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Review

Alzheimer's Disease and tauopathy studies in flies and worms

Jill Wentzell, Doris Kretzschmar *

Center for Research on Occupational and Environmental Toxicology, Oregon Health and Science University, Portland, OR 97239, USA

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ABSTRACT

Progressive dementias like Alzheimer's Disease (AD) and other tauopathies are an increasing threat to human health worldwide. Although significant progress has been made in understanding the pathogenesis of these diseases using cell culture and mouse models, the complexity of these diseases has still prevented a comprehensive understanding of their underlying causes. As with other neurological diseases, invertebrate models have provided novel genetic approaches for investigating the molecular pathways that are affected in tauopathies, including AD. This review focuses on transgenic models that have been established in *Drosophila melanogaster* and *Caenorhabditis elegans* to investigate these diseases, and the insights that have been gained from these studies. Also included are a brief description of the endogenous versions of human "disease genes" (like tau and the Amyloid Precursor Protein) that are expressed in invertebrates, and an overview of results that have been obtained from animals lacking or overexpressing these genes. These diverse models can be used to advance our knowledge about how these proteins acquire a pathogenic function and how disrupting their normal functions may contribute to neurological pathologies. They also provide powerful assays for identifying molecular and genetic interactions that are important in developing or preventing the deleterious effects.

Contents

Introduction

Alzheimer's Disease (AD) is the most common form of dementia, accounting for 60–80% of all cases and it is currently the 6th leading cause of death (Alzheimer's Association; www.alz.org). Worldwide, approximately 26 million people suffer from this devastating disease, and with the increase in life span this number is predicted to quadruple over the next four decades (American Health Assistance Foundation; www.ahaf.org). Starting with slight memory loss and confusion, AD eventually leads to severe mental impairment, often accompanied by changes in personality. Although a number of drugs have been developed for treatment to slow the progression of cognitive decline, there is currently no cure for AD.

Histologically, AD is characterized by the formation of amyloid plaques and neurofibrillary tangles (NFTs). The latter primarily consist of tau, a microtubule-binding protein that, when hyperphosphorylated aggregates into insoluble fibrillar deposits in the form of NFTs (Mandelkow and Mandelkow, 1998). These pathological tau inclusions are also found in several other neurodegenerative diseases, including frontal temporal dementia with Parkinsonism linked to

* Corresponding author. CROET, OHSU, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA. Fax: +1 503 494 6831.
E-mail address: kretzsch@ohsu.edu (D. Kretzschmar).
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chromosome 17 (FTDP-17), corticobasal degeneration, progressive supra nuclear palsy, and Pick's disease, accordingly this group of diseases is referred to as tauopathies (Gendron and Petrucelli, 2009). In contrast, the main component of amyloid plaques is, as the name implies, Aβ amyloid, which are small peptides consisting of 40 or 42 amino acids (Selkoe, 2000). These peptides are cleaved from the larger Amyloid Precursor Protein (APP) by proteases called β-secretase and γ-secretase (De Strooper and Annaert, 2000; Turner et al., 2003). Whereas β-secretase activity is encoded by a single protein, called the β-site APP-cleaving enzyme or BACE, γ-secretase is a protein complex consisting of presenilin (Psn), nicastrin, aph.1, and pen2 (De Strooper, 2003). That the cleavage of APP and the production of Aβ is indeed a crucial factor in AD was shown by the identification of mutations in Psn-1, Psn-2, and APP that promote the production of Aβ and lead to early-onset familial AD (FAD) (Haass and De Strooper, 1999). Alternatively, APP can be cleaved by α- and γ-secretase, which does not result in the production of toxic peptides (De Strooper and Annaert, 2000).

Although studies on patients afflicted with FAD have contributed significantly to our understanding of the pathogenesis of AD, it has also become apparent that other genes contribute to the more prevalent sporadic forms of the disease. However, studies to identify such factors in humans have obvious limitations, due to methodological difficulties and ethical concerns. Therefore, animal models are an essential experimental system to identify and understand the function of candidate genes and delineate the genetic pathways in which they play a role. In addition, animal models can be used to systematically investigate the disease mechanisms from the earliest stages, whereas human studies must rely on postmortem tissue that typically represents late stages of the disease.

Invertebrates, and especially the well-established model organisms *Drosophila* and *Caenorhabditis elegans*, provide many experimental advantages for this type of analysis. Their anatomy, development, and behavior have been thoroughly studied and they are amenable to a variety of genetic and molecular methods, including the relatively easy generation of transgenic animals and the possibility of performing large-scale mutagenesis screens. Therefore, it is not surprising that they have also successfully been used in recent years to investigate the mechanisms of neurodegenerative diseases, including AD and other tauopathies (Bilen and Bonini, 2005; Wu and Luo, 2005; Lu and Vogel, 2009; Teschendorf and Link, 2009).

Modeling amyloid toxicity

As noted above, accumulation of Aβ into senile plaques is one of the hallmarks of AD (Glenner et al., 1984; Wong et al., 1985) and together with findings that mutations leading to increased Aβ production have been described in familial AD (Chartier-Harlin et al., 1991; Goate et al., 1991; Levy-Lahad et al., 1995; Sherrington et al., 1996) this provided the basis for the amyloid hypothesis, which proposed that Aβ accumulation is the driving factor for disease progression and pathology. For the vast majority of AD cases, however, the cause of amyloid plaque formation is still undefined, and the intrinsic pathogenicity of Aβ peptides remains controversial.

To investigate the toxic function of Aβ peptides in *Drosophila*, several transgenic fly models have been created that specifically express either Aβ40 or Aβ42 (Finelli et al., 2004; Iijima et al., 2004; Crowther et al., 2005). Both of these peptides are found in human plaques, but the Aβ42 peptide has been found to be the more toxic species (Findeis, 2007). Confirming the increased toxicity of this peptide, only expression of Aβ42 resulted in amyloid deposits and degeneration in the fly eye (Finelli et al., 2004) and brain (Iijima et al., 2004; Crowther et al., 2005). Surprisingly however, both peptides induced defects in an associative olfactory learning assay when expressed pan-neuronally (Iijima et al., 2004). Whereas the performance of young flies expressing either protein was not different from controls, their performance became increasingly worse when aged for 6–7 d, although there was no significant difference between flies expressing Aβ42 versus Aβ40. These experiments clearly showed that Aβ-peptides alone are sufficient to induce AD-like phenotypes. In addition, they suggested that learning defects do not require visible plaque formation, which is similar to results obtained by Crowther et al. in a climbing assay (Crowther et al., 2005). In this study the deficits were also observed before the occurrence of large extracellular deposits and instead correlated with the intracellular accumulation of Aβ (in this case Aβ42 and the arctic mutation which is found in patients with early-onset familial AD). More recently, Iijima et al. used the fly model to investigate the effects of different aggregation rates by expressing Aβ42 with the arctic mutation, which increases aggregation, or an artificial mutation shown to decrease aggregation (Iijima et al., 2008). Expressing the arctic mutation resulted in higher levels of Aβ42 oligomers compared to the normal Aβ42, while the artificial mutation reduced the formation of oligomers. Notably, these differences in aggregation tendency correlated with the deleterious effects on life span and locomotion. However, both mutations increased short-term memory deficits compared to flies expressing normal Aβ42, with the artificial mutation causing an even earlier onset than the arctic mutation. This further supported the earlier results by this group, which suggested that the aggregation propensity does not determine the severity of memory deficits. Interestingly, each form of Aβ42 had distinct defects on neuronal degeneration, with the arctic mutation causing mostly vacuoles in the cortex (where all neuronal cell bodies are located, similar to the gray matter in vertebrates). In contrast, the artificial mutation induced vacuolization in the neuropil, which is comprised of neuritis (equivalent to white matter). The specificity of these pathologies correlated with the localization of aggregates, because Aβ42arctic showed large deposits in the cell bodies, whereas the artificial mutation primarily resulted in deposits in neurites. This finding suggested that although aggregation levels can affect some phenotypes, differences in aggregation rates alone do not determine pathogenicity.

Similar results were obtained in *C. elegans*, where expression of Aβ42 in muscle cells induced the formation of amyloid containing inclusions (Link, 1995). As in flies, however expressing variants of Aβ42 with mutations that prevented deposit formation did not reduce toxicity (Fay et al., 1998), providing additional support for a model in which their pathogenicity is not solely due to the levels of aggregation. Although transgenic worm models expressing Aβ42 in neurons also exhibit amyloid aggregates, the resulting phenotypes are very subtle (Link, 2006; Wu et al., 2006). Although expression of Aβ peptides in these models does certainly not completely recapitulate the disease process, these studies have provided important insights about the toxicity of specific peptide species. However, a caveat of inducing only the expression of Aβ peptides is that features requiring the expression of the entire APP protein cannot be recapitulated nor can these models be used to investigate genetic factors or therapeutic drugs that affect processing.

For these purposes the fly lines that express full-length human APP_{695} (Fossgreen et al., 1998), the predominant form in the nervous system, might prove more useful. To ensure β-cleavage of APP in this model, a human BACE construct was co-expressed with APP_{695}, which together with endogenous fly γ-secretase produced toxic Aβ fragments (Greeve et al., 2004). Histological analysis revealed the formation of amyloid deposits and age-dependent degeneration in these flies, in addition to a decreased life span (Fig. 1). Surprisingly, the same phenotypes were induced after expression of APP_{695} alone, suggesting that flies possess an endogenous BACE-like enzyme. Indeed Western blots confirmed the production of an Aβ fragment in these flies, though it was slightly larger than the Aβ produced by co-expression of human BACE. Flies expressing full-length APP were also used to investigate the effects of altering the processing pattern of APP by either genetic or pharmacological means (Greeve et al., 2004).