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Modeling the complex pathology of Alzheimer's disease in *Drosophila*

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ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia and the most common neurodegenerative disorder. AD is mostly a sporadic disorder and its main risk factor is age, but mutations in three genes that promote the accumulation of the amyloid-β (Aβ42) peptide revealed the critical role of amyloid precursor protein (APP) processing in AD. Neurofibrillary tangles enriched in tau are the other pathological hallmark of AD, but the lack of causative tau mutations still puzzles researchers. Here, we describe the contribution of a powerful invertebrate model, the fruit fly *Drosophila melanogaster*, to uncover the function and pathogenesis of human APP, Aβ42, and tau. APP and tau participate in many complex cellular processes, although their main function is microtubule stabilization and the to-and-fro transport of axonal vesicles. Additionally, expression of secreted Aβ42 induces prominent neuronal death in *Drosophila*, a critical feature of AD, making this model a popular choice for identifying intrinsic and extrinsic factors mediating Aβ42 neurotoxicity. Overall, *Drosophila* has made significant contributions to better understand the complex pathology of AD, although additional insight can be expected from combining multiple transgenes, performing genome-wide loss-of-function screens, and testing anti-tau therapies alone or in combination with Aβ42.

1. Introduction

1.1. Etiology and pathology of Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and the leading cause of dementia among the elderly. The main risk factor for AD is age, with one in nine people above 65 and one-third over 85 suffering AD (Thies et al., 2013). At least 5.2 million Americans had AD in 2013, most of them over 65 years old. Since the risk for AD is the same across racial groups and geographic location, aging populations in Western Europe and Japan currently face similar economic, social, and medical burdens. Moreover, the AD burden is expected to grow for many emerging economies in Asia and Latin America over the next decades, reaching the level of a global epidemic. At a pathological level, the AD brain is characterized by the shrinkage of several regions, starting with those implicated in memory and cognition — hippocampus and cortex, which explains the early symptoms of memory loss and confusion. In addition to cell loss, the AD brain accumulates two characteristic protein deposits: amyloid plaques enriched in the amyloid-β (Aβ42) peptide and neurofibrillary tangles (NFTs) enriched in hyperphosphorylated tau (Overk and Masliah, 2014). Both Aβ42 and hyperphosphorylated tau are prone to misfold and form soluble assemblies (oligomers and protofibers), which have shown to be highly toxic in some experimental settings (Benilova et al., 2012; Hardy, 2009). These oligomers continue to aggregate over time into highly organized, insoluble amyloid fibers that have a diagnostic value for AD. Despite the recent emphasis on understanding the role of oligomers in neurotoxicity, both soluble and insoluble Aβ42 and tau assemblies may contribute to disease in the complex environment of the human brain; thus, neutralizing the toxicity of different aggregates remains an unmet challenge in the field (Golde et al., 2013).

AD is a complex neurological disorder with sporadic etiology, except for a small fraction of cases with familial inheritance. Familial forms of AD are linked to mutations in three functionally related genes: the *amyloid precursor protein* (*APP*) and *presenilins* (*PS*) *1 and 2*, two key γ-secretase components (Karch et al., 2014). APP encodes a transmembrane protein that is cleaved by combinations of α-, β-, and γ-secretases, which results in the release of several proteolytic fragments, including Aβ40 and Aβ42 (Fig. 1). Mutations in APP, PS1, and PS2 favor the production of the Aβ42 peptide, thus supporting a central role for Aβ42 in AD. The presence of Aβ42 deposits in the brain of AD patients and the genetic links that favor the production of Aβ42 led to the *amyloid cascade hypothesis*, which posits that the accumulation of Aβ42 is the triggering event in AD (Hardy and Higgins, 1992). Although this hypothesis was recently revised to recognize the role of both Aβ42 and tau oligomers in synaptic toxicity and neuronal loss, its basic

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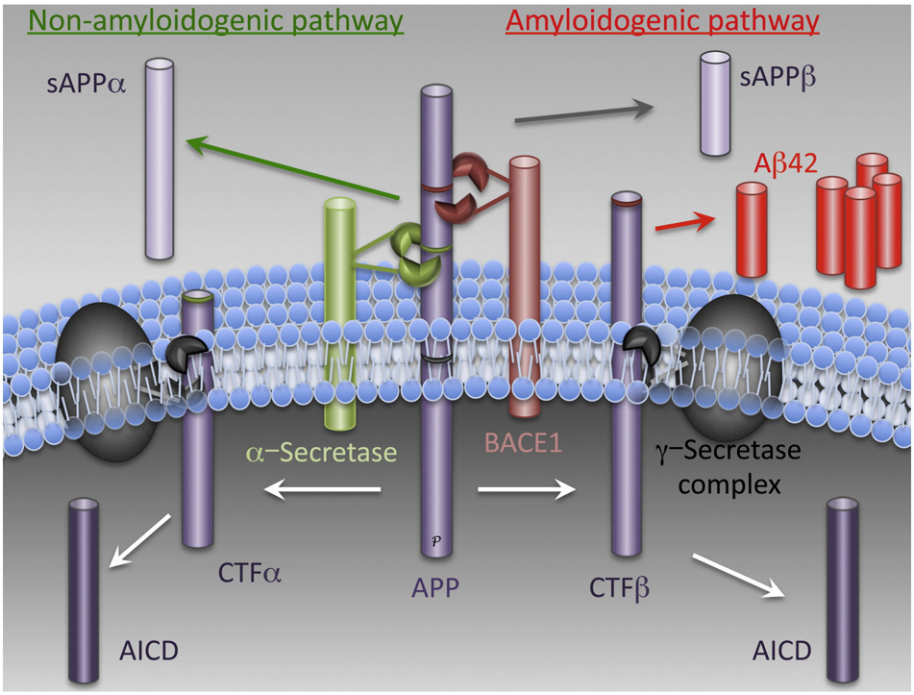

Fig. 1. APP processing. APP is a single-pass transmembrane protein (purple) that is cleaved by three types of proteases. Cleavage by of α-secretase (green) releases the secreted APPα (sAPPα) fragment. The C-terminal fragment α (CTFα) is then cleaved by the γ-secretase complex (black) to produce the amyloid intracellular domain (AICD). This is considered the non-amyloidogenic pathway because the activity of α-secretase breaks the Aβ fragment. Cleavage of APP by the β-secretase activity of BACE1 releases a smaller secreted fragment (sAPPβ) and CTFβ, which is a substrate for γ-secretase. This final cleavage releases AICD and Aβ fragments of different sizes, form 36 to 43 amino acids, with Aβ42 being the pathogenic trigger.

premise still drives AD research and the search for elusive therapies (Hardy, 2009). In our current understanding, tau acts downstream of Aβ42 and plays a key role in promoting neuronal toxicity and neuronal loss. The experimental evidence for this connection came from the study of mice expressing mutant APP, PS1, and tau, in which clearance of Aβ42 reduced tau aggregation, but increasing tau burden had no effect on Aβ42 pathology (Oddo et al., 2007). In addition, reduction of tau levels rescued the memory deficits induced by Aβ42, supporting the downstream role of tau in AD pathogenesis (Roberson et al., 2007). Recent work has identified the molecular mechanisms linking extracellular Aβ42 and intracellular tau (see below), although more work is still needed to define in more detail all the factors implicated in this key pathogenic process. Overall, despite tremendous advances in our understanding of AD pathogenesis, this knowledge has not yet led to the development of disease-modifying therapies. Thus, more research is needed to better understand APP processing and the cellular pathways disrupted by Aβ42 and tau.

1.2. The winged supermodel

You may be thinking about the Victoria's Secret angels. But the real winged supermodel is a small fruit fly (*Drosophila melanogaster*) with genetic superpowers. To the untrained eye, fruit flies share little anatomical similarity to humans. But the conservation at the cellular, genetic, and molecular levels is striking based on the common evolutionary origin of all animals. The two Nobel prizes awarded to *Drosophila* researchers in the last 20 years on early development and innate immunity underscore the universality of basic biological principles. Moreover, the tripartite organization of the *Drosophila* brain (proto-, deuto-, and tritocerebrum) is homologous to the forebrain, midbrain, and hindbrain of humans, supporting the ancient origin of the brain (Reichert, 2005). *Drosophila* has driven genetics and developmental studies for over 100 years due to its easy handling in the lab, compact genome distributed in four chromosomes, and short generation time of just ten days from fertilized embryo to adult fly. *Drosophila* was one of the first organisms to be fully sequenced, at last providing the blueprint for mapping thousands of existing mutations (Adams et al., 2000). Comparisons with the human genome revealed that 74% of human genes causative of diseases are conserved in *Drosophila* (Chien et al., 2002), indicating the relevance of studying gene function in flies. The innovative resources for generating mutant strains has helped elucidate many complex biological processes, including the development, differentiation, and function of the nervous system, as well as evolutionarily conserved behaviors such as sleep, learning and memory, foraging, and aggression (Bellen et al., 2010).

In addition, fruit flies are a hotbed for technology development and innovation, which now allow the most sophisticated animal manipulations (Kondo, 2014; Mohr et al., 2014; Venken et al., 2008). The main advantage of *Drosophila* is the ability to manipulate gene expression with extraordinary precision. Despite the slow penetration of gene knock-in technology, recent developments with the fast and flexible CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) system adds another powerful weapon to the genetic arsenal of the fruit fly (Bassett et al., 2013; Gratz et al., 2013; Yu et al., 2013). Since its introduction in 1993, massive resources based on the UAS/Gal4 system are now available, including genome-wide RNAi libraries and thousands of Gal4 strains to map single neurons and circuits (Brand and Perrimon, 1993; Jenett et al., 2012). Two new expression systems, Q and LexA, provide added flexibility for creative and sophisticated gene manipulation (Lai and Lee, 2006; Potter et al., 2010). Finally, *Drosophila* has introduced many genetic-based tools to study neuronal circuits and function, including optical tracers, calcium sensors, and engineered channels to regulate neuronal function like the Transient receptor potential A [TrpA1] and Channelrhodopsin. Overall, the wealth of genetic tools for manipulating gene activity and neuronal function make *Drosophila* the most versatile model organism for the discovery of conserved physiological and pathological conditions.