

The Paradoxical Cell Biology of α -Synuclein

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Abstract Synucleinopathies are a group of neurodegenerative diseases characterized by accumulation and aggregation of the protein α -synuclein in neuronal perikarya and processes. In contrast to the proximal localization of α -synuclein in diseased states, under physiologic conditions, the bulk of α -synuclein is present in distant presynaptic terminals. Thus, pathologic conditions lead to mislocalization and aggregation of α -synuclein in neuronal cell bodies, and an outstanding question relates to the cell-biological mechanisms that can lead to such mislocalization. Like most other synaptic proteins, α -synuclein is synthesized in the neuronal perikarya and then transported into axons and synaptic domains. Accordingly, it has been hypothesized that disturbances in biogenesis/axonal transport or presynaptic targeting of α -synuclein can lead to its mislocalization in diseased states. In this chapter, key observations that lead to this hypothesis are presented in addition to a review of some recent literature that has directly addressed this issue. Finally, conflicting results that have resulted from such studies are also highlighted, and a view is offered to reconcile these controversies.

1 Introduction

Synucleinopathies or Lewy body (LB) diseases are a heterogenous group of neurodegenerative conditions characterized by movement disorders and/or dementias, and are second only to Alzheimer's disease (AD) in its prevalence. Common examples of these conditions include Parkinson's disease (PD) and dementia with Lewy bodies (DLB). A key neuropathologic hallmark of these diseases is the perikaryal and neuritic accumulation and aggregation of the small, 14-kDa protein α -synuclein into insoluble, fibrillar structures called Lewy bodies (LBs) and Lewy neurites (LN), where the wild-type (wt) protein undergoes posttranslational modifications.

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However, while the accumulation/aggregation of α -synuclein is seen in proximal neuronal compartments, under physiologic conditions, the bulk of α -synuclein is localized to distant presynaptic terminals. Thus, pathologic events lead to both accumulation/aggregation and mislocalization of presynaptic α -synuclein in diseased states. The paradoxical localization of α -synuclein in diseased states gives rise to an interesting problem in neuronal cell biology; namely, how does the presynaptic protein α -synuclein mislocalize and accumulate/aggregate proximally in these diseases? Considering that α -synuclein is synthesized in the neuronal perikaryon and is transported along the axons, much like other presynaptic proteins, subsequently localizing to presynaptic terminals, a leading hypothesis is that impairments in biogenesis/axonal transport of α -synuclein cargoes, and/or disruptions in presynaptic targeting may be early events leading to mislocalization, and subsequent accumulation of α -synuclein in synucleinopathies. While recent studies have attempted to resolve this issue, the results have been controversial, and often contradictory. In this chapter, the pathologic features of LB diseases and the evidence linking α -synuclein to these diseases are reviewed. It is then followed by a review of our current knowledge of the mechanisms underlying the biogenesis/axonal transport/targeting of wt and pathologically altered α -synuclein, and a discussion of the controversies in the field.

2 LB Diseases and the Role of α -Synuclein

LB diseases encompass a variety of conditions and a reasonable view of the diverse disease entities is briefly offered here. Patients with LB diseases often present with variable symptoms, ranging from a pure “Parkinsonian” movement abnormality (“pure” PD) to full-blown dementias (“pure” DLB) that resemble AD. The entities defining LB diseases are complex, largely a result of the heterogeneity in the clinical and pathologic presentation of these patients, and the diversity of nomenclature attributed to these diseases by clinicians over the years. The focus here is on the common neuropathologic feature in all of these diseases; namely, the proximal accumulation of α -synuclein, and the main issue is that perikaryal α -synuclein accumulations in diseased states is almost universal, and the mislocalization of α -synuclein does not depend on how these entities are segregated.

Substantial evidence links α -synuclein to disease pathogenesis in LB diseases. First, as mentioned above, perikaryal aggregates of α -synuclein are diagnostic hallmarks of LB diseases (McKeith et al. 2004). Though many other proteins are also found in LBs, including neurofilaments and other synaptic proteins, α -synuclein is one of its most abundant constituents. In addition to the aggregates in the cell bodies, most cases have marked “neuritic” pathology, and several studies have now shown that many of these “neurites” are axonal accumulations of α -synuclein (Braak et al. 1999; Saito et al. 2003; Orimo et al. 2008). Examples of α -synuclein pathology in a human brain with LB disease are shown in Fig. 1. In addition, autosomal-dominant mutations in the α -synuclein gene itself are found in familial

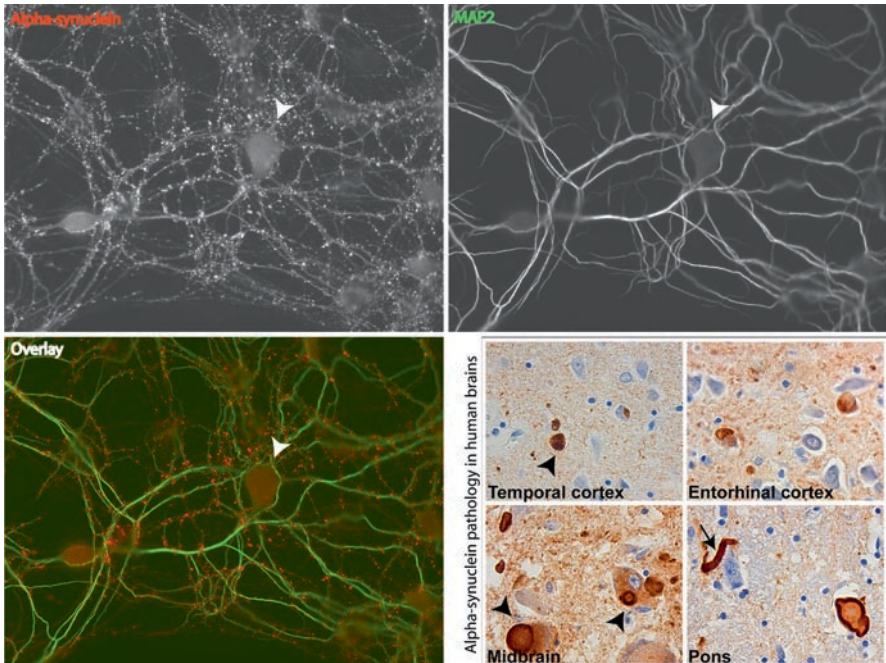


Fig. 1 Localization of α -synuclein in mature (14 DIV) cultured hippocampal neurons and in a human brain with LB disease. Cultures were stained with an antibody to α -synuclein and microtubule associated protein – MAP2 (see *red/green* overlay in ebook). Note that the vast majority of α -synuclein is localized to presynaptic boutons in these cultures, and very little protein can be discerned in the perikarya (*white arrowhead*). The *lower right panel* shows α -synuclein immunohistochemistry in various brain regions of a patient with LB disease. Note the presence of large inclusions of α -synuclein within the neuronal cell bodies (*black arrowheads*), as well as the pathology within neuritic processes (*black arrow*)

forms of LB diseases. Three of these (A30P, A53T, and E46K) representing mutations in the respective amino acid positions have been described in several families (McKeith and Mosimann 2004). Though rare, combined with the other evidence linking α -synuclein to these diseases, the mutants offer a window into the specific mechanisms by which changes in the α -synuclein protein can lead to pathology. Besides these mutations, it was recently found that some families with these diseases have a duplication or triplication of the α -synuclein gene itself (Singleton et al. 2003). Such triplication leads to a doubling of the protein levels of α -synuclein, suggesting that even a modest increase in protein levels can lead to pathology.

In addition to this compelling evidence from human genetics implicating α -synuclein in the pathogenesis of such dementias, several mouse models expressing wt, posttranslationally modified α -synucleins, as well as familial LB disease mutants replicate key features of these diseases (Masliah et al. 2000; Kahle et al. 2001; Giasson et al. 2002; Lee et al. 2002; Tofaris et al. 2006), and

neurodegeneration occurs in fly and other α -synuclein animal models as well (Feany and Bender 2000; Link 2001; Lakso et al. 2003; Kuwahara et al. 2006). Thus, collectively, the immunohistochemical, biochemical, genetic, and animal model data have established α -synuclein as a major player in the pathogenesis of LB diseases.

3 The Neuronal Cell Biology of α -Synuclein

In mature neurons growing under physiologic conditions, the bulk of α -synuclein localizes to presynaptic regions. Accordingly, in dissociated cultured hippocampal neurons, the vast majority of α -synuclein in mature neurons is localized to the presynaptic terminals (Fig. 1), while in immature neurons, punctate α -synuclein staining is seen in both axons and dendrites. It was reported by the Banker laboratory that in neurons obtained from embryonic (E17–18) mice, the expression of α -synuclein is selectively suppressed until about 7 days in vitro (DIV), a time when such neurons begin to robustly generate new synapses (Withers et al. 1997). Based on this, it was speculated that α -synuclein may have a role in synapse maturation and synaptic plasticity, and not necessarily in synapse biogenesis. Indeed, the notion that α -synuclein may be involved in synaptic plasticity is also supported by the profound increase of α -synuclein expression in brains of songbirds, precisely at a time when they are actively learning new songs. However, we have found that the expression of α -synuclein in hippocampal cultures is largely dependent on the maturity of neurons at the time of plating. While E18 neurons only express α -synuclein after 7 days in culture as described by Withers et al., hippocampal neurons cultured from postnatal (P1–2) mice express α -synuclein immediately after plating, and α -synuclein expression can be seen even in neurons that grow in complete isolation (see Fig. 2). Thus, it is likely that the absence of α -synuclein expression in 1–7 DIV neurons seen by Withers et al. reflects the immature state of these cells themselves, and not the effects of synaptogenesis on the expression of α -synuclein. Curiously, in addition to synapses, α -synuclein expression is usually seen in the nucleus as well (arrowhead in Fig. 2). Though the nuclear localization has been less well studied, α -synuclein has been proposed to play a role in inhibiting histone acetylation (Kontopoulos et al. 2006).

Previous studies have revealed many key biochemical features of wt and disease-associated α -synuclein. Briefly, monomeric wt- α -synuclein is a small (140 aa), natively unfolded protein that binds to acidic phospholipids (Uversky 2007). Though α -synuclein is known to bind to certain vesicles, it lacks a transmembrane domain or a GPI anchor, and though the precise mechanisms by which α -synuclein binds to vesicles is still debated, it most likely involves the α -helical N-terminus. In diseased states, however, α -synuclein is thought to self-associate, and this self-association appears to stabilize the protein, leading to formation of oligomers and subsequent fibrillar forms, as seen in LBs and LNs in neurons. Within such LBs and LNs α -synuclein is found to undergo extensive changes, including posttranslational

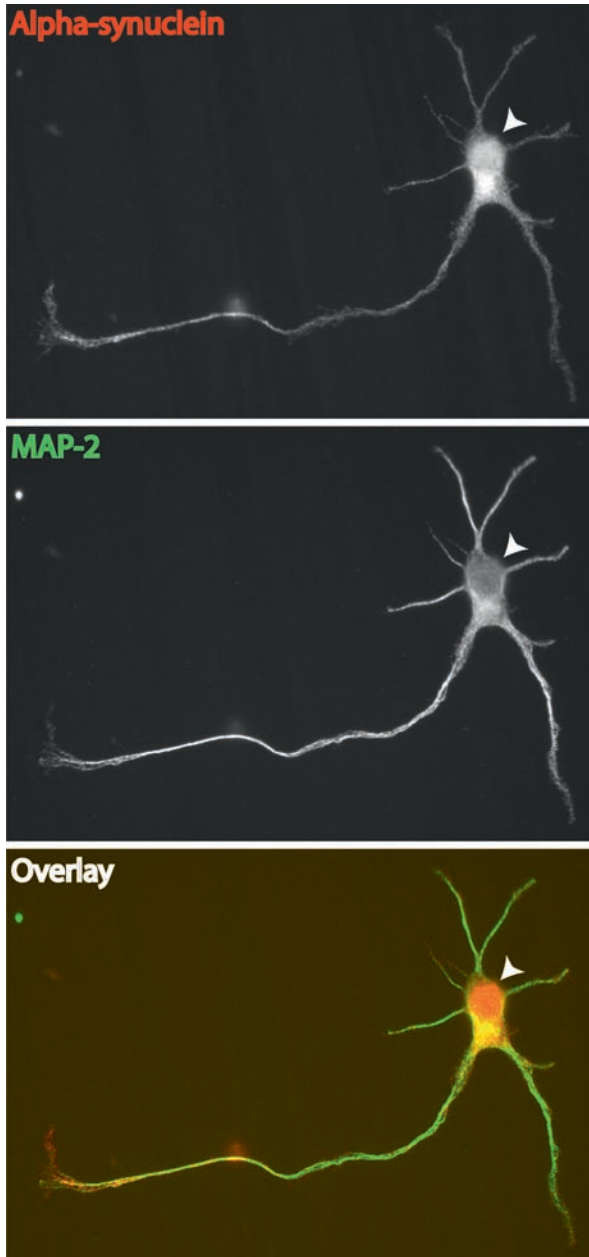


Fig. 2 Localization of α -synuclein in immature hippocampal neurons. A 3-DIV hippocampal neuron obtained from postnatal (P1) mouse brain is stained with an antibody to α -synuclein and MAP2 (see red/green overlay in ebook). Note that at this stage of development, punctate α -synuclein staining is seen in the axon as well as the dendrites. Also note that the α -synuclein expression in these neurons is not dependent on the connectivity of the cells or the presence of synapses. The arrowhead depicts the nuclear localization of α -synuclein in these neurons

modifications like phosphorylation and nitration, as well as C-terminal truncations. Many of these modifications lead to an acceleration in the rate of aggregation of the protein in *in vitro* biochemical experiments (reviewed in Mukaetova-Ladinska and McKeith 2006). These disease-associated modifications may play a pathogenic role, as animal models expressing these disease-associated modifications replicate some features of the disease including neuronal loss, LB-like α -synuclein accumulations, and behavioral abnormalities (Chen and Feany 2005; Tofaris et al. 2006; Gorbatyuk et al. 2008; Zhou et al. 2008). Thus, collectively, existing data suggest that specific alterations of wt- α -synuclein may play an important role in the transformation of α -synuclein into fibrillar LBs and LNs. Similarly, familial disease-associated mutations in α -synuclein also lead to acceleration in the fibril formation of the protein, and animal models expressing these mutations replicate some features of the disease.

4 Mislocalization of Presynaptic α -Synuclein in LB Dementias

Despite all this available information on the steps involved in the transformation of α -synuclein into fibrillar LBs and LNs, an outstanding question in the field is how α -synuclein, a protein that is normally localized to distal presynaptic terminals/boutons (Withers et al. 1997; Kahle et al. 2000), accumulates and aggregates into LBs and LNs that are seen in proximal neuronal compartments. One possibility is that in diseased states, pathologically altered α -synucleins fail to localize to presynaptic domains, leading to its gradual proximal accumulation, subsequent self-association, and eventual aggregation. This model of time-dependent accumulation is supported by studies in sympathetic axons and their cell bodies that are affected in LB diseases, suggesting that α -synuclein accumulation occurs in a centripetal fashion, with the axonal accumulation preceding the perikaryal accumulation/aggregation (Orimo et al. 2008). The notion that α -synuclein accumulation in the neuronal perikarya occurs gradually is also supported by immunohistochemical data from human brains with LB diseases showing that α -synuclein staining in these cases ranges from a mild diffuse perikaryal staining to a more robust pattern of staining (called “pale bodies”), in addition to the typical donut-shaped LBs that are strongly positive for α -synuclein (Wakabayashi et al. 2007). These data suggest that the variable patterns of α -synuclein pathology may represent evolving stages of LB formation.

What are the specific mechanisms that lead to the mislocalization of α -synuclein in proximal neuronal compartments? Considering that α -synuclein is synthesized in the neuronal perikarya and then transported into the axons, eventually localizing to presynaptic terminals or boutons (Jensen et al. 1999; Li et al. 2004; Saha et al. 2004), it seems reasonable to hypothesize that defects in either the presynaptic targeting and/or axonal transport/biogenesis of the protein can lead to its accumulation in diseased states. Another possibility is that the proximal accumulation of α -synuclein is a result of the failure of degradation of α -synuclein in diseased

states, as suggested by other groups (Cuervo et al. 2004). This idea is not necessarily mutually exclusive of the notion of transport/targeting abnormalities in LB diseases. For example, it is conceivable that the abnormally targeted/transported α -synuclein may present the cell with problems of degrading the stalled α -synuclein, or abnormally targeted/transported α -synuclein may facilitate the formation of pathologic α -synuclein species that are difficult to degrade.

5 Biogenesis and Axonal Transport of wt and Pathologically Altered α -Synuclein

In this section, our current knowledge about mechanisms involved in the biogenesis and axonal transport of α -synuclein in physiologic and pathologic states is reviewed. As a first step, it is important to understand if α -synuclein localizes to a specific subcellular structure/organelle within the neuron. While α -synuclein's presynaptic localization suggests that it may be localized to synaptic vesicles (at least within synapses), α -synuclein's colocalization with vesicles has been difficult to demonstrate biochemically. While α -synuclein can associate with isolated purified vesicles *in vitro*, it behaves as a largely soluble protein in extracts from brains (Kahle et al. 2000). Ultrastructurally, while α -synuclein is clearly present in synaptic domains, it does not appear to be tightly associated with synaptic vesicles (Clayton and George 1998). Thus, the consensus view is that α -synuclein is transiently associated with vesicles at synapses, and that this interaction is dynamically regulated. Though α -synuclein is present as puncta along axons (see Fig. 2), the subcellular structure/organelle to which it binds during transport, if any, is unknown.

The biogenesis of α -synuclein is not well studied. Being largely a cytosolic protein, α -synuclein is not likely to traffic via the endoplasmic reticulum (ER) \rightarrow Golgi secretory pathway. Surprisingly, however, when expressed in yeast, wt- α -synuclein localizes robustly to the plasma membrane, and this localization is likely to involve the secretory pathway (Dixon et al. 2005). Notably, in the yeast model, although the A53T mutation also localizes to the plasma membrane like the wt protein, the A30P mutation behaves like a soluble protein (Cooper et al. 2006; Soper et al. 2008). Though this result has always been interpreted as indicating the failed localization of the A30P mutant to the membrane, it could also reflect defects in ER \rightarrow Golgi \rightarrow plasma membrane trafficking of the mutant protein. Some evidence from nonneuronal cells also suggests a Golgi localization of α -synuclein (Tompkins et al. 2003), but we have seen that the localization of α -synuclein in neuronal cell bodies is unchanged by experimental manipulations that tend to cluster, or disperse other Golgi-derived proteins (our unpublished observations). Thus we feel that data from yeast model systems that seems to process α -synuclein very differently from neurons, must be interpreted with caution. Biogenesis of cytosolic proteins is difficult to study, and it is still unclear how α -synuclein is synthesized or how pathologic forms of α -synuclein may contribute to defects in this process.

In contrast, the overall mechanics of axonal transport of α -synuclein are better understood. In general, axonal transport conveys proteins synthesized in the neuronal cell bodies to distant sites, including presynaptic terminals. This is a constitutively active mechanism that occurs throughout the life of the neuron. Previous studies have shown that proteins are transported along axons in two overall groups. While one group of proteins was rapidly transported along axons at speeds of 50–400 mm/day, the other was transported much more slowly, at rates of only 0.2–8 mm/day. These two groups were called the fast component and the slow component (SC), respectively (reviewed in Roy et al. 2005). Closer examination of the SC revealed that it consisted of two, fairly nonoverlapping groups of proteins – one consisting of the cytoskeleton, namely, the microtubules and the neurofilaments, and the other, a slightly faster-moving group consisting of a heterogeneous collection of over 200 diverse proteins. They were called SCa and SCb, respectively.

Previous studies using the classic pulse-chase paradigm have also shown that the bulk of α -synuclein moves in slow axonal transport, in the sub-group called SCb using the classic pulse-chase paradigm (Jensen et al. 1999; Li et al. 2004). In these studies, radiolabeled amino acids were injected in the vicinity of neuronal cell bodies of living animals. The injected label was incorporated into the newly synthesized proteins in the perikarya, and these pulse-labeled proteins, including labeled α -synuclein, were transported along the axons. As the wave of radiolabeled α -synuclein moved along the axon, the composition of the labeled α -synuclein at any given time along the axon varied according to time and distance from the cell body. Thus, by studying the α -synuclein composition at various points along the axon, serial “snapshots” of the transported α -synuclein were obtained, showing that α -synuclein specifically moved in SCb. It was also reported that a small fraction ($\approx 15\%$) of α -synuclein moves in the fast component as well (Jensen et al. 1999), though this was later refuted by a subsequent study (Li et al. 2004). However, it is noteworthy that small fractions (10–15%) of other SCb proteins like synapsin and heat shock proteins are also conveyed in the fast component, and the reasons for this peculiar kinetics of SCb proteins is unclear.

A limitation of the radiolabeling technique is that it can only detect bulk transport of the entire population of proteins moving in slow or fast transport and individual cargoes cannot be visualized. Thus, while the overall movement of wt- α -synuclein in SCb was well established by the above-mentioned studies, it was not clear how individual α -synuclein cargoes were transported. Recently, we designed a live-cell model-system where it is possible to visualize individual α -synuclein and SCb cargoes in living neurons (Roy et al. 2007). Using this model, we found that individual α -synuclein, and other SCb cargoes move rapidly in axons, at instantaneous velocities similar to those of the fast component. However, when compared directly to various fast component proteins, like synaptophysin and amyloid precursor protein, slow moving α -synuclein cargoes often underwent pauses during transit, and the movements were infrequent, unlike the fast component proteins that were transported much more frequently and persistently. These pauses in transit of individual slow cargoes made movement of the entire population slow, when averaged over time. Using this model, we also showed that α -synuclein cargoes were cotransported

with other SCb proteins in axons (Roy et al. 2007), and that this movement was microtubule-dependent (Roy et al. 2008). More recent unpublished work from my laboratory shows that the movement of SCb proteins is an interplay of diffusion and motor-driven transport, leading to a biased transit of bulk cytosolic cargoes. These findings will be reported elsewhere shortly.

While the aforementioned studies have focused on the axonal transport of wt- α -synuclein, two previous studies have specifically looked at axonal transport of disease-associated familial α -synuclein mutants with seemingly contradictory results. While one study reported that the two familial disease-associated α -synuclein mutants A30P and A53T were transported more slowly than the wt protein (Saha et al. 2004), a subsequent study reported no differences in the transport of the wt and the mutant protein (Li et al. 2004). Notably, different methods were used to study axonal transport in these two reports. In Saha et al., the authors determined transport of wt and mutant α -synuclein in cultured cortical neurons by looking at bulk transport in fixed cultured neurons. Specifically, cultured neurons were transfected with the wt- or mutant green fluorescent protein (GFP)-tagged α -synuclein constructs, and then fixed at varying times posttransfection, allowing for transport of the transfected GFP- α -synucleins into the axon. The authors reported that they could locate the advancing “front” of the GFP- α -synuclein fluorescence in these fixed axons and measure the distance of this “front” from the cell body at varying times after transfection, as a measure of overall transport of the transfected wt- or mutant α -synuclein. Using these methods, they reported that the axonal transport of the α -synuclein “front” was significantly reduced when the protein had the A30P or A53T mutations. We note that these methods to study axonal transport are unconventional and are not yet validated by other laboratories (or in a variety of fast/slow cargoes). In our opinion, these techniques could lead to uncontrolled variability due to vagaries of transfection and/or protein expression, nevertheless, the results reported in these studies are intriguing.

In the study by Li et al. (2004), the authors studied axonal transport of the A30P and A53T mutant α -synuclein *in vivo* by the more conventional pulse-chase type of radiolabeling methods in peripheral sensory and motor nerves, and failed to see any difference in the axonal transport rates of the two mutants, compared to the wt protein. The reasons for such discrepancies in the transport of mutant α -synuclein in these studies are difficult to explain. However, differences in the methodology or the presence of endogenous mouse α -synuclein in these studies may account for some of the differences. Alternatively, differences in the synuclein proteins in the central and peripheral nervous systems may also account for these discrepancies. Indeed, the distribution of synucleins is quite different in the two systems, and some synuclein family members (e.g., persyn) are only present in the peripheral nervous system (Buchman et al. 1998). Saha et al. (2004) also reported that phosphorylation of α -synuclein at Ser-129 also led to a reduction in the transport of α -synuclein in their system, but this modification was not studied by Li et al. in their system. Though the underlying reasons for these discrepancies are unclear, the lack of consensus in the literature highlights the need for further studies with model-systems that can (a) directly visualize the

transport of pathologic α -synuclein cargoes, (b) perform such studies in a α -synuclein null background, and (c) focus on α -synuclein targeting/transport in central nervous system neurons.

6 Mechanisms of Targeting wt and Pathologic α -Synuclein to Synapses

Proteins synthesized in the perikarya are transported into axons and synapses. Recent data clearly indicates that neurons have evolved specific mechanisms to “capture” the transiting vesicular cargoes into synapses when needed, so physiologic functioning of synapses that are distally situated would not be dependent on perikaryal protein synthesis, (reviewed in Levitan 2008). Though such mechanisms have not been shown for SC synaptic proteins yet, it is likely that these do exist for SC proteins, including α -synuclein as well. In that regard, one possible mechanism of mislocalization of α -synuclein in diseased states is that the disease-associated mutant proteins fail to localize to presynaptic domains after reaching their ultimate destination. To test this idea, many studies have compared the membrane interactions of the familial disease-associated α -synuclein mutants A30P and A53T to that of the wt protein, in *in-vitro* preparations, where such binding can be documented readily. However, the results have been controversial. While some studies showed decreased membrane-binding of the human A30P and the A53T mutants (Iwai et al. 1995; Jensen et al. 1998; Jo et al. 2000, 2002), others reported an increase in membrane-binding with the A53T mutant (Sharon et al. 2001; Lotharius et al. 2002), or no change in membrane-binding of these mutants at all (McLean et al. 2000; Perrin et al. 2000).

Most of these studies measured the ability of purified human α -synuclein to insert spontaneously into lipids, and did not take into account cytosolic factors that can regulate this interaction, including chaperones/cofactors normally present in the cell. However, a recent study adopted a novel biochemical approach by incubating membrane preparations from mice expressing wt and the A30P and A53T mutant human α -synucleins (donor) with brain cytosol from α -synuclein null mice (acceptor) (Wislet-Gendebien et al. 2006). The rationale was to evaluate the interactions of the mutant α -synucleins with membranes in the presence of cytosolic factors, thereby mimicking the *in vivo* situation. The rationale for using the α -synuclein null brains as the “acceptor” was to eliminate possible confounding factors introduced by endogenous mouse α -synuclein. These studies showed that both the human mutations decreased the association of α -synuclein with membranes compared to the wt protein, but remarkably, *only in the presence of brain cytosol*. These studies suggest that under conditions that mimic the *in-vivo* situation in the brain, both human α -synuclein mutants tend to show reduced association with synaptic structures, conferring a pathologic signature common to both human mutants.

Two studies have also evaluated A30P and A53T mutant human α -synucleins in cultured hippocampal neurons from wt mice, expressing endogenous mouse α -synuclein (Fortin et al. 2004, 2005). Using GFP-tagged human α -synucleins transfected in cultured hippocampal neurons from wt mice, these studies showed that while the A30P mutant was mislocalized to synapses, there was no difference in the presynaptic targeting of the A53T mutant, compared to the wt protein. These results in hippocampal cultures seem to contradict the biochemical studies by Wislet-Gendebien et al. (2006) mentioned above, though both seemingly replicated an “in vivo like” situation. Intriguingly, however, the one difference between these two studies is that while the biochemical study used cytosol from α -synuclein null mice, the experiments with cultured hippocampal neurons were done in wt-neurons that express the endogenous mouse α -synuclein as well. Thus, it is possible that the presence of endogenous mouse α -synuclein may have confounded some of the observations in the cultured neurons (Fortin et al. 2004). Further studies in α -synuclein null neurons are likely to resolve this outstanding issue. Besides these mutants, little is known about the presynaptic targeting of other pathologic α -synucleins *in-vivo*.

7 Conclusions and Perspectives

In this chapter, current understanding of the mechanisms underlying defective biogenesis/axonal transport/targeting of α -synuclein in diseased states are discussed. It is clear that despite significant effort by researchers towards understanding the mechanisms of α -synuclein in health and disease, many basic questions regarding its biology and pathology remain unanswered. One of the major problems has been the chameleon-like nature of the α -synuclein protein itself, which not only behaves diversely in *in vivo* and *in vitro* settings, but also tends to localize, and behave anomalously in non-neuronal cells. Unfortunately, the majority of the cell biological studies to date have been performed in a variety of non-neuronal cells, making it difficult to interpret and synthesize the available data. Finally, as noted above, some of these controversies may be due, at least in part, to the confounding effects of the endogenous mouse α -synuclein. In summary, though there is tantalizing data supporting the hypothesis that axonal transport and/or targeting of α -synuclein is disrupted in diseased states, the lack of systematic studies of transport/targeting of wt and disease-associated α -synucleins in a suitable model-system is lacking. Future studies focusing on these outstanding issues may eventually resolve these controversies and provide a mechanistic basis for mislocalization of this protein in diseased states.

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