Capturing Aβ42 aggregation in the cell

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Novel imaging techniques with ever-increasing resolution are invaluable tools for the study of protein deposition, as they allow the self-assembly of proteins to be directly investigated in living cells. For the first time, the acceleration in Aβ42 aggregation induced by the Arctic mutation was monitored in cells, revealing a number of distinct morphologies that form sequentially. This approach will help discriminate the impacts of mutations on amyloid protein processing, Aβ aggregation propensity, and other mechanistic outcomes.

Approximately 50 million people worldwide live with some form of dementia; according to the Alzheimer's Association, two-thirds of this number suffer from Alzheimer's disease, a progressive neurodegenerative disorder affecting the association cortices and hippocampus of the brain and leading to an inexorable decline of cognitive abilities and memory. Genetic evidence and time courses of a variety of brain-imaging modalities and fluid-based biomarkers available today show that the abnormal formation, aggregation, and accumulation in the form of extracellular amyloid plaques of a short peptide (Aβ40 or Aβ42)[2] are early causative events of the disease, which has been termed the amyloid cascade hypothesis (1). Considerable knowledge has been obtained on the aggregation process of the Aβ peptide *in vitro*, including the detection of various forms of oligomeric intermediates and fibrillar forms of the peptide, as well as characterization of their morphology and structure, kinetics of formation and conversion into other species, and toxicity (2). However, limited information is available on the aggregation process *in vivo*, mainly due to our technical limitations in monitoring the various steps of the process and in the elucidation of its mechanism.

Recently, key innovations in light microscopy have bypassed the earlier limits of optical resolution and made optical microscopy one of the most powerful and flexible diagnostic tools in cell biology (3). New super-resolution technologies can monitor tailored illumination, nonlinear fluorophore responses, or the precise localization of single molecules and aggregates (4). Overall, these cutting-edge approaches have created new possibilities to investigate the complex structure and function of macromolecules in living cells. Using highly specific fluorescence labeling techniques such as immunocytochemistry, *in situ* hybridization, or fluorescent protein tags, the spatial distribution and dynamics of subcellular compartments, proteins, or genomic sequences of interest are currently being analyzed in chemically fixed or living samples to answer the most challenging experimental designs (4).

The paper by Lu *et al.* (5) provides a welcome demonstration of these advances in their study of the aggregation propensity and stability of the "Arctic" mutant (E22G or E693G) Aβ42 aggregates in engineered human embryonic kidney cells. The authors were able, in particular, to characterize the nature and dynamics of the Aβ42 peptide assemblies in living cells using fluorescence-lifetime imaging microscopy (FLIM) to monitor the conversion of soluble protein fragments into amyloid fibrils. The structural heterogeneity of the protein aggregates was also examined using 3D structural illumination microscopy (SIM), which reveals morphological and structural details of aggregated protein species by reconstructions from a series of 2D SIM sectioning images. The high-resolution images revealed distinct morphologies of intracellular protein aggregates at different stages of maturation, reflecting the assembly of the monomeric peptides into small oligomeric species, oligomers into fibrils, and further assembly of these species into fibril bundles, larger clusters, and aggresomes. The authors were additionally able to show that the aggregates were not degraded as quickly as WT aggregates and that this was likely due to more tightly bound fibrils rather than a consequence of the protein degradation machinery being overwhelmed, in line with the expected biophysical outcome of the E22G mutation. The main strength of this study was the live-imaging capability, such that protein aggregates at high resolution in a physiological environment could be observed. This paper paves the way for future studies targeting the effect of additional mutations, chaperones, and inhibitors of the aggregation process.

Another strength of the study is that, although the Arctic E22G mutation has previously been shown to promote self-assembly of Aβ42 (6), here the authors dissect the whole process of E22G Aβ42 aggregation into its various steps in the cells, revealing that both oligomer and fibril formation were faster for the mutant protein relative to the WT counterpart. This is an important point, as it is generally assumed that mutations involving the precursor protein of Aβ are pathogenic because they promote cleavage at either the β-secretase or γ-secretase sites, resulting in increased formation of Aβ or of Aβ42 relative to Aβ40. However, the Arctic mutation seems to be solidifying a new class of mutations rather than serving as a lone exception to the cleavage rule. A detailed survey of the literature indicates

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[1] To whom correspondence should be addressed. E-mail: fabrizio.chiti@unifi.it.
[2] The abbreviations used are: Aβ, amyloid-β; FLIM, fluorescence-lifetime imaging microscopy; SIM, structural illumination microscopy; APP, amyloid precursor protein.

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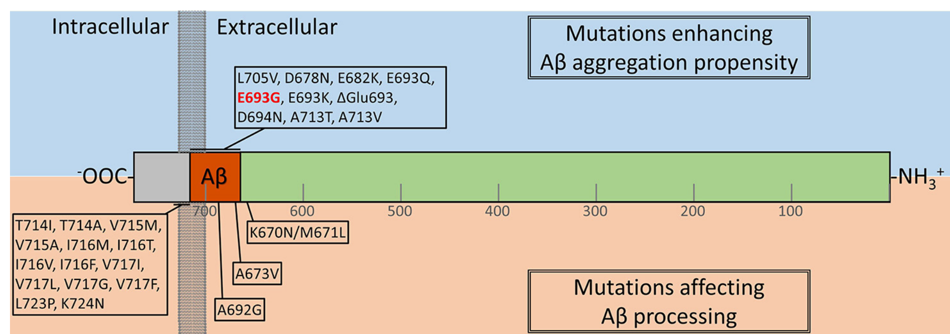

Figure 1. Sequence and mutations of APP. The extracellular N-terminal domain (residues 18–671), the Aβ42 sequence (residues 672–713), and the intracellular C-terminal domain (residues 714–770) are shown in *green*, *orange*, and *gray*, respectively. The transmembrane domain encompasses residues 700–723. Mutations that have been shown to induce an enhancement of the Aβ aggregation propensity are reported above. Mutations that alter the processing of the APP sequence are reported below. The Arctic mutation (E22G or E693G) is labeled in *red*.

that a considerable number of the mutations do not have such an effect on APP processing. In fact, in addition to the various mutations causing a duplication of the APP protein (7), 27 mutations have been found to be associated with the gene coding for the APP protein and to cause early onset Alzheimer's disease or related disorders (http://www.molgen.ua.ac.be/ADMutations/default.cfm?MT=0&ML=1&Page=AD and references therein).[3] Of these, 14 mutations are downstream of the cleavage sites of γ-secretases (Fig. 1) and increase the specificity of γ-secretase for the cleavage after residue 713, increasing the concentration ratio of the Aβ42 and Aβ40 forms (8). One double mutation (known as the Swedish mutation, K670N/M671L), immediately before the N terminus, and two mutations located inside the region of the APP sequence coding for Aβ42, the A673V mutation and the Flemish A692G mutation, render the APP protein a better substrate for β-secretases with consequent overproduction of both forms of Aβ (9, 10). The remaining 10 mutations involving the Aβ42 sequence, including the E693Q, E693G, E693K, ΔGlu693, D694N, and A713T mutations, were not found to increase the specificity of any secretase, resulting in unchanged or even decreased levels of Aβ (2). All of the 10 mutations are expected on theoretical grounds to increase Aβ aggregation rate, and for a few of them this hypothesis was also experimentally confirmed (2).

These considerations further enhance the importance of the study by Lu *et al.* (5) and urge a more systematic study of all the various APP mutations on Aβ aggregation *in vivo*. The technical advancements that allow the investigators to quantify the rate of formation of the various forms of deposits are undoubtedly of great value in this research field. It would also be valuable to test these new methods on primary neurons and extend the analysis to the formation of the initial oligomeric species in its different steps.

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