Protest-70: Protecting protein homeostasis in synucleinopathies and tauopathies by modulating the Hsp70/co-chaperone network

The gradual accumulation of α-synuclein (α-Syn) and tau aggregates is characteristic of many neurodegenerative diseases. This consortium aimed to determine how molecular chaperones, specifically the heat shock protein 70 (Hsp70) chaperone and its co-chaperones, impact the aggregation of structurally distinct assemblies of α-Syn and tau. The goal was to identify particular chaperone networks targeting α-Syn and tau, characterize their mechanisms of action (e.g., prevention of aggregation, disaggregation, degradation) and relate these effects to cellular toxicity and associated phenotypes to assess their therapeutic potential.

Results

The Hsp70 disaggregation machinery, consisting of the core Hsp70, the J-domain protein DNAJB1 and the nucleotide exchange factor (NEF) HSP110, is able to dissemble aggregated tau *in vitro* (Figure 1A and B) [1]. Different tau isoforms [2] [3] were dissolved by this particular combination of chaperones, although to varying degrees. DNAJB4, another class B J-domain protein, was able to target aggregated tau as well as DNAJB1, but not class A DNAJs (such as DNAJA1 or DNAJA2), substantiating the client specificity of the J-domain family of co-chaperones. However, the tau species liberated from fibrils were still able to induce the aggregation of tau in a cell culture model (Figure 1C).

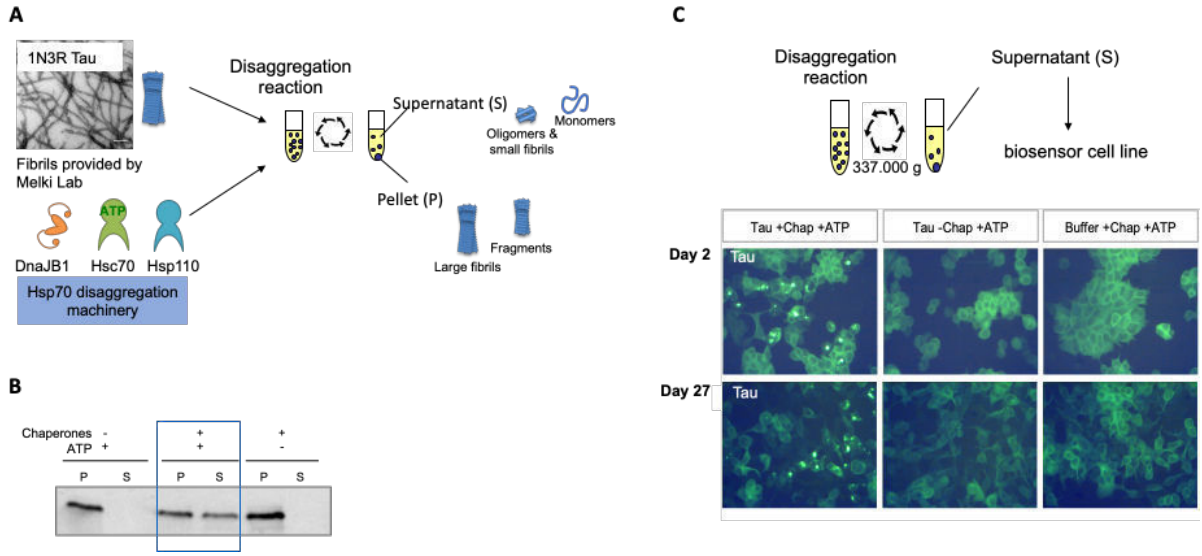

Figure 1 The chaperone-mediated disaggregation of amyloid tau fibers liberates seeding-competent species A: Experimental setup of the disaggregation reaction and subsequent sedimentation assay. B: Disassembly of tau fibers incubated with chaperones separated by centrifugation into supernatant (S) and pellet (P) fractions. C: TauP301S-Venus HEK293 cells were seeded with the supernatant fractions of the indicated disaggregation reactions and further passaged.

The physiological effects of the Hsp70 disaggregation activity was furthered characterized using a *C. elegans* model. Knockdown of the Hsp70 NEF HSP110 revealed that reduction of disaggregation activity leads to a reduction in foci formation and rescue of toxicity (Figure 2A and B). The age-dependent spreading of α-Syn from the muscle to the hypodermis was also reduced with the KD of HSP110 (Figure 2C and D) [4].

Both studies suggest that by generating seeding-competent species, the Hsp70-mediated disaggregation activity may potentiate the self-templated amplification of amyloid-type aggregates and thus play a role in the progression of associated diseases.

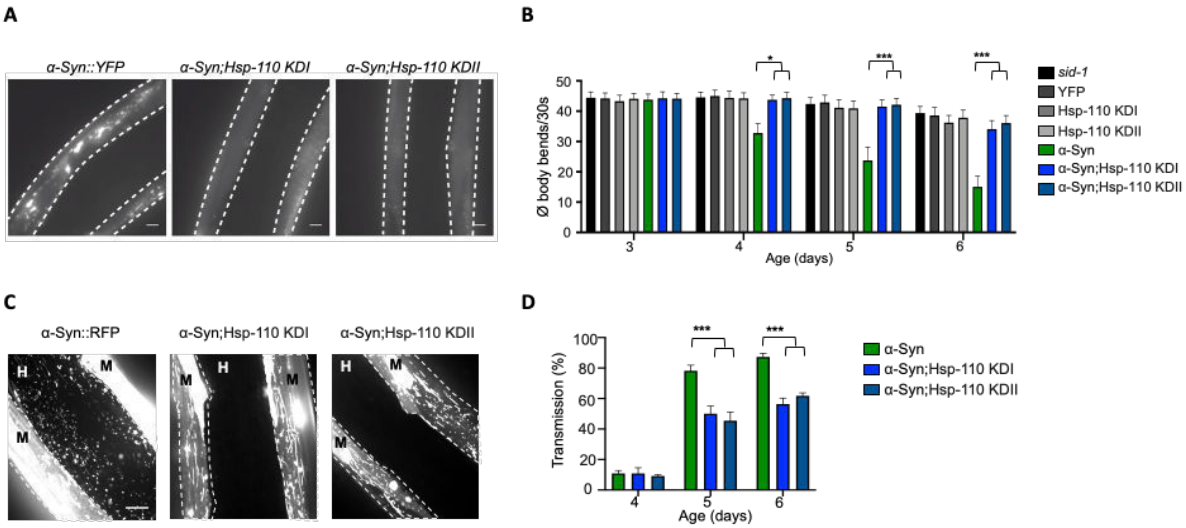

Figure 2 KD of Hsp110 impairs Hsp70-mediated disaggregation and reduces aggregation, toxicity and spreading of α-Syn A: Maximum intensity projections of fluorescent microscopy z-stacks of 5-day-old nematodes expressing the indicated transgenes are shown. White dashed lines outline the borders of muscle cells. B: Motility assay as a measure for transgene toxicity. Displayed is the mean number of body bends per 30 s. Statistical analysis was done using two-way ANOVA with Dunnett's multiple comparison test. C: Maximum intensity projections of fluorescent microscopy z-stacks of 5-day-old nematodes expressing the indicated transgenes. White dashed lines outline the borders of muscle cells. M: muscle, H: hypodermis. Signal outside of muscle cells reveals spreading of a-Syn. D: Quantification of animals showing a-Syn transmission at indicated ages. Statistical analysis was done using two-way ANOVA with Dunnett's multiple comparison test.

Fibrillar α-Syn is able to adopt distinct conformations, called polymorphs, that differ in their structure and thus amino acids exposed to the fiber surface, which impacts their interactome [5]. These distinct α-Syn polymorphs also display a unique fluorescence lifetime, which was exploited to monitor the dynamic process of amyloid remodeling by the cellular environment [6]. α-Syn polymorphs were differentially processed by cellular clearance pathways, including the Hsp70 disaggregation system, yielding fibrillar species with increased seeding capacity. These results again suggest that disaggregation of amyloid aggregates may accelerate their prion-like propagation rather than lead to their removal.

Hsp70 co-chaperones can also prevent aggregation of proteins. One such J-domain protein is DNAJB6, which can prevent the aggregation of amyloidogenic proteins, such as polyglutamine peptides [7]. Therefore, the role of DNAJB6 in altering disease progression in α-Syn transgenic rodents was explored. Strikingly, dopaminergic cell death caused by the overexpression of α-Syn was reduced by co-expression with DNAJB6 [8].

Significance and impact of the work on the field

The cellular chaperone network represents a double edge sword in neurodegeneration, while over expressing DNJAB6 could rescue neuronal cell death caused by α-Syn, the Hsp70 disaggregase generates seeding and spreading-competent species that could be linked to the exacerbation of disease progression. While the chaperone network is already an intricate network, the ability of amyloidogenic proteins to adopt conformationally distinct polymorphs adds another degree of complexity. A deeper understanding on the role the specific chaperone network plays in the disease progression is vital to understand targeted therapeutics approaches.

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[6] J. Tittelmeier, S. Druffel-Augustin, A. Alik, R. Melki, and C. Nussbaum-Krammer, "Dissecting aggregation and seeding dynamics of α-Syn polymorphs using the phasor approach to FLIM," *bioRxiv*, p. 2022.02.09.479740, Feb. 2022.

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