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Testing whether alpha-synuclein binds specific DNA conformations

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Keywords

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Abstract

The abnormal accumulation of the intracellular protein α-synuclein has been implicated in the pathogenesis of several neurodegenerative disorders, including Dementia with Lewy Bodies (DLB), Lewy Body variant Alzheimer's Disease (LBVAD) & Parkinson's Disease (PD). In these diseases, known as "synucleinopathies", the accumulation of aggregated α-synuclein in the cytoplasm, known as Lewy inclusions, is correlated with cellular dysfunction and death. Although the presence of Lewy inclusions are used as a marker for a definitive diagnosis of synucleinopathies, their specific role in neurodegeneration still remains unclear. The Unni lab has discovered a previously unrecognized function for the protein α-synuclein in repairing nuclear DNA damage. Based on this discovery, we have proposed a new hypothesis that during disease, α-synuclein protein is sequestered in cytoplasmic Lewy bodies, decreasing its nuclear DNA damage repair function and potentially leading to cell death of Lewy body-containing neurons. We have examined which confirmations of DNA α-synuclein preferentially binds for further insight into α-synuclein's normal function in the nucleus.

We utilize electrophoretic mobility shift assays with subsequent fluorescence imaging to visualize the biochemical interactions between α-synuclein and DNA, both in the presence and absence of small molecule reagents known to alter DNA conformations. Through the use of DNA bending dyes (DAPI, Hoescht), which both bind to the minor groove of DNA, we have characterized a shift in synuclein's concentration-dependent binding to DNA. Increasing amounts of DAPI and Hoescht in a 10% polyacrylamide gel system, shifted the α-synuclein bound DNA to higher apparent molecular weights. Our data suggests that α-synuclein binds preferentially to non-linear DNA conformations and may have a role in DNA bending. Understanding the mechanism by which α-synuclein binds DNA will help to elucidate the mechanistic role α-synuclein plays in DNA repair, as well as potentially inform the creation of new therapies that could ameliorate cell death caused by loss of α-synuclein function.