CHAPTER

26

Lipids and Lipid Signaling in *Drosophila* Models of Neurodegenerative Diseases

Kyoung Sang Cho, Se Min Bang and Amanda Toh

INTRODUCTION

The fruit fly *Drosophila*, has been used for approximately 100 years in the laboratory setting, making it one of the oldest animal models to be utilized for scientific research. It is considered to be the most useful animal genetic model, owing to several advantages: its high fecundity (800 eggs per female), short life cycle (eggs grow to become fertile adults within 2 weeks), and low maintenance (small body size and cheap cost to maintain). Moreover, more than 10,000 genetic mutants of *Drosophila* have been developed, which can be employed to discover the function of such mutated genes. In 2000, the full *Drosophila* genome sequence was released and compared with the human genome. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster* has revealed that about 75% of the human disease genes have a *Drosophila* ortholog. On the basis of its genetic similarity to humans, the use of *Drosophila* has since been extended from the study of development to the modeling of human diseases.

One of the major objectives in the quest to find cures for human diseases is to understand the molecular and cellular mechanisms of the disease processes. Although frequently appropriate, cell culture systems and mouse models are time-consuming and expensive. In contrast, fruit flies can be generated quickly and are more cost-efficient. Additionally, *Drosophila* provides an impressive array of available genetic tools that facilitate the screening for interacting proteins, and allow foreign genes to be expressed in tissue-specific and temporally regulated patterns. Using the powerful genetic and cell biological tools available in *Drosophila*, much has been revealed about the pathophysiology of several neurodegenerative diseases. Furthermore, the *Drosophila* models can also be used to rapidly screen for dietary components, drugs, and regimens of administration. In this chapter, the use of several *Drosophila* models for studying neurodegenerative diseases is introduced, and studies that have been carried out using these models that show the function of lipids and dietary oils in neurodegenerative diseases are presented.

DROSOPHILA AS A MODEL SYSTEM OF NEURODEGENERATIVE DISEASES

Genetic Tools for Making Neurodegenerative Disease Models in *Drosophila*

P-Element-Mediated Mutagenesis

P-element-mediated mutagenesis is a powerful genetic method that allows for the creation of genetic mutants on a genome-wide scale. The P-element is a transposon that is found specifically in *Drosophila* (Figure 26.1A). Transposons are also known as 'jumping genes' owing to their ability to excise and insert themselves in various locations within the genome. The hallmark of a transposon lies in its 31-bp inverse terminal repeat ends—the enabling factor for transposition. Autonomous (complete) P-elements are 2.9 kb in size and contain 4 exons (Ryder and Russell, 2003). An important feature of these elements is the functional encoding of the transposase gene. Transposases are enzymes that identify the inverse terminal repeat sequences within the DNA and proceed to bind and excise the DNA transposons in between the terminals. Subsequently, the transposons can be re-inserted elsewhere through the identification of the same inverse terminal repeats, while the donor site in the DNA is then repaired. Insertions result in the generation of an 8-bp duplication at the target sites (5′ end and 3′ end).

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Experimentally, the P-element used is specially engineered, with the intron between the 3rd and 4th exons being spliced ($P\{\triangle 2{-}3\}$) so that the stop codon is removed and complete alternative splicing can occur, resulting in a somatically active functional mRNA from the P-element transcript. This functional P-element transcript does not occur naturally in the soma, as the stop codon in the intron between exon 2 and exon 3 prevents complete alternate splicing from happening. With this engineered P-element, transposition can occur in the somatic cells. In *Drosophila*, the non-autonomous (incomplete) transposon and transposase are kept within separate fly lines, which can then undergo crossing. The resultant F_1 generation will then have an autonomous transposon, allowing for transposition to occur in their germ cells. In the F_2 generation, a variety of the P-element insertion mutants can be obtained, in which the P-elements are inserted in various loci of their genome. On the basis of their insertion sites in the genome, the P-element can disrupt a specific gene (Figure 26.1B). For example, if the P-element is inserted in the regulatory regions or exons of a gene *X*, the expression of gene *X* will be affected. Several genome-wide P-element insertion projects have been carried out in different laboratories. Therefore, currently, a large majority of the *Drosophila* genes are associated with at least one P-element mutant. The information relating to the mutants exists in the gene data bank Flybase (http://flybase.org), and the insertion mutants of genes of interest can be purchased from the fly stock centers.

However, the insertion is often unable to disrupt gene expression completely; that is, they generate hypomorphic mutants. Therefore, to disrupt the gene expression completely (i.e., generate null mutants), further genetic crosses, termed imprecise excision, are needed. In this process, the fly with a P-element surrounding a gene *X* is again crossed with the fly with the transposase. Precise or imprecise excision of the P-element occurs, which can be reflected in the eyes of the *Drosophila* fly. Usually, this engineered P-element contains eye pigment genes such as *miniwhite*, and so the eyes of the flies in which transposition has occurred will be white, whereas the eye of the flies with the P-element will remain red. To determine the exact location of excision, a polymerase chain reaction is usually carried out. Imprecise excision occurs randomly in each transposition, and the genes involved are usually those that flank the target gene.

Using these techniques, the mutants of most of the neurodegenerative disease-associated genes have been generated and characterized in *Drosophila*.

UAS-GAL4-*Based Gene Expression System*

The *UAS-GAL4* system is a method of activating gene expression in *Drosophila* (Figure 26.2). The GAL4

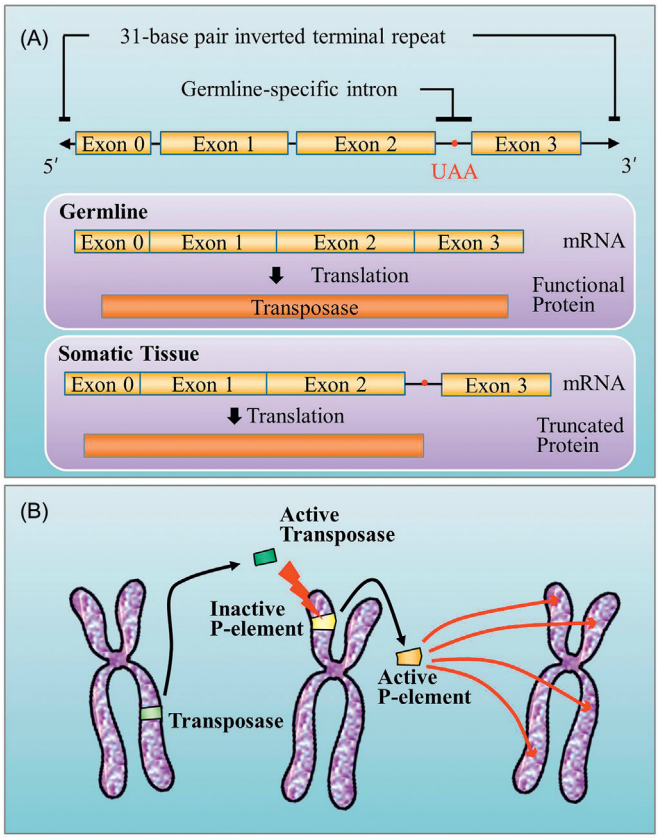

FIGURE 26.1 The structure of P-element and P-element-mediated mutagenesis. A. P-element structure and its tissue-specific alternative splicing. B. A cartoon showing P-element-mediated mutagenesis. *A, adapted with permission from Laski and Rubin 1989.*

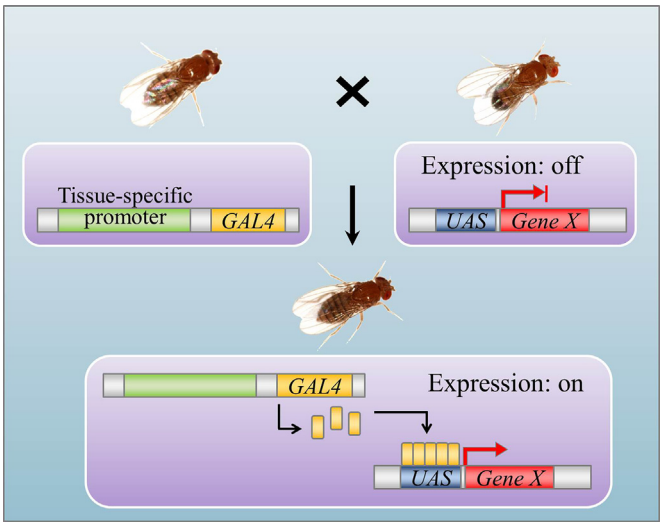

FIGURE 26.2 A schematic diagram of the *UAS-GAL4* system. Induction of the expression of the gene of interest, *X*, at the specific tissue of interest is enabled. *Adapted with permission from Brand and Perrimon 1993.*

protein, derived from yeast, serves as the transcriptional activator in this system. Its lack of endogenous targets within *Drosophila*, together with the ability to activate transcription within the fly, makes it a favorable tool. The *upstream activation sequence* (*UAS*) is an enhancer that is specific to the GAL4 protein. The *UAS-GAL4* system is an efficient bipartite approach in the activation of gene expression (Duffy, 2002). *UAS*, together with a specific gene of interest, is kept in one fly line, and *GAL4* with a tissue-specific promoter is kept in another. When flies of these two lines undergo crossing, the GAL4 protein will bind to the *UAS* and activate the gene at the tissue that the promoter is specific for. One of the advantages of this system is that toxic genes will only be expressed when bound to the GAL4 protein. This allows flies carrying the inactivated form of a toxic gene to survive normally. Another advantage of this system is that the effects of various genes can be studied through their overexpression or misexpression at various sites in the body using the array of tissue-specific promoters available.

Reporters for Amyloid Precursor Protein γ-secretase Activity in Drosophila

As the *Drosophila* genome contains genes for all of the γ-secretase complex components that show well conserved γ-secretase activity, it is considered to be a suitable model to study the regulation of amyloid precursor protein (APP) cleavage activity of γ-secretase. Several reporter systems were developed for measuring APP γ-secretase activity *in vivo* in *Drosophila*. Among these, a reporter system that can be applied as a powerful genetic screening for isolating the γ-secretase activity-regulating molecules is introduced here.

Using the transgenic system expressing human *APP-GAL4* under the eye-specific *glass multimer reporter* (*GMR*) promoter, Guo et al. (2003) generated a living reporter for APP γ-secretase activity in *Drosophila* (Figure 26.3). The transgene, known as *APP-GAL4*, encodes a fusion protein with a fragment of human APP, together with β- and γ-secretase cleavage sites as well as GAL4. The *APP-GAL4* is expressed in the developing eye under the control of the eye-specific *GMR* promoter. The genome of the reporter fly also contains *UAS-GRIM*, which results in cell death when induced by GAL4. If γ-secretase activity is absent, GAL4 remains tethered at the membrane, unable to activate the transcription of *GRIM*. However, if γ-secretase activity is present, the intracellular domains of APP and GAL4 enter the nucleus and induce *GRIM* expression, which results in cell death in the eye. As the eye phenotype of the reporter fly is easily visible and quantifiable, this method can be used for *in vivo* genetic screening.

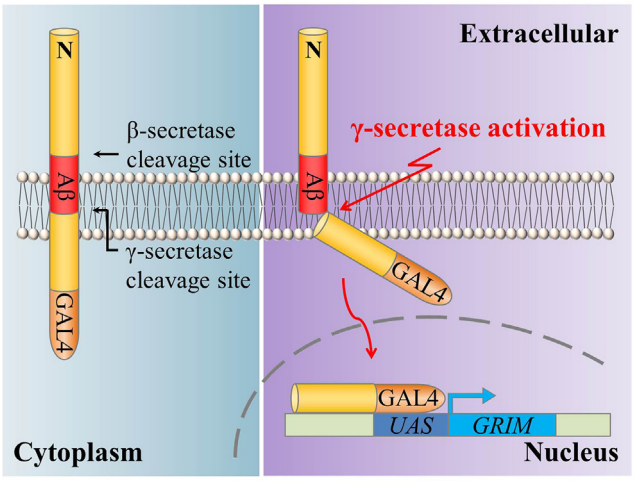

FIGURE 26.3 A fly reporter system for gamma-secretase activity. N, amino terminus of protein. *Adapted with permission from Guo et al. 2003.*

Representative *Drosophila* Models for Neurodegenerative Diseases

Using powerful genetic modification techniques, many human disease models have been generated in *Drosophila*. In particular, neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and polyglutamine diseases are considered suitable to be modeled with *Drosophila*, as its neuronal system is well conserved and extensive studies have been done. Indeed, a variety of neurodegenerative diseases have been modeled with *Drosophila*, and together with powerful genetic systems, *Drosophila* geneticists have successfully used these models to identify many novel disease-associated genes, shedding light on our understanding of the pathology of these diseases.

AD is the most common neurodegenerative disease, which causes a deficiency in memory and other cognitive functions. As AD-associated genes are largely conserved in its genome, *Drosophila* is considered to be a relevant model system for determining the molecular mechanisms underlying AD. The *Drosophila* genome contains genes that encode orthologs of β-APP (APPL) and Tau, which are involved in the most important pathogenic event of AD. Moreover, the genes of 4 major protein components of γ-secretase (presenilin, Nicastrin, APH-1, and PEN-2) are also well conserved. As anticipated, transgenic flies expressing human *Aβ42* or *Tau* using the *UAS-GAL4* system, resulted in AD-like neuronal degeneration and early death (Iijima et al., 2004; Wittmann et al., 2001). To investigate the molecular mechanisms responsible for the neurodegeneration, several genetic modifier screenings have been conducted in the *Drosophila* models of AD. As a result, it was found that several biochemical processes

such as secretion, cholesterol homeostasis, and regulation of chromatin structure and function were involved in mediating toxic Aβ42 effects (Cao et al., 2008). Moreover, kinases and phosphatases comprised the major class of modifiers of the tauopathy (Shulman and Feany, 2003). Interestingly, the modifiers of *Tau*-expressing models are largely distinct from those of polyglutamine toxicity, despite some clinical and pathological similarities among neurodegenerative disorders (Shulman and Feany, 2003). However, from the genetic screening study with *A*β*42*-expressing flies, a few candidate mutations of the genes involving vesicular trafficking, autophagy, and metal homeostasis have been identified to mediate common mechanisms of neurodegeneration by Tau, polyglutamine, and Aβ42 (Rival et al., 2009).

Toward the end of the 1990s, *Drosophila* models of polyglutamine diseases including spinocerebellar ataxia type 3 (SCA3/MJD) and Huntington's disease (Hashimoto et al., 2002) were developed (Jackson et al., 1998; Warrick et al., 1998). Targeted expression of a segment of the SCA3/MJD or human huntingtin protein with an expanded polyglutamine repeat induced neuronal degeneration, which suggested that cellular mechanisms of human polyglutamine disease are conserved in invertebrates. These models have been used to identify a variety of genetic modifiers of polyglutamine degeneration (Bilen and Bonini, 2007; Fernandez-Funez et al., 2000; Li et al., 2008), as well as some beneficial drugs, such as histone deacetylase inhibitors, the transglutaminase inhibitor cystamine, and mTOR inhibitor rapamycin (Karpuj et al., 2002; Lai et al., 2008; Ravikumar et al., 2004; Sang et al., 2005; Steffan et al., 2001).

In addition to AD and HD, many other neurodegenerative disease models have been generated in *Drosophila*. Among them, X-linked adrenoleukodystrophy (X-ALD) is closely associated with lipid metabolism. A genetic screening with P-element-mediated mutagenesis identified a very long-chain acyl coenzyme A (CoA) synthetase mutant as being a brain degeneration mutant that showed a similar phenotype to that of human patients with X-ALD (Min and Benzer, 1999). This model provided a good opportunity to test the effect of dietary oil on the neurodegeneration in the metazoan. Mutations of the *trp* gene, which encodes a transient receptor potential (TRP) channel, are also good examples of the neurodegeneration induced by 1 gene deficiency in *Drosophila*. Interestingly, TRP channels have been implicated in several neurodegenerative diseases, such as AD, PD, stroke, and hypoxia (Leonelli et al., 2011). TRP channel activities have been extensively associated with fatty acid function in both mammalian and *Drosophila* studies (Chyb et al., 1999; Leonelli et al., 2011).

EFFECTS OF LIPIDS AND LIPID SIGNALING ON *DROSOPHILA* MODELS OF NEURODEGENERATIVE DISEASES

Although *Drosophila* is one of the most studied animal models, surprisingly, only a small number of studies about the roles of lipids and lipid signaling in *Drosophila* models of neurodegenerative diseases have been performed to date. This might be due to the difficulty in the study of the lipid system in insects, as the biochemical knowledge of insect lipid metabolism is largely unknown. In addition, disparity in lipid metabolism between insects and mammals makes it challenging to study the effect of lipids in *Drosophila*. However, several recent studies that will be described in this chapter demonstrate the potential of *Drosophila* as a useful model for lipid biology in relation to neurodegenerative diseases.

Effects of PUFA and Cholesterol Levels on *Drosophila* AD Models Expressing Human Aβ42

The effects of hempseed meal (HSM) intake and linoleic acid on the human Aβ42-expressing *Drosophila* AD model were studied (Lee et al., 2011). Hempseed is a rich source of oil, composed of more than 80% polyunsaturated fatty acids (PUFAs). The fatty acids in hempseed oil include a variety of essential fatty acids, including linoleic acid (LA, 18:2n6) and α-linolenic acid (ALA, 18:3n3), as well as γ-linolenic acid (GLA, 18:3n6) (Callaway, 2004).

HSM shows a strong antioxidant effect, which was tested by rearing flies on either HSM or cornmeal-soybean standard media, together with H_2O_2, and then comparing their survival rates (Lee et al., 2011). Intriguingly, the survival rates of flies reared on HSM were much higher, indicating that HSM exerts a protective effect from the toxicity of H_2O_2, which suggests that HSM has antioxidant properties. LA, a major non-polar component of hempseed, also showed a protective effect against the toxicity of H_2O_2 when supplemented in standard medium containing H_2O_2. However, because the degree of increase in the survival rate of LA-fed flies was lower than that observed in the flies fed on HSM, the antioxidant activity of HSM is probably not caused solely by LA, but rather, a result of the complex effects of various HSM components, such as other PUFAs and phytosterols.

HSM intake also showed a protective effect against the cytotoxicity of Aβ42. When Aβ42 was ectopically expressed in the fly eye, it induced profound eye degeneration. The defective eyes could be divided into 2 groups (mild and severe) according to their size

(Lee et al., 2011). Eighty percent of the flies reared in standard medium showed severe phenotypes. However, feeding with HSM reduced the rate of occurrence of the severe defects to 50%, indicating that HSM intake suppressed Aβ42 cytotoxicity. In an effort to find the molecular components of hempseed that mediate the protective effect against eye degeneration, four major components of HSM – LA, ALA, GLA, and campesterol – were tested in the fly AD model. Interestingly, LA and ALA, but not GLA or campesterol, ameliorated the eye degeneration phenotypes in a dose-dependent manner.

At the moment, the molecular mechanism by which HSM, LA, and ALA exert their protective effects have yet to be clarified. As oxidative stress is an important mediator of Aβ42 toxicity, the antioxidant property of the HSM and fatty acids could be the main factor for the protective effects. Indeed, some studies have shown that the supplementation with PUFAs decreased oxidant parameters such as lipid peroxide and reactive oxygen species levels in mammalian animal models including the rat AD model (Hashimoto et al., 2002; Sarsilmaz et al., 2003). However, other studies reported that PUFA treatment does not prevent amyloid-β-mediated oxidative stress (Florent et al., 2006; Florent-Béchard et al., 2009). Consistently, HSM feeding does not affect the disease-like phenotypes of the *Drosophila* model of PD and HD, two diseases that have been extensively associated with oxidative stress. Therefore, it is unlikely that the suppression of oxidative stress is a plausible way to explain the protective effect of HSM against Aβ42 cytotoxicity. Alternatively, HSM and PUFAs could exert their protective activity by regulating cholesterol level (Figure 26.4). Although its role in AD pathology is still not fully understood, cholesterol has been implicated in AD at various aspects of pathology. Aβ42 is produced from APP through the amyloidogenic pathway, which occurs in the cholesterol-enriched lipid rafts, while APP is alternatively cleaved by α-secretase in the non-raft region (Florent-Béchard et al., 2009). Accordingly, cholesterol depletion has been found to suppress Aβ42 production in hippocampal neurons (Simons et al., 1998). In *Drosophila*, a screening of genetic modifiers of Aβ42 cytotoxicity identified *loechrig* mutants, in which the cholesterol homeostasis-associated protein AMP kinase γ is deficient (Cao et al., 2008). HSM and LA intake reduced the cholesterol uptake level in *Drosophila*. When flies were reared in high-cholesterol food with HSM or LA, the body cholesterol level was reduced to a nearly normal level; on the contrary, the body cholesterol level of control flies reared on high-cholesterol food without HSM and LA was greatly increased (Lee et al., 2011). Consistently, LA intake affects *Drosophila* development by decreasing the cholesterol level

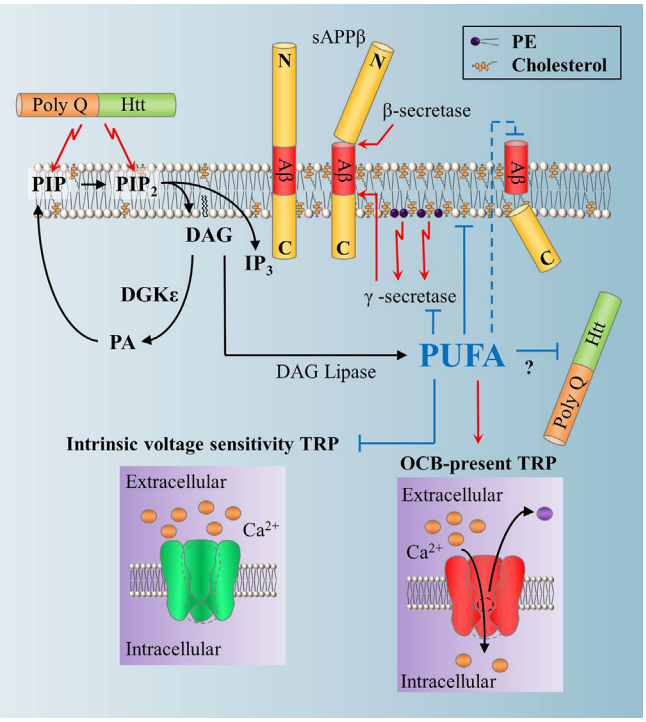

FIGURE 26.4 The molecular mechanisms of the lipid functions in *Drosophila* models of neurodegenerative diseases. C, carboxyl terminus; DAG, diacylglycerol; DGK ε, diacylglycerol kinase ε; Htt, huntingtin; IP_3, inositol trisphosphate; N, amino terminus; OCB, open channel block; PA, phosphatidic acid; PE, phosphatidylethanolamine; PIP, phosphatidylinositol monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; Poly Q, polyglutamine; PUFA, polyunsaturated fatty acid; sAPPβ, soluble beta amyloid precursor protein.

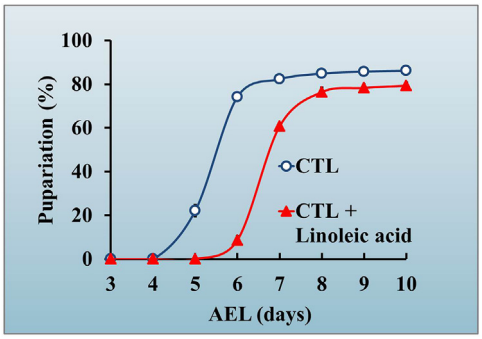

FIGURE 26.5 The effect of linoleic acid on *Drosophila* development. *Adapted with permission from Lee et al. 2011.*

(Figure 26.5), which is crucial for metamorphosis, as cholesterol is a precursor of the molting hormone ecdysone. *Drosophila* larval growth is delayed by intake of a PUFA mixture or LA, and is enhanced by cholesterol. Interestingly, the delayed larval growth induced by LA feeding is almost completely rescued by an intake of cholesterol. These results suggest that linoleic

acid can act antagonistically with cholesterol during *Drosophila* development. Therefore, PUFAs may exert their beneficial effects on *Drosophila* AD models by reducing the brain's cholesterol level, which causes the deleterious effects of AD at high levels.

Effect of Phosphatidylethanolamine Depletion on γ-secretase-Mediated APP Processing in Transgenic *Drosophila*

Recently, the role of membrane lipids on the generation of Aβ was studied in mammalian cells and *Drosophila* (Nesic et al., 2012). Brain phospholipid (PL) metabolism has been implicated in the pathogenesis of AD. The level of PLs was significantly reduced in some gray areas of patients with AD (Svennerholm and Gottfries, 1994). In particular, among the PLs, phosphatidylethanolamine (PE) was shown to be reduced in the plasma membranes of the synaptosome, glial, and neuronal cell bodies (Wells et al., 1995). APP-cleaving machinery such as β- and γ-secretases are present in detergent-resistant microdomains (DRMs), in which the level of cholesterol is important, despite that APP and α-secretase are localized in detergent-soluble (non-DRM) microdomains, which are PL-enriched membrane domains. Therefore, the lipidic components of the membrane microdomains, such as cholesterol and PL, are likely to be important in the pathology of AD, by influencing the production of Aβ.

Interestingly, PE, the major membrane PL in both *Drosophila* (Jones et al., 1992) and mammalian cells, influences Aβ production (Figure 26.4). Using the fly reporter system for γ-secretase activity (Figure 26.3), Nesic et al. (2012) showed that a depletion of PE by the deficiency of *easPC80*, a *Drosophila* ethanolamine kinase (Etkn), decreased γ-secretase−mediated APP processing. The rough eye phenotype and reduced eye size in the *GMR > C99-GAL4/UAS-GRIM* reporter line were ameliorated by the *EasPC80* homozygous mutant, suggesting that the PE level is important for γ-secretase activity in *Drosophila*. This is coincident with the data in mammalian cells, where the knockdown of *Etkn* by siRNA reduced the Aβ level, by both promoting the cleavage of α-secretase and decreasing the γ-secretase activity to process APP. It was suggested that the perturbed membrane PL content reduced the γ-secretase activity in DRM by inhibiting the assembling of its complex or the accessibility to its substrate APP in non-DRM. However, the altered PL content could increase the accessibility of α-secretase to APP. These results highlight the importance of the lipidic environment of the membrane in the pathology of AD.

Effects of Lipid Signaling Enzyme Diacylglycerol Kinase ε Inhibition on Mutant Huntingtin Toxicity

Membrane lipids are also associated with huntingtin (Htt)-induced toxicity in *Drosophila* and mammalian cell models of HD. In *Drosophila*, HD models are well established and have been used for screening the genetic components that could be associated with HD (Jackson et al., 1998). When the N-terminal of Htt with a 128Q expansion was ectopically expressed in the fly nervous system using the *elav-GAL4* driver, the flies showed a locomotive defect compared with the normal control. On the other hand, decreasing the diacylglycerol kinase ε (DGKε) level by shRNA reduced the toxic effects of the mutant Htt, which implicates DGKε-associated lipid signaling in the pathology of HD (Zhang et al., 2012) (Figure 26.4). DGK catalyzes the phosphorylation of diacylglycerol (DAG) to produce phosphatidic acid (PA), and is an important regulator of lipid metabolism. DGK-deficient mice showed a decreased polyphosphoinositide level, and treatment of mutant Htt-expressing cells with a DGK inhibitor reduces the level of phosphatidylinositol monophosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) (Zhang et al., 2012). Therefore, altered lipid metabolism by DGK inhibition is supposed to be a critical mechanism for the rescue of HD cytotoxicity.

The huntingtin peptide is associated with the membrane through its interactions with PLs. The region of huntingtin interaction with the membrane was predicted by structural analyses to be at the N-terminus (Kegel et al., 2009). Moreover, over-expressed and endogenous human huntingtin proteins in cells are associated with PLs. In particular, endogenous wild-type huntingtin from the mouse brain associates more with multivalent phosphoinositides such as PI(3,4)P2, PI(3,5)P2, PI(4,5)P2, and PI(3,4,5)P3, as compared to monovalent PLs or to PE and phosphatidylcholine (PC) (Kegel et al., 2009). Interestingly, an altered interaction of mutant huntingtin with phosphatidylinositols was revealed. That is, the interaction with PE and monovalent PLs such as PI(3)P and PI(4)P is greatly increased in the mutant huntingtin from the brain of mice with HD ($Hdh^{140Q/140Q}$ mouse). This suggests that the alteration of lipid metabolism could be a therapeutic treatment for HD, which is currently an incurable disease.

Drosophila Mutant of Very Long-Chain Acyl Coenzyme a Synthetase and Glyceryl Trioleate Oil

X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease that causes a rapidly progressive

inflammatory demyelination within the brain, or non-inflammatory distal axonopathy in the spinal cord (Kemp et al., 2012). In patients with X-ALD, various tissues, including adrenal and cerebral white matter and the testis, have lipid inclusions that contain cholesterol, phospholipids, and gangliosides esterified with saturated very long-chain fatty acids (VLCFAs), suggesting that the aberrant metabolism of VLCFAs is the key factor in the pathogenesis of X-ALD (Kemp et al., 2012). The *ABCD1* gene has been identified as a causative gene of X-ALD and encodes adrenoleukodystrophy protein (ALDP), which transports VLCFA acyl-CoA into peroxisomes, after which beta-oxidation of VLCFAs occurs. Therefore, the lack of ALDP results in the abnormal accumulation of VLCFAs, mainly hexacosanoic acid (26:0), due to a defect in VLCFA peroxisomal beta-oxidation.

Presently, the only available therapeutic treatment is Lorenzo's oil, which is a dietary intake of a 4:1 mixture of glyceryl trioleate oil (GTO) and trierucin. Treatment with this mixture decreases plasma VLCFAs to nearly normal levels, probably through competitive inhibition of the elongase that forms VLCFAs. However, the clinical efficacy of this oil and the clinical indications for its use are still controversial.

In the genetic screening for *Drosophila* brain degeneration mutants, *bubblegum*, a *Drosophila* VLCFA acyl CoA synthetase (VLCS) was isolated (Min and Benzer, 1999). The optic lobe of *bubblegum* mutant flies showed a bubbly appearance by degeneration on light microscope sections, and electron micrographs revealed that the mutant photoreceptor axons had become greatly expanded in the diameter. Because the β-oxidation of VLCFAs is catalyzed in the peroxisomes after activation to thioester derivatives by VLCS, the first enzyme in the beta-oxidation pathway, a lack of this enzyme may result in the accumulation of VLCFAs, as observed in the cells of patients with X-ALD. Indeed, mutant male flies showed increased levels of VLCFAs, and dietary treatment with GTO restored the accumulation of VLCFAs to a normal level. Moreover, the degeneration of the optic lobes and the phototactic behavior of the *bubblegum* homozygous mutant were rescued by feeding with GTO. Although *VLCS*-deficient flies showed X-ALD-like phenotypes, *VLCS* knock-out mice failed to mimic the X-ALD phenotype. Therefore, the implication of VLCS in X-ALD is not clear. However, the study with the *bubblegum* mutant provides a good example of how the efficacy of dietary lipids on neurodegenerative diseases can be tested using the *Drosophila* model.

Lipids, TRP Channels, and Neurodegeneration in *Drosophila*

A number of human diseases including neurodegenerative diseases have been associated with the dysfunction of transient receptor potential (TRP) channels, most of which are non-selective Ca^{2+}-permeable cation channels. Lipids possibly affect neurodegeneration by modulating TRP channels. There are 13 family members of TRP in *Drosophila*, which are involved in various biological pathways including light sensing, pain transduction, and temperature preference. Among them, two members of the TRP protein family, proteins encoded by the *trp* gene and the closely related *trpl* gene, appear to account for all light-activated channel activities in *Drosophila* photoreceptors. TRP channels are localized within microvilli of the *Drosophila* eye, forming a rhabdomere. The canonical G protein-coupled signaling cascade, involving rhodopsin, Gαq protein and phospholipase C, is responsible for the activation of the light-sensitive TRP channels.

Both *trp* gain-of-function or loss-of-function mutants undergo retinal degeneration, which is closely related with Ca^{2+} homeostasis. One of the *trp* mutants, Trp^{P365} showed an increase of spontaneous Ca^{2+} entry into the eye, which resulted in a constitutive current development. Interestingly, the Trp^{P365} mutant showed extremely rapid and massive photoreceptor degeneration (Yoon et al., 2000). In Trp^{P365} heterozygotes at 2 days after eclosion, raised in light-dark cycles, the photoreceptors began to show evidence of degeneration. From the genetic complementation tests that examined the electroretinogram phenotypes of Trp^{P365} heterozygotes and several *trp* alleles, it was suggested that Trp^{P365} was an allele of the *trp* gene. This was confirmed by rescuing experiments in which the wild-type *trp* gene was reintroduced to the Trp^{P365} background. As TRP channels of Trp^{P365} are constitutive active, ensuing accumulation of Ca^{2+} would lead to cell death.

Interestingly, loss-of-function mutants of *trp* also showed retinal degeneration. As the cell death in these mutants is suppressed by a loss-of-function mutation of Na^{+}/Ca^{2+} exchanger, the retinal degeneration in the *trp* mutants is due to reduced light-dependent Ca^{2+} influx caused by disruption of TRP channel activity (Wang et al., 2005). $PI(4,5)P_2$ depletion followed by delocalization of phosphorylated Moesin, a PIP_2-regulated membrane-cytoskeleton linker, was suggested to be an underlying mechanism of the retinal degeneration in *Drosophila trp* loss-of-function mutants. Consistently, a deficiency in phospholipase C, which is responsible for the hydrolysis of PIP_2, completely rescued the *trp* degeneration under red light. Moreover, the depletion of PIP_2 by PIP_2 phosphatase was sufficient to induce retinal degeneration. As TRP channels are implicated in various biological processes in the nerve system, disruption of PIP_2 homeostasis could be associated with the neurodegenerative diseases caused by TRP channel dysfunction.

The TRP channel has been known to be regulated by PUFAs in *Drosophila* (Figure 26.4). PUFAs such as arachidonic acid and ALA have been shown to activate *Drosophila* light-sensitive channels, TRP and TRPL, in whole-cell recordings from a photoreceptor (Chyb et al., 1999). Moreover, recombinant TRPL channels were also activated by ALA. The activation of the TRP and TRPL channels may occur by the direct binding of PUFAs with the channels, rather than through the metabolites of PUFAs or through the increasing membrane fluidity by PUFAs. Indeed, TRP channels were also activated by monounsaturated fatty acids, which are not substrates for the major PUFA-metabolizing enzymes, or trans-isomers of ALA. Moreover, inhibitors of enzymes that metabolize PUFAs, such as lipoxygenase inhibitors nordihydroguaiaretic acid and cinnamyl-3,4-dihydroxy-α-cyanocinnamate, suggest that an increase in endogenous PUFAs results in excitation of the photoreceptors through TRP channel activation (Chyb et al., 1999). Consistently, DAG lipase, which generates PUFAs from DAG, and DAG kinase, have been shown to be involved in TRP channel regulation (Leung et al., 2008; Raghu et al., 2000). Recently, a molecular mechanism suggesting how PUFAs activate TRP has been proposed. PUFAs such as LA activate TRP channels by alleviating open channel block (OCB), which is a process that blocks the flow of ions through the channel by the binding of ions to the inside of a channel pore (Parnas et al., 2009a). *Drosophila* TRP and TRPL channels require OCB removal in order to be activated (Parnas et al., 2009a). On the contrary, LA inhibits TRP channels with intrinsic voltage sensitivity (Parnas et al., 2009b). This type of channel does not show OCB and includes the heat-activated TRPV1 and the cold temperature-activated TRPM8 of mammals. As the proper level of TRP channel activity is very important in maintaining neuronal health, PUFAs could influence the maintenance of nervous system by modulating the activity of TRP channels.

POINTS TO CONSIDER WHEN *DROSOPHILA* MODELS ARE USED FOR STUDYING THE ROLE OF LIPIDS

Many studies have shown that supplementation of PUFAs are beneficial in patients with neurodegenerative diseases, as well as in animal models of the diseases (Das, 2006). In particular, the majority of the studies have been focused on the effects of long-chain fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), since they are major PUFAs found in the human brain. However, unlike the human brain, the *Drosophila* brain lacks C20 and C22 long-chain fatty acids. Moreover, genes encoding for Δ-6/Δ-5 desaturases, the key enzymes for the synthesis of C20/C22 PUFAs, do not exist in *Drosophila* (Shen et al., 2010). Furthermore, GLA is also absent in the head of *Drosophila*, including the brain and compound eyes (Yoshioka et al., 1985). This suggests that the lipid metabolism of insects is different from that of the mammalian system. Therefore, *Drosophila* should be cautiously employed for the study of lipid metabolism and related diseases.

Another point that should be considered when studying lipid functions in the *Drosophila* model is the role of cholesterol in *Drosophila* development. As *Drosophila* cannot synthesize cholesterol in its body, it relies completely on a dietary intake of whole cholesterol for maintaining its proper development, especially since cholesterol is also an essential component of the molting hormone ecdysone. It is highly probable that most PUFAs, such as ALA and LA, delay fly development through the suppression of cholesterol uptake (Lee et al., 2010) (Figure 26.5). Supporting this hypothesis, a previous study showed that LA significantly reduced the cholesterol uptake in flies fed on both LA and cholesterol (Lee et al., 2011). Based on the crucial role of cholesterol in insect development, the effect of PUFAs on the developmental phenotype of a disease model should be carefully interpreted.

PERSPECTIVE

An excellent genetic model system, *Drosophila* has been used extensively in studies on most of the biological processes, including the pathology of human diseases. Together with the availability of various powerful tools in both genetics and molecular biology, the fly system may be a useful alternative model for the investigation of the role of lipids in the pathology of neurodegenerative diseases. Yet surprisingly, only a limited number of studies in this field have been carried out using the *Drosophila* model. This may be due to the difficulty in studying the lipid biochemistry in *Drosophila*. In addition, the contrast in lipid metabolism between insects and mammals also makes it more complicated to study the effects of lipids in *Drosophila*. Despite these obstacles, a growing body of evidence supports the notion that a large portion of lipids and lipid signaling are well conserved and play a crucial role in the neuronal health of *Drosophila*. Furthermore, as *Drosophila* contains many lipid metabolism-associated genes in its genome, application of the powerful genetic tools together with *Drosophila* mutant models of the lipid metabolism-associated genes may provide invaluable insights into the roles of lipids and lipid signaling in the maintenance of neuronal health.

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