

two unrelated proteins. We now have a huge parts store in our backyard waiting for creative scientists to assemble the pieces and realize new devices that never existed in nature. We need design protocols that are appropriate for the soft, nanoscale systems that are folded proteins. As we begin to design these new systems, it is becoming increasingly clear that genetic approaches to engineering represent the future of soft, nanoscale protein design.

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Chemistry & Biology, Vol. 11, November, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.11.003

Targeting α -Synuclein in Parkinson's Disease

α -Synuclein aggregation into fibrils is associated with the pathogenesis of Parkinson's disease (PD). Li et al. provide strong evidence that rifampicin interacts with α -synuclein and inhibits its fibrillization [1]. Rifampicin could be a promising candidate for therapeutic application for PD.

Sometimes an observation may produce a hypothetical link between two apparently unrelated events. For example, the observation by McGeer et al. and Namba et al. that anti-leprosy-treated elderly patients have less dementia and senile plaques in their brains than non-treated patients has created a link between the anti-leprosy drug rifampicin and neurodegenerative diseases [2, 3]. How this hypothesis has been pursued and what might be the potential consequences for PD and other cell-degenerative diseases will be discussed here.

In vivo protein aggregation into fibrillar deposits is strongly associated with cell degeneration and the pathogenesis of a number of progressive cell-degenerative diseases. These include fatal neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), the transmissible spongiform encephalopathies (TSEs or prion diseases), the pancreatic β cell degenerative disease type II diabetes (T2D), and several other localized or systemic amyloidoses [4]. In all these conditions, a disease-specific polypeptide or protein aggregates into fibrillar deposits. Recent evidence suggests that common molecu-

lar events may underly the pathogenesis of the different "protein aggregation" diseases.

Parkinson's disease is the most common human neurodegenerative movement disorder and affects $\sim 1\%$ of the elderly population. Although symptomatic treatment strategies are available, PD has remained a noncurable disease [5]. Primary clinical symptoms of PD are bradykinesia, resting tremor, muscular rigidity, and difficulty with balance. PD is neuropathologically characterized by a marked and progressive degeneration of dopaminergic neurons and by the presence of fibrillar cytoplasmic inclusions (Lewy bodies [LBs]) and dystrophic neurites (Lewy neurites [LNs]) in the *substantia nigra* region of the brain [6]. Although the loss of dopamine neurons is certainly related to the major clinical symptoms of PD, the causes and the pathogenesis of this multifactorial disease as well as that of related "synucleinopathies" are still largely unknown.

The major components of both LBs and LNs are fibrillar aggregates of α -synuclein [6, 7]. α -Synuclein is a widely expressed, neuronal presynaptic protein that appears to play a role in membrane-associated processes and synaptic plasticity and has been linked to learning and development processes [6]. While the mechanism(s) of formation of LBs and LNs and their association with PD is (are) yet not understood, several lines of evidence suggest that α -synuclein fibrillization is associated with PD [6, 8]. Similarly to other protein aggregation diseases, both neurotoxic and neuroprotective roles have been attributed to the endproducts of α -synuclein aggregation, the fibrillar α -synuclein deposits [6, 8]. α -Synuclein fibril formation in vitro proceeds via the conversion of the 140 amino acid residue protein, that appears to be "natively unfolded," into ordered, β sheet-rich oligomers also termed "protofibrils" [8]. α -Synuclein protofibrils or alternatively folded/assembled oligomers

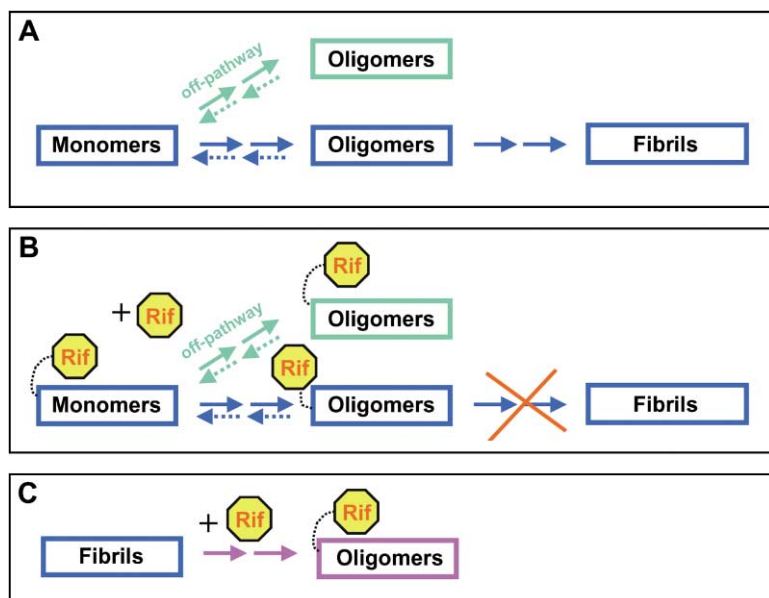


Figure 1. Potential Pathways for α -Synuclein Fibrillization and Its Inhibition by Rifampicin
(A) Simplified schematic representation of possible molecular events in the fibrillization pathway of α -synuclein and possibly of other amyloidogenic polypeptides or proteins. Monomeric α -synuclein aggregates—possibly following formation of a partially folded intermediate state—into soluble, β sheet-rich oligomers (i.e., protofibrils) that will subsequently transform into fibrils [1, 8, 9, 15]. Alternatively, formation of oligomers may also proceed *off-pathway* (green arrows).

(B) Possible effects of rifampicin on fibrillization of α -synuclein: According to the findings and suggestions of Li et al. [1], rifampicin (Rif) may tightly and, possibly, covalently (dashed line between Rif and the protein) bind monomeric α -synuclein and its oligomeric form(s) (in-, or off-pathway oligomers). The oligomers may thus become “stabilized” and unable to transform into fibrils.

(C) Possible effects of rifampicin on fibril disassembly of α -synuclein: According to the findings of Li et al. [1], rifampicin disassembles α -synuclein fibrils and may bind—

possibly covalently—to the oligomers that are generated during disassembly of fibrils. Both the quaternary structure and the potential neurotoxicity of the oligomeric and fibrillar α -synuclein forms are still unknown. The different colors of the boxes around the oligomers indicate potential differences between the oligomers with regard to quaternary structure and neurotoxicity.

have been suggested to be neurotoxic [8, 9]. Protofibrils gradually transform into fibrils that may thus act as sequesters of neurotoxic species [9]. Independently of the nature of the neurotoxic species, the process of α -synuclein fibrillization appears to be strongly linked to neurodegeneration. Therefore, identifying molecular strategies/factors that may interfere with and/or inhibit this process is a reasonable approach to both understand the molecular causes of PD and to develop novel treatment concepts. Li et al. describe in this issue their efforts toward these targets [1].

In the course of their studies to investigate a potential link between senile dementia and rifampicin, Tomiyama et al. earlier found that rifampicin inhibits fibrillization and neurotoxicity of β -amyloid peptide ($A\beta$) [10–12]. $A\beta$ is the major constituent of senile plaques, and its fibrillization is strongly associated with neurodegeneration in AD. In addition, rifampicin inhibited cell toxicity of islet amyloid polypeptide (IAPP), which is the amyloidogenic polypeptide in T2D. As oxidative stress is associated with cell degeneration, these effects were first attributed to the potential antioxidative effect of rifampicin, which possesses a naphthohydroquinone or naphthoquinone structure. Later on, it was suggested that rifampicin binds to $A\beta$ and IAPP fibrils and inhibits their contact to the neighboring cells [11, 12].

The findings of Li et al. provide strong biochemical and biophysical evidence that rifampicin interferes with the fibrillization pathway of α -synuclein in substoichiometric amounts, inhibits formation of fibrils, and can disaggregate already formed fibrils (Figure 1) [1]. When following the conformation of α -synuclein in the presence of rifampicin at various time points by circular dichroism spectroscopy (CD), no changes in overall conformation were detected. By contrast, in the absence of rifampicin, α -synuclein aggregated into soluble β sheet

oligomers and fibrils. These results suggest that rifampicin prevents the formation of β sheet aggregates of α -synuclein. Size-exclusion chromatography (SEC), Thioflavin T binding, and electron microscopy suggest that the interaction of rifampicin with soluble α -synuclein results in the stabilization of α -synuclein monomers and soluble oligomers. In addition, rifampicin was found to be able to dissociate α -synuclein fibrils into soluble, β sheet-rich oligomers. As rifampicin easily degrades and/or oxidizes into quinone products in aqueous solution, rifampicin solutions incubated under aerobic or anaerobic conditions were also studied, and solutions containing oxidative degradation products were significantly more “potent.” SEC analysis indicated that rifampicin tightly binds α -synuclein mono- and oligomers. The authors propose that in fact rifampicin might covalently bind α -synuclein, possibly, via reaction of its naphthoquinone form with amino groups of lysine side chain to form a Schiff base.

This hypothesis and the results of the rifampicin studies presented here (Figure 1) are in line with the results of other studies: Zhu et al. have very recently shown that the flavonoid baicalein also inhibits α -synuclein fibrillization, stabilizes a partially folded oligomer, and disaggregates fibrils [13]. The baicalein quinone was suggested to be the most “potent” baicalein form and mass spectroscopy indicated the formation of a covalent baicalein quinone- α -synuclein adduct [13]. Conway et al. [14] have suggested that dopamine kinetically stabilizes a, potentially neurotoxic, α -synuclein protofibril (oligomer) by oxidative ligation to α -synuclein via its orthoquinone form. Moreover, a very recent study by Li et al. has shown that dopamine can disaggregate both α -synuclein and $A\beta$ fibrils, suggesting the formation of covalently modified α -synuclein oligomers by catecholamine quinone(s) [15].

Taken together, the findings by Li et al. as published in this issue of *Chemistry & Biology* are exciting and offer new mechanistic insight and potential therapeutic strategies for PD. At the same time, these results in conjunction with the above discussed findings give rise to a number of questions that need to be addressed. For example, what are the chemical and quaternary structure(s) of the α -synuclein oligomers that are potentially stabilized by rifampicin, baicalein, or dopamine? What is the morphology and what are the biochemical, biophysical, and cell viability-associated properties of these species? Are they neurotoxic oligomers or are they nontoxic oligomers? Are the oligomers that are stabilized by rifampicin (or the other compounds) via interaction with soluble α -synuclein different from the oligomeric species that are generated from fibril disaggregation? If β sheet-rich α -synuclein oligomers or protofibrils were in fact neurotoxic [8, 9] and the stabilized oligomers had properties similar to them, this would possibly result in a rifampicin/baicalein/dopamine-enhancing effect on α -synuclein-associated neurotoxicity. On the other hand, if the "fibril disassembly" oligomers and the stabilized partially ordered oligomers were noncytotoxic and because conditions of oxidative stress promote α -synuclein fibrillization and neurotoxicity by rifampicin, baicalein, and related compounds would offer a reasonable perspective for the development of drugs to combat PD and, possibly, AD and other protein aggregation diseases [16, 17].

Finally, for the potential long-term use of rifampicin and related compounds in a novel disease situation, i.e., its use as a therapeutic in cell degenerative diseases, it appears important to find out if and how efficiently these compounds may unwisely modify cellular proteins other than the amyloidogenic proteins. However, these potential drawbacks could well be counterbalanced, because the medical use of rifampicin is long established in principle from its application in infectious diseases.

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Microbe Manufacturers of Semiconductors

Synthesis of cadmium sulfide (CdS) semiconductor nanoparticles within a prokaryotic organism is reported for the first time by Sweeney et al. [1]. This paper demonstrates the utility of microorganisms to perform chemistries outside the scope of their "normal" metabolism and offers an environmentally benign synthesis of CdS nanoparticles.

Semiconductor nanoparticles are an extremely important class of materials with properties that can be finely tuned through composition, size, and particle morphol-

ogy. This is illustrated by the technological drive toward the single electron transistor and in the use of semiconductor nanoparticles for bioimaging. It is well known that the properties of semiconductors change as a function of size, shape, and crystallinity. Cadmium sulfide (CdS) is a semiconductor material that has been used for such applications as fluorescent labels and optoelectronic transistor components, where particles of approximately 4–5 nm in diameter behave as so-called quantum dots (QD) [2, 3]. These particles when embedded within an appropriate matrix act as potential wells that confine and stabilize electrons in discrete energy levels. The technologically useful properties of CdS QDs are due in part to the fact that the band-gap is tunable over a range of 1.5–3.5 eV (i.e., visible to UV).

Many synthetic routes to semiconductor nanoparticles involve highly toxic solvents, explosive precursors,