of interacting with lipid membranes, indicated by the amino acid sequence and subcellular localization. Due to the apolipo-protein-like class-A2 helix, the repeat region can mediate reversible binding to acid phospholipids (especially phosphatidic acid, PA), which in turn is associated with a large shift in protein secondary structure from around 3% to about 80% α-helical (Davidson et al., 1998). Interestingly, alpha-synuclein has been identified as highly specific inhibitor of phospholipase D2 (PLD2). This enzyme is localized to the plasma membrane and submembranous vesicles, where it by means of hydrolysis of phosphatidylcholine, produces PA (Jenco et al., 1998). Since PA metabolism has been specifically implicated in vesicle budding, alpha-synuclein, through their action on PLD2, may be involved in synaptic membrane biogenesis.

Several observations showed the role of alpha-synuclein in membrane-associated processes: first, mice with an alpha-synuclein knock out have an increase dopamine release at nigrostriatal terminals in response to paired electrical stimuli. This observation suggests that alpha-synuclein is an activity-dependent negative regulator of dopamine neurotransmission (Abeliovich et al., 2000). Secondly, a decrease in the distal pool of presynaptic vesicles as visualized by electron microscopy was observed after depletion of alpha-synuclein from the primary hippocampal neurons with antisense oligonucleotide treatment (Murphy et al., 2000). Finally, alpha-synuclein
plays a role in synaptic plasticity; this was showed with an experiment with songbirds. During a period of song acquisition, alpha-synuclein is specifically upregulated in a discrete population of presynaptic terminals (George et al., 1995).

The interaction with lipid membranes and the inhibition of PLD 2 activity are not the only properties of alpha-synuclein it seems to interact with several other proteins (Dev et al., 2003) Synphilin-1 is a protein, which is identified by yeast two-hybrid screening. It is a 90-kDa cytoplasmic protein and the function of this protein is unknown, but it appears to bind to alpha-synuclein as an adapter molecule. It is suggested that alpha-synuclein is anchored by synphilin-1 to intracellular proteins that are involved in vesicle transport and cytoskeletal function (Engelender et al., 1999).

Besides the interaction with synphilin, alpha-synuclein also binds to 14-3-3 proteins. These proteins are a family of ubiquitous cytoplasmic chaperones, and alpha-synuclein shares physical and functional homology with these proteins. Alpha-synuclein binds to these proteins, as well as to protein kinase C and BAD, which are known to associate with 14-3-3 proteins. It is suggested that increased expression of alpha-synuclein could be harmful in relation to this interaction (Osterrova et al., 1999). This suggestion is supported by an observation made by inducible neuro2a cell lines. Alpha-synuclein was reported to inhibit MAP kinase signaling and accelerate cell death, following serum reduction (Iwata et al., 2001).

These observations show that increased expression of alpha-synuclein could be harmful, however this is not always the case. Several observations show that overexpression of alpha-synuclein also protects different processes in cells. It has been reported that alpha-synuclein wild type overexpression protects the neuronal cells from apoptotic stimuli and to delay cell death induced by serum withdrawal (Alves da Costa et al., 2000). Alpha-synuclein also plays a role in protection against oxidative stress by inactivation of the c-jun N-terminal kinase. This kinase plays an important role in the stress response (Hashimoto et al., 2002). Finally, recent data suggest that full length alpha-synuclein is involved in dopaminergic cell differentiation and survival in that cells from transgenic mice overexpressing truncated protein seem to be more sensitive to environmental conditions (Michell et al., 2007).

It should be clear that alpha-synuclein plays a complex role in different cell processes, both negative and positive. However, in the case of neurodegeneration such as Parkinson’s disease, alpha-synuclein seems to have a mainly negative role. Two observations support this presumption: first is the identification of point mutations and gene duplication in a small number of families with autosomal-dominant early-onset Parkinson’s disease and secondly the fact that alpha-synuclein is a major component of Lewy body filaments.

**Alpha-synuclein and Lewy bodies**

The most defining neuropathological characteristics of Parkinson’s disease are the Lewy bodies and Lewy neurites. Aggregates of alpha-synuclein are the main constituent of these proteinous inclusions. Due to the ability of interacting with lipid membranes and inhibiting PLD2 activity, like mentioned earlier, alpha-synuclein is bound to synaptic vesicles or to membranes rich in acid phospholipids. Normally, alpha-synuclein is located in both synaptic boutons and the axon (Perrin et al., 2000). But in the case of Parkinson’s diseases, for unknown reasons, alpha-synuclein leaves their binding site and assumes a β-pleated sheath formation. It does this together with other components such as phosphorylated neurofilaments and ubiquitin. These misfolded proteins then aggregate with each other and transform into virtually insoluble Lewy bodies and Lewy neurites (Kopito, 2000).

In relation to this aggregation Polymeropoulos et al, first discovered a point mutation in familiar Parkinson’s diseases. This mutation swaps an alanine residue 53 to threonine (A53T) and is
known to be an accelerator of the alpha-synuclein aggregation. A30P and E46K are two other mutations that were described in unrelated families (Krüger et al., 1998; Zarranz et al., 2004). These mutations are responsible for an increase of aggregation of alpha-synuclein, and therefore very important in generation of Lewy bodies and Lewy neurites.

Once alpha-synuclein is aggregated and the Lewy bodies and Lewy neurites arose, the affected neurons cannot manage to eliminate these proteineous inclusions and will degenerate.

Substantia nigra

Not all parts of the brain are equally vulnerable for developing Lewy bodies and Lewy neurites, it seems that some parts are more susceptible than others. The substantia nigra is the most affected area. Postural imbalance, rest tremors, rigidity and bradykinesia are the symptoms of Parkinson's disease, caused by Lewy bodies and Lewy neurites in the dopaminergic neurons of this area.

The substantia nigra is located in the mesencephalon and plays an important role of movement control. It contains high levels of dopaminergic neurons and therefore an important source of the neurotransmitter dopamine. It is unknown why this area is so more affected than others. But it seems that certain brain areas and cell types that are more vulnerable share some properties.

First, all of the vulnerable cells belong to the class of neurons whose axons project to distant regions of the brain, the so-called projection neurons. Within this class, only the cells that generate axons that are disproportionately long in relation to their stoma, seems to develop the Lewy bodies and Lewy neurites. Projection neurons with a normal length of the axon, showed to withstand these Lewy bodies and Lewy neurites development. Secondly, projection neurons that have axons that are unmyelinated or only partially myelinated, have been demonstrated to be more vulnerable than projection neurons with a thick myelinated axon (Braak et al., 2002). An earlier study suggested that projection neurons with an unmyelinated axon are more susceptible to pathological sprouting (Kapfhammer and Schwab, 1994). These neurons have an inordinately high energy turn over and therefore encounter a high amount of oxidative stress, which is known to be an important factor in the pathogenesis of idiopathic Parkinson's disease.
Projection neurons with long unmyelinated axons are more vulnerable than neurons with short myelinated axons. Dopaminergic neurons are indeed projection neurons with a long unmyelinated axon (Björklund and Dunnett, 2007). So this could be the reason that these neurons are so affected in Parkinson's disease, but to ensure this, further research is needed.

**Braak staging of Parkinson's disease**

For several decades, Parkinson's disease was thought to comprise a relative simple neuropathology, with the processes described above as main cause of the symptoms. However, recent new studies changed this view and suggest that Parkinson's disease has a very complex and more widespread neuropathology. Braak and colleagues suggested that the Lewy bodies first appear in the brain stem and olfactory bulb and then gradually spread throughout the brain to involve eventually large parts of the central nervous system (Braak et al., 2002). This theory can be compared with a falling row of dominos, where the major component of the Lewy bodies, aggregates of alpha-synuclein, spreads from an infected neuron to a yet healthy neuron. This theory is supported by several observations from autopsies that were performed on Parkinson's disease patients. These patients received implants of embryonic tissue in the 1980-1990s. In these patients, Lewy bodies were not only present throughout the brain, but also in the previously grafted neurons (Kordower et al., 2008). These findings support the idea that the grafted neurons were infected by the patients’ own neurons, because the grafted neurons were relatively young (10-15 years beyond embryonic stage) that it seems very unlikely that they have developed aggregates through an independent cell-autonomous process. Prion-like mechanisms like these are also found in other neurodegeneration diseases for instance Alzheimer disease (Frost et al., 2009). According to Braak this is not the only similarity that Parkinson’s disease shares with Alzheimer disease. Alzheimer disease follows a stereotypic pattern in all patients, where the distribution of tau-containing neurofibrillary tangles is coupled to the clinical disease stage (Braak et al., 1993). Braak and colleagues suggested that this is also the case in Parkinson’s disease, where the distribution of Lewy bodies and Lewy neurites progress in a largely caudo-rostral direction over time. They proposed that the neuropathology of Parkinson’s disease develops in a characteristic, non-random manner. According to them, the
neuropathology follows six stages that represent presymptomatic and symptomatic phases (Braak et al., 2003a).

**The six stages of the Braak staging concept for Parkinson's disease**

The process starts in the dorsal motor nucleus of the vagus nerve dmX in the lower brain stem, where it goes upwards through vulnerable regions of the medulla oblongata, tegmentum pontis, mid- and forebrain until it reaches the cerebral cortex (fig 4). This process is subdivided in six stages (Braak et al., 2003b).

![Neocortex](Image)

**Stage 1**

The first Lewy pathology appears at two sites: the olfactory bulb, in the anterior olfactory nucleus and in the dorsal motor nucleus of the vagus nerve (dmX). Due to the fact that the pathology in anterior olfactory structures makes fewer incursions into related areas than the pathology in the lower brain stem, the dmX is probably the starting point of the disease process, which takes an essentially ascending path (Braak et al., 2002, 2003b).

**Stage 2**

In stage 2, Lewy pathology is more widespread within the medulla, including the lower raphe nuclei, the gigantocellular reticular nucleus and the locus coeruleus. These three nuclei work together as constituents of the gain setting system (Braak and Braak, 2000). The gain setting system receives major input from components of the limbic and motor systems, such as the central subnucleus of the amygdala. It is capable of limiting the conduction of incoming pain signals in stress situations and ensures that the motor neurons are in a heightened state of preparedness for action (Randich and Gebhart, 1992). The descending tracts of the gain setting system form a sensory control system for both the somato- and visceromotor efferents, enabling adaptation to the organism’s momentary demands.
Stage 3

In stage 3, progress of the Lewy pathology moves caudo-rostrally from the brain stem to the mesencephalic tegmentum and basal portions of the prosencephalon. In the central subnucleus of the amygdala, the magnocellular cholinergic nuclei of the basal forebrain and the substantia nigra pars compacta, massive neuronal destruction develops. The central subnucleus of the amygdala projects to the gain system and dmX. It regulates these nuclei grays and brings supervening limbic influences to them. The central subnucleus in its turn, receives projections from the amygdalar baso-lateral complex, which receives strong input from the magnocellular nuclei of the basal forebrain (Braak et al., 1994).

The connection between these different parts of the brain and the fact that all these parts are infected by one after the other supports the hypothesis that the pathology develops in a non-random manner.

During stage 1, 2 and 3, the individuals do not exhibit noticeable motor symptoms (fig 5).

Stage 4

Individuals experience symptoms for the first time during stage 4 (fig 5). In this stage, the pathology processes in a specific portion of the cerebral cortex: the temporal mesocortex. In higher primates, the temporal mesocortex is a unique and highly developed part of the cerebral cortex, which acts as a transitional zone between allo- and neocortex (Braak and Braak, 2000). The temporal mesocortex projects all signals from the neocortex to the centres of the limbic loop (amygdala, hippocampal formation, entohirnal region) and prefrontal cortex. Of all cortical sites, the temporal mesocortex experiences the most pathology during the following stages (Braak et al., 2003b).

Stage 5 and 6

At these stages, the pathology reaches the neocortex and the motor symptoms are severe. Also the cognitive dysfunction becomes apparent. In stage 5, Lewy bodies and Lewy neurites develop in the high-order sensory association and prefrontal areas of the neocortex. The first order sensory association areas, premotor fields and finally the primary sensory and motor fields are infected during stage 6 (Braak et al., 2003b).
The dual-hit theory

Braak and colleagues proposed a theory about how this pathology begins in the first place. Based on the location of the first appearances of Lewy bodies and Lewy neurites, which are the visceromotor projection neurons of the dmX that give rise to unmyelinated preganglionic fibers of the parasympathetic nervous system and thereby connecting the brain with the postganglionic neurons of the enteric nervous system, they suggested that this pathology starts with an infiltration of a neurotropic pathogen. According to this ‘dual-hit theory’ an unknown pathogen enters via the respiratory pathway, through the nasal passages, and via the gastric pathway, due to swallowing of saliva containing nasal secretions. Loss of the sense of smell and gastrointestinal disturbances, including constipation, during early stages of Parkinson's disease is regularly observed in patients (Abbott et al., 2001; Müller et al., 2002). These observations support this theory.

The nasal route is used to explain the early involvement of olfactory structures, but Braak and colleagues don't think this is the starting point for the Lewy pathology. Instead, they propose that the gastric system is point of departure for the Lewy pathology (Braak et al., 2003b).

The pathogen is likely capable of crossing the epithelial and mucus membranes thereby effecting nearby neural structures. In axon terminals, the uptake of exogeneous substances from the extraneuronal space occurs, and from there transported to the cell soma via retrograde axonal transport. In this way, neurotropic viruses may be capable of entering nerve cells (Helke et al., 1998). It may therefore be that an unknown pathogen enters the body and gains access to the gastrointestinal tract and invades vulnerable neurons in the enteric nervous system. From there, the pathogen is transported via retrograde axonal transport to the central nervous system through the unmyelinated preganglionic fibers (Braak et al., 2003b).

This pathogen could possess unconventional prion-like properties and might consist of misfolded alpha-synuclein fragments (Liautard, 1991). How these alpha-synuclein fragments are transferred from one cell to another will be discussed in a later section.

The enteric nervous system has neurons that can develop idiopathic Parkinson's disease associated Lewy bodies, not only in symptomatic patients but also in incidental cases (Wakabayashi et al., 1990). These neurons are VIP neurons and they are prominently present in the Auerbach plexus of the enteric nervous system. The preganglionic fibers of the dmX terminate not only at the postganglionic excitatory cholinergic neurons, but also at the inhibitory visceromotor and secretomotoric VIP neurons of the Auerbach plexus (Costa et al., 1986). So once in the gastric system, there is, anatomically speaking, a neuronal and fiber pathway, which the pathogen can use to overcome the distance from the mucous membrane of the digestive tract to the central nervous system.

The gastric mucosa has several properties that contribute for the entry of an unknown pathogen. First, the gastric mucosa is innervated by a very large segment of the dmX (Karim et al., 1984). Secondly, the chymus stays in the stomach for a long period of time. Thirdly, the epithelium of the stomach consists of only a single cell layer and is vulnerable to lesions. Finally, large numbers of Lewy bodies have been found previously in the enteric nervous system of the stomach (Wakabayashi et al., 1993).

The dual-hit theory is supported by the observation that if the neuronal pathway is not present, presumably no pathogenic transfer could take place. As described above, Lewy bodies and Lewy neurites consistently develop in the dmX, which gives rise to unmyelinated preganglionic axons and has connection to the enteric nervous system. The ambiguous nucleus, which is a second motor nucleus belonging to the vagus nerve, gives rise to thick and solidly myelinated axons and its projection neurons establish no direct connections to the viscero- and secretomotor neurons; and so it remains unaffected in idiopathic Parkinson's disease (Braak et al., 2003b).

Also the finding of Lewy bodies in the gastrointestinal tract is a great support for this theory.
Transfer of alpha-synuclein

As mentioned earlier, alpha-synuclein is the major component of Lewy bodies and Lewy neurites. So the spread of the neuropathology during Parkinson’s disease is related to the spread of alpha-synuclein. For several years it has been known that alpha-synuclein is secreted and can be detected in cerebrospinal fluid, plasma and saliva (Jang et al., 2010). However, the exact pathway of secretion of alpha-synuclein is poorly understood. Experimental evidence is still growing to support the fact that alpha-synuclein does indeed transfer from one cell to another. This evidence is important for the Braak staging concept, because this explains how an infected neuron can infect a yet healthy neuron with alpha-synuclein aggregation. There are six possible mechanisms of alpha-synuclein transfer (fig 6).

Cell injury and leaking (1), Transmembrane intrusion (2)

Alpha-synuclein can leak from injured cells into the extracellular space. From there, alpha-synuclein could then directly translocate over the cell membrane and gain access to other neurons.

Endocytosis and exocytosis (3)

Exocytosis

Vesicular exocytosis is involved in transporting alpha-synuclein out of the cell. Experiments with SH-SY5Y human neuroblastoma cells overexpressing alpha-synuclein showed that a small percentage of the protein was packaged into the lumen of vesicles and rapidly secreted from cells. Remarkably, the ER/Golgi exocytic pathway is not involved in this process, because inhibition of the secretion of alpha-synuclein is only possible with low temperature and not with BFA, which is an inhibitor of the ER/Golgi pathway (Lee et al., 2005). Overexpression of alpha-synuclein results in increased aggregation and secretion of alpha-synuclein is enhanced when cells are subjected to various stress conditions, such as mitochondrial inhibition, along with induction of protein misfolding (Jang et al., 2010).
Endocytosis

When recombinant alpha-synuclein is added to cultured cells, it can be taken up by the recipient cell (Danzer et al., 2009). Normally, the recombinant protein needs a lipid-based agent to enter neurons (Nonaka et al., 2010). But recombinant alpha-synuclein does not require these lipid-based agents. Volpicelli-Daley and colleagues showed seeding of endogenous alpha-synuclein by pre-formed fibrils and that as a consequence of this, neurons showed a loss of synaptic proteins, reduction in neuronal excitability and connectivity and eventual death. Also when neurons had been maintained in culture for a longer period of time, the uptake and seeding was enhanced. This is probably due to an increased amount of alpha-synuclein in older neurons (Volpicelli-Daley et al., 2011).

Cellular entry of extracellular alpha-synuclein is also affected by its state. Monomeric forms of alpha-synuclein are able to interact with membranes and lipids and enter via passive diffusion (Ahn et al., 2006). But the uptake of oligomeric and fibrillar forms is dependent on the assembly of oligomers. Danzer and colleagues found that some forms enter the cell and increased intracellular aggregation of alpha-synuclein. These forms that enter the cell, were comprised of primarily high order oligomers, rather than monomers (Danzer et al., 2007). So oligomerization/aggregation is very important in cell-to-cell transfer. Probably the classical endocytic pathway is involved in transporting alpha-synuclein into the cell. This is suggested by the fact that the uptake is reduced at low temperature and when dynamin inhibitors are applied to cells (Hansen et al., 2011). The endocytic pathway is probably evolved as a protective mechanism for high levels of extracellular alpha-synuclein. A healthy cell can process these high levels via normal proteolysis or the lysosomal pathway. In an infected cell, these pathways are defective and the cell cannot clear these high levels. This results in a built up of alpha-synuclein and aggregation of the protein (Winslow et al., 2010).

Exosome release (4)

Exosomes were to believed to be involved in the removal of unwanted proteins from cells, but new insights showed that they play a role in much more processes including signalling in immune cells and having virus-like properties that allow gene regulation in the recipient cell (Shorey and Bhatnagar, 2008). The small membrane vesicles of endocytic origin contain mRNA, miRNA and protein and have previously been shown to be involved in the secretion of prion protein from cultured cells. The secreted prion was able to act as seed for prion propagation in the uninfected cell (Fevrier et al., 2004). It was found that alpha-synuclein is also secreted via exosomes in SH-SY5Y cells (Emmanouilidou et al., 2010). So alpha-synuclein is transferred between cells via exosomes and that transmission is enhanced under conditions associated with Parkinson’s disease pathology, including aggregated alpha-synuclein.

Nanotube (5)

Intercellular transfer of alpha-synuclein could occur between neighbouring neurons via tunnelling nanotubes (TNTs). TNTs are long thin extensions comprising F-actin between 50 and 200 nm in diameter and often with a length of several cells. They play a role in signalling between cells via intercellular transfer of organelles, as well as vesicles of endocytic origin and cytoplasmic molecules (Gerdes et al., 2007). If TNTs form between neurons in vivo, this process could mediate the spread of alpha-synuclein and support Braak’s theory of the spread of the neuropathology during Parkinson’s disease.

Transynaptic (6)

Recently, it has been found that alpha-synuclein can transfer trans-synaptically from one neuron to another at axonal terminals. In this paradigm, heat shock protein 70 is associated with extracellular alpha-synuclein and when it is overexpressed it appears to reduce alpha-synuclein oligomerization (Danzer et al., 2011). Heat shock 70 protein is also secreted from several cells as a response to stress (De Maio, 2011), and is present in exosomes (Lancaster and Febbraio,
2005). It might play a role in chaperoning a number of aggregation-prone proteins in the extracellular space.

**Prions**

The hypothesis that alpha-synuclein acts in a prion-like manner is supported by these transfer mechanisms. But not only the ability of being transferred between cells is enough to act as a prion. The term prion is derived from the words proteinaceous and infectious. They are composed of a misfolded form of endogenous PrPc, the PrPsc protein. This PrPsc protein acts as a template upon a native PrPc, which is refolded into PrPsc (Prusiner, 1982). Studies showed that alpha-synuclein is also capable of this seeding action. They demonstrated that alpha-synuclein, that had been released from co-cultured cells could be taken up and act as a seed for aggregation in the recipient cell (Hansen et al., 2011). They used differentiated SY-SH5Y cells, where a small amount of imported alpha-synuclein-GFP was detected surrounded by alpha-synuclein-DsRed (derived from recipient cell). This observation provides clear evidence of imported alpha-synuclein to act as a seed for propagation of alpha-synuclein aggregation in the recipient cell. Although the transfer and seeding mechanisms of alpha-synuclein are not completely understood, it seems clear that alpha-synuclein has prion-like properties and is capable of spreading the neuropathology during Parkinson's disease.

**Conclusion**

Clearly there is increasing evidence demonstrating that extracellular alpha-synuclein can infiltrate surrounding cells and initiate a Parkinson's disease like pathological response in a prion-like manner. However, it remains unclear what initiates this process and whether it can account for disease progression in the human brain. The propagation patterns of alpha-synuclein pathology described by the Braak model indicate indeed a prion-like spread of alpha-synuclein. However it remains controversial, whether the theory that the progression of the pathology can be generalised to the predictable, sequential involvement of vulnerable sites, as described by Braak. Several studies showed that this predictable route of the pathology not always occurs, they reporting cases with inclusions throughout the brain but not in the medullary nuclei. In fact recent reports suggest that the Braak system fails to classify upwards 50% of alpha-synuclein immunoreactive cases (Jellinger, 2008). This suggests that the Braak staging scheme is not the only possible route of spread of the pathology. According to Braak the severity of the lesions in the affected regions will increase as the disease progress (Braak et al., 2003a). So, the first region that is affected would have more severe pathology than the regions that get infected in a later stadium. Therefore, the dmX would be more affected than all other regions. However, this is not true in all cases. Attems and Jellinger found in their study that 65% of the cases had alpha-synuclein loads in the substantia nigra and locus coeruleus that were equivalent to that found in dmX (Attems and Jellinger, 2008).

These inconsistencies between the Braak model and these studies might be explained by the fact that the spread of the pathology can occur in both an anterograde and retrograde direction. Braak and colleagues suggest that departure point of the spread of the pathology is not the olfactory system, while these structures are affected very early in the disease process. Several studies, however, suggest that the Lewy pathology may also start in the olfactory structures (Hubbard et al., 2007). This possible anterograde direction of the spread of the pathogen through the olfactory system may reconcile some discrepancies with the Braak model (Lerner and Bagic, 2008).

Another explanation for the contradiction with the Braak model and de reported observations might be the clinical diversity of Parkinson's disease. There are different subtypes of Parkinson's disease, and these different types have different underlying pathological patterns. Halliday et al found that the patients with older onset and short disease duration had pathology that did not fit with the Braak model. However, patients with a younger age of onset and longer disease duration had pathology that fit with Braak model (Halliday et al., 2008).
Despite the ongoing controversy over Braak’s staging concept, the evidence grows that alpha-synuclein is capable of transferring between cells and can act in a prion-like manner in Parkinson’s disease. Debate is still open over how this process starts in the first place and what kind of neurotrophic pathogen is responsible for it. Braak and colleagues never suggest an option for this neurotropic pathogen in their studies. According to them, this pathogen is responsible for initiating the disease process. It seems contradictory that they never suggest what kind of pathogen this could be. For therapy, this knowledge is essential. So more research about this unknown pathogen is necessary.

If Parkinson’s disease indeed develop and progress via non-cell autonomous means, spreading by transcellular mechanisms in a prion-like manner, this could have important therapeutic implications. A possible treatment could be a drug that blocks the release or uptake of the pathogenic protein, in this case alpha-synuclein (Konno et al., 2012). However, it also seems clear that this prion-like pathological process not always occurs. It may therefore be that a prion-like spread of alpha-synuclein described by Braak indeed occurs in Parkinson’s disease but only in certain subtypes of the disease.
References


