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Probing Mechanisms That Underlie Human Neurodegenerative Diseases in *Drosophila*

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Abstract

The fruit fly, *Drosophila melanogaster*, is an excellent organism for the study of the genetic and molecular basis of metazoan development. *Drosophila* provides numerous tools and reagents to unravel the molecular and cellular functions of genes that cause human disease, and the past decade has witnessed a significant expansion of the study of neurodegenerative disease mechanisms in flies. Here we review the interplay between oxidative stress and neuronal toxicity. We cover some of the studies that show how proteasome degradation of protein aggregates, autophagy, mitophagy, and lysosomal function affect the quality control mechanisms required for neuronal survival. We discuss how forward genetic screens in flies have led to the isolation of a few loci that cause neurodegeneration, paving the way for large-scale systematic screens to identify such loci in flies as well as promoting gene discovery in humans.

Keywords

oxidative stress; autophagy; protein degradation; Alzheimer’s disease; Parkinson’s disease; amyotrophic lateral sclerosis

INTRODUCTION

Neurodegenerative diseases (NDs) comprise a large, diverse group that characteristically involves a progressive worsening of neuronal symptoms, including loss of sensation, motor control, and memory as well as cognitive impairment. Some of these diseases, such as Alzheimer’s and Parkinson’s disease (AD and PD, respectively), are common. Current data

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from the longitudinal Framingham study indicate that dementia, which includes AD, has a lifetime risk of 1 in 7 men and 1 in 5 women, whereas PD has a lifetime risk of 1 in 50 men and 1 in 75 women (162). With an aging world population, these diseases are becoming more prevalent, and we currently have little treatment recourse. Besides the affected patients, these diseases also place a tremendous burden on family members, society, and the health-care system. Hence, many scientists are trying to answer basic questions related to these diseases: What are the primary causes of these diseases? What are the molecular mechanisms that trigger their onset? Can we delay or cure these diseases?

To tackle these questions, a thorough understanding of the molecular and cellular mechanisms that are at the basis of the primary pathology can provide key insights that can be exploited later to cure or delay these diseases. We must therefore resort to manipulation of multicellular model organisms that possess a nervous system and that allow sophisticated genetics: These typically include worms (*Caenorhabditis elegans*) (47, 205), flies (*Drosophila melanogaster*) (189, 190), and mice (*Mus musculus*) (66, 100). Although the molecular nature of the neurodegenerative pathways is often unknown in patients, some NDs are familial in nature and are caused by mutations in specific genes. These model organisms are quite suitable for studying the functions of these genes in vivo to provide us with a better understanding of the molecular pathology. For example, numerous causative mutations have been identified for NDs, including peripheral neuropathies such as Charcot-Marie-Tooth disease, many spinocerebellar ataxias (SCA), as well as Leigh syndrome (a mitochondrial disease) (161). For AD, PD, and amyotrophic lateral sclerosis (ALS), 90% of the patients have no known genetic lesions (94), whereas approximately 10% carry mutations in known genes (161). Finally, for some diseases, such as multiple sclerosis, gene association has been limited (26). As the vast majority of the genes implicated in NDs are evolutionarily conserved in higher eukaryotes, studies in model organisms provide us with a tool set to tackle basic biological questions related to the function and pathways of these genes.

Although model organism studies have provided us with a better understanding of the genes and molecular events involved, they do not exactly mimic the phenotypes and consequences of human NDs associated with mutations in specific genes. Hence, we should steer away from certain phrases like “a fly model for PD.” Instead, we should focus on how “the molecular and cellular mechanisms by which mutations in PD-causing genes affect model organisms.” Indeed, genetic model organisms often display few, if any, symptoms found in human patients for PD. Yet, notwithstanding this caveat, model organisms have provided very valuable insights into the function of PD-associated genes (61).

In flies, two fundamentally different strategies can be used to study the homologs of human genes that cause NDs. The first is based on a reverse genetic strategy, sometimes called the directed-expression approach because it heavily relies on expression of human proteins in transgenic flies (21). In this approach, a human wild-type or mutant protein is overexpressed in a fly tissue, usually the eye, and the corresponding phenotypes are assessed. These phenotypes form a starting-point for further studies. However, to understand the normal endogenous functions of the gene, one begins with the generation of null mutations in the fly ortholog and assesses the associated phenotypes (188). This is followed by rescuing this null mutation with a transgene containing a wild-type copy of the locus or ubiquitous/tissue-specific expression of the corresponding fly complementary DNA (cDNA). This rescue should then be further complemented by expression of the corresponding human cDNA in the fly null mutant to demonstrate that both human and fly homologs affect the same biological processes and have similar functions. These data then provide a backdrop to study specific human mutations (e.g., point mutations) that can then be introduced into the genomic or fly/human cDNA rescue constructs to obtain a detailed phenotypic comparison

of the loss and gain-of-function phenotypes associated with the fly gene. These strategies, combined with numerous other experimental paradigms, permit determination of the basic function of the gene, help determine the function of the human gene, assess the nature of the human mutations, and allow for the development of strategies to suppress mutant phenotypes. The second approach is more typical of fly research but has not been used much in the study of NDs specifically.

Another approach is the use of unbiased forward genetic screens designed to isolate mutations that result in neurodegenerative phenotypes. This is then followed by mapping and gene identification. Following gene identification, studies can be initiated to define the function of the in vivo gene/protein. Interestingly, although this approach has been the mainstay of fly biology research, it has not yet been systematically carried out to isolate loci that cause neurodegeneration (see Forward Genetics section below), probably because a direct screen for neurodegenerative phenotypes was tedious to run. However, these strategies should permit the identification of novel human ND-causing genes and provide valuable insights into the molecular and pathogenic mechanisms of numerous existing diseases that have not yet been modeled in flies. In this review, we focus mainly on recent progress achieved through genetics research in fruit flies, specifically on those areas where, in our opinion, fly research has been the most probing.

MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN NEURODEGENERATIVE PROCESSES

Elevated levels of oxidative stress have been increasingly linked to aging and neurodegenerative processes. The free radical/oxidative stress hypothesis of aging states that the accumulation of molecular oxidative damage induced by reactive oxygen species (ROS) is the principal factor in age-associated loss of physiological function (64, 171). Oxidative damage is now implicated not only in aging, but also in numerous NDs (95). ROS is primarily produced by the mitochondria's respiratory complexes I and III. Hence, mitochondrial defects often result in excess ROS that ultimately leads to cell death. Importantly, elevated ROS/oxidative stress has been documented in AD, Huntington's disease (HD), and PD (80, 160).

Parkinson's Disease

Some of the most investigated neurodegeneration- causing disease genes in *Drosophila* are implicated in PD. PD patients display progressively increasing levels of muscle rigidity combined with repetitive, uncontrollable tremors, a progressive loss of facial expression, and impaired balance. As the disease progresses, dementia is not uncommon. Pathologically, the dopaminergic (DA) substantia nigra becomes severely atrophied, and this is believed to contribute to patients' motor difficulties (104). In most, but not all, patients, an abundance of Lewy bodies composed primarily of α-synuclein is also evident. Although most cases of PD are believed to be sporadic in nature, an increasing number of genes have been implicated in familial cases. These include Parkin (83), Pink1 (184), DJ-1 (20), leucine-rich repeat kinase 2 (LRRK2) (211), and α-synuclein (139).

The role of mitochondrial and oxidative stress in PD is one of the primary models for disease onset and progression. Indeed, exposure to the chemical MPTP, a compound that inhibits mitochondrial Complex I, results in parkinsonism (86). Similarly, other Complex I–inhibiting agents including the insecticide rotenone and the herbicide paraquat also induce PD-like phenotypes in animal models (120).

Reverse genetics has generated a number of useful flies to study PD-related features for Parkin (*PARK2*), Pink1 (*PARK6*), DJ-1 (*PARK7*), and LRRK2 (*PARK8*) (138). In

Drosophila, loss of *parkin* (an E3 ubiquitin ligase) (164) or *pink1* (a serine/threonine kinase) results in reduced life span, locomotor defects, and male sterility (37, 59, 137). Striking features of both *parkin* and *pink* fly mutants are their muscle degeneration and male sterility, which may be due to abnormal mitochondrial morphology and function (37, 59). Moreover, *pink1* mutants have lower ATP production (37) and defects in respiratory complexes I (97, 113) and IV (97). Genetic analysis in *Drosophila* has also elegantly documented that Pink1 acts upstream of Parkin, supporting the phenotypic similarities observed in mutant flies (37).

The study of mitochondrial dynamics (fission/ fusion) in *parkin* and *pink1* fly mutants provided the first clues into their protein functions. Overexpression of Drp1 (a fission inducer) or knockdown of mitofusin or opa1 (fusion inducers) rescues the phenotypes of muscle degeneration, cell death, and mitochondrial abnormalities in *pink1* and *parkin* mutants (44, 140). However, loss of *pink1* or *parkin* causes mitochondrial morphologies different from those associated with loss of *drp1*. Moreover, flies carrying one copy of *drp1* display an enhancement of the *pink1* phenotype, resulting in lethality (44, 140). These data suggest that Pink1/Parkin can alter mitochondrial morphology, but how they regulate the mitochondrial fission/fusion machinery remains to be elucidated.

Pink1 and Parkin have also been proposed to play a role in mitochondrial function and maintenance by regulating mitophagy and the activity of respiratory chain complexes. In mammalian cells, the Pink1/Parkin protein duo targets dysfunctional mitochondria for degradation via autophagy (mitophagy) through ubiquitination (118). Moreover, recruitment of Parkin to the mitochondria in *Drosophila* promotes mitofusin ubiquitination followed by its degradation (141, 213), suggesting that loss of *pink1* or *parkin* prevents the cell from degrading Mitofusin and the timely removal of dysfunctional mitochondria. This in turn may lead to altered mitochondrial morphology, impaired mitochondrial energy production, and locomotor defects.

As mentioned above, *pink1* mutants have defects in respiratory Complexes I and IV, but findings from rescue experiments of the respiratory chain complexes are conflicting. One group demonstrated that overexpression of Drp1 partially rescues Complex I and IV defects of *pink1* mutants (97). Another group showed that the *pink1* mutant Complex I defect is not rescued by Drp1 overexpression (192), although the *pink1* mutant mitochondrial morphology phenotype is rescued by overexpression of Drp1 (192). Interestingly, bypassing respiratory chain Complex I can rescue the *pink1* mutant–associated defects (male fertility, flight, and ATP production). Moreover, bypassing respiratory chain complexes III and IV does not rescue *pink1* mutant–associated phenotypes (192). This suggests that Pink1 primarily alters Complex I and not Complex IV. Furthermore, a genetic modifier screen for *pink1* flight defects identified UBIAD1/*heix*, which converts vitamin K1 to vitamin K2 (193). *heix* mutants showed severe mitochondrial defects that were restored with administration of vitamin K2, which acts in the electron transfer within the mitochondrial respiratory chain similar to ubiquinone. Addition of vitamin K2 also restored mitochondrial function in *pink1*, *parkin*, and complex I mutant flies (193). Vitamin K2 may provide a promising strategy to treat PD patients suffering from Pink1 or Parkin deficiency.

Furthermore, the subsequent accumulation of dysfunctional mitochondria in *pink1* and *parkin* mutants results in an increase in ROS/oxidative stress (29). As a result, both *pink1* and *parkin* mutants may be more susceptible to chemicals like paraquat and rotenone (37, 137), whereas antioxidants or overexpression of Cu/Zn superoxide dismutase (SOD) extends life span in *parkin* mutants (158). Although the roles of Pink1/Parkin in mitochondria have not been completely elucidated, studies in *Drosophila* have already provided important insights.

DJ-1 (*PARK7*) is a member of the ThiJ/PfpI family of molecular chaperones, and PD associated mutations in DJ-1 result in an unstable and defective protein (112). DJ-1 is expressed widely throughout the body and is subcellularly localized to the cytosol, mitochondrial matrix, and intermembrane space (209). DJ-1 is a redox-sensitive molecular chaperone that regulates redox-dependent kinase signaling pathways and antioxidant-related gene expression (79, 112). DJ-1 is also an atypical mitochondrial peroxidase that protects against oxidative stress (5). Whereas mammals possess a single *DJ-1* gene, *Drosophila* possesses two orthologs, the male germ-line-expressed *DJ-1α* and the ubiquitously expressed *DJ-1β* (106).

Multiple DJ-1 *Drosophila* models have been generated to explore the contribution of DJ-1 dysfunction to neuropathology, but the results relating to degeneration of DA neurons have so far been conflicting. We therefore focus on the role of DJ-1 in providing oxidative-stress protection. *DJ-1α*/*β* double-knockout flies display a striking and selective sensitivity to paraquat and rotenone (63, 106). Similar to the findings in the study of *DJ-1α*/*β* mutants by Hao et al. (63), *DJ-1β* mutants exhibit hypersensitivity to MPTP and oxidative stress (paraquat) (88, 130). Moreover, *DJ-1β* mutants display reduced life spans and motor impairment (88, 130). Loss of *DJ-1β* function also results in ROS accumulation, increased lipid peroxidation, and catalase activity, suggesting that the DJ-1β protein may protect against oxidative insults (87). Consequently and consistent with this model, dietary supplementation with antioxidants and anti-inflammatory drugs confer life span extension to *Drosophila DJ-1β* mutants (87). Moreover, DJ-1β is localized to mitochondria (130), and *DJ-1β* mutants exhibit poorly coupled mitochondria and reduced ATP production (63). Furthermore, DJ-1β overexpression can ameliorate *pink1* mutant phenotypes (63). These data indicate that DJ-1 prevents mitochondria-generated oxidative stress.

LRRK2, the most frequently mutated gene in familial PD, contains leucine-rich repeats toward the N-terminal portion of the protein and a kinase domain (MAPK) toward the C-terminal end (41). *Drosophila* has a single ortholog, the LRRK-like protein (*dLRRK*). Loss of *dLRRK* causes resistance to H2O2 or paraquat-induced oxidative stress (75), possibly due to reduced ROS levels. In contrast, overexpression of human LRRK2 containing the pathogenic PD mutations or overexpression of dLRRK alone in a wild-type background causes DA neuron death, supporting the idea that the pathogenic mutations act via a gain-of-function mechanism (75, 98). Although further studies are needed to elucidate the role of LRRK2, both human LRRK2 and dLRRK phosphorylate eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP), a negative regulator of eIF4E-mediated protein translation and a key mediator of various stress responses (75). This suggests that chronic inactivation of 4E-BP by a pathogenic mutation–possessing LRRK2 protein results in deregulated protein translation and eventually an age-dependent loss of DA neurons (75). Impaired protein translation is thus another pathway that can lead to neurodegenerative phenotypes.

α-synuclein or *SNCA* (*PARK1/4*) was the first identified familial PD gene (139). Although the *α-synuclein* gene has thus far been identified only in vertebrates, *Drosophila* expressing human α-synuclein also exhibit neurodegenerative phenotypes. Interestingly, expression of either wild-type or mutated α-synuclein is neurotoxic and causes DA neuronal loss and locomotor dysfunction, as evidenced by a progressive loss of climbing ability (51). An interesting toxic role for dopamine in this process was documented when depletion of cytoplasmic dopamine by α-methyl-*p*-tyrosine was found to rescue the early stages of α-synuclein-mediated DA cell loss (131). In addition, overexpression of a *Drosophila* vesicular monoamine transporter that sequesters dopamine into synaptic vesicles also rescued α-synuclein-mediated neurodegeneration (131). These data suggest that elevated levels of cytoplasmic dopamine can precipitate α-synuclein-mediated neurodegeneration.

In agreement with the hypothesis that ROS plays an important role in PD pathogenesis, oxidative stress is elevated in α-synuclein expressing flies. This was confirmed by the finding that reducing oxidative stress could suppress the phenotypes and promote life span extension by expressing either methionine sulfoxide reductases (201) or phase II detoxification enzymes involved in glutathione metabolism (180) as well as by feeding antioxidant-rich grape extracts (99). Sustained upregulation of the Nrf2 pathway, a conserved global regulator of cellular antioxidant responses, could also restore the locomotor activity of α-synuclein-expressing flies and suppress α-synuclein-induced DA neuronal loss (8). Finally, expression of SOD protects against the DA neuronal loss induced by mutant α-synuclein overexpression (23). Together, these data provide a strong case for ROS playing an important role in α-synuclein-mediated PD-related phenotypes in flies.

Exposure to environmental toxins such as pesticides is now believed to play an important contributory role in the pathogenesis of diseases like PD (134). *Drosophila* has been used to study the mechanisms by which some of these toxins, like paraquat and rotenone, cause neurodegeneration. Rotenone exposure induces a PD-like phenotype in the rat and induces cytoplasmic inclusions similar to Lewy bodies (15). Similarly, rotenone-treated flies present with locomotor impairment and DA neuronal loss (39). Interestingly, overexpression of HSP27 alleviates paraquat-induced locomotor impairment and neuronal loss (93), suggesting that chaperones can enhance stress resistance and prevent apoptosis in the fly.

In summary, *Drosophila* studies of PD gene function provide a robust system to test the effects of mitochondrial dysfunction, ROS toxicity and suppression by measuring life span, locomotor ability, and neuronal cell loss. Reducing ROS toxicity by increasing antioxidants or ROS scavengers often ameliorates the phenotypes. Moreover, altering mitochondrial dynamics and restoring respiratory chain functions also rescue *parkin* and *pink1* phenotypes. However, how all these observations will translate to PD therapies remains a nagging question.

Alzheimer's Disease

AD is characterized by progressive dementia in which a person's ability to remember and recognize familiar objects and other people declines significantly with age. Distinct pathological changes in the brains of patients with AD include tau tangles in neuronal cell bodies and β-amyloid plaques in the extracellular matrix (65). An early indication of a genetic component came from the observation that people with a first-degree relative with AD are much more likely to develop the disease (25, 30). Genes with evidence for causality of early-onset familial AD are *Amyloid Precursor Protein (APP)*, *Presenilin 1*, and *Presenilin 2*. However, there are numerous other genes associated with AD (13). Accordingly, the scientific focus has been on the β-amyloid and tau proteins, because not only are their pathologies hallmarks of the disease, but the *APP* gene and its cleavage enzyme presenilin are also mutated in some patients with AD (65). Early on, it was observed in *Drosophila* that tau and β-amyloid overexpression in the retina result in rough, smaller eyes (40, 203). This robust phenotype has been further utilized in numerous ways to identify modifiers and pathological mechanisms, as reviewed elsewhere (22).

Although the *Drosophila* genome encodes an APP homolog *Appl*, some of the machinery involved in APP cleavage is not evolutionarily conserved (22). Our focus is on the function of Aβ42, a cleavage product of APP and the main component of amyloid plaques (175) in *Drosophila* neurons. Aβ42 expression in the fly CNS results in reduced life span, brain and photoreceptor degeneration, and impaired locomotion (56, 74). Toxicity from Aβ42 expression may come from the suppression of genes involved in reducing oxidative stress (149). A subsequent genetic screen revealed that the iron-binding ferritin and ROS scavenger catalase are potent suppressors of the toxicity associated with Aβ42 expression,

and treatment with the iron-binding compound clioquinol rescued the toxicity (149). Moreover, in the presence of Aβ42 accumulation, clioquinol rescues the toxicity, suggesting that the toxicity is ROS-related (149).

Aβ-42 plaques induce JNK signaling in neurons, which contributes to Aβ-42-mediated cell death (176). This is consistent with ROS directly activating JNK signaling (124). The level of JNK activation correlates with the degree of tau-induced neurodegeneration (45). Moreover, the neurotoxicity observed in *Drosophila* models by overexpression of human tau is suppressed by SOD overexpression and pharmacological manipulations using antioxidants such as vitamin E, suggesting that oxidative stress plays a role in tau toxicity (45). Hence, both Aβ42 and tau induce stress signaling pathways that increase oxidative stress, which, when reduced, ameliorates the corresponding neurodegenerative phenotypes.

In *Drosophila*, a full understanding of the effects of Aβ42 and tau toxicity on mitochondrial dysfunction is still lacking. Extensive examination of the mitochondrial size and fusion/fission balance in *Drosophila* models expressing Aβ42 is needed, but on the basis of the published data, mitochondria are fragmented and mislocalized, their transport along microtubules is defective, and reduced numbers of mitochondria in the axons and dendrites have been documented (73). In addition, overexpression of tau disrupts axonal transport, causing vesicle aggregation, which is associated with loss of locomotor function (114). Overall, these studies suggest that overexpressed tau and Aβ42 fragment mitochondria and disrupt their subcellular distribution, causing a loss of synaptic mitochondria at synapses (148).

Apolipoprotein (Apo) D (115) and ApoE (191) have also been implicated in human AD pathogenesis. Known as *Glial Lazarillo* (*GLaz*), ApoD, but not ApoE, is conserved in *Drosophila. GLaz* mutants have reduced resistance to oxidative stress and starvation, impaired fat storage, and shortened male life spans (159). In contrast, overexpression of *GLaz*, the related *Neural Lazarillo* (*NLaz*), as well as human ApoD, produce extended life spans and increased resistance to hyperoxia and starvation (71, 116, 195). These proteins thus have protective roles when the organism is stressed, and their deficiency results in reduced life span and more rapid neurodegeneration (159, 195). Furthermore, *NLaz* is transcriptionally regulated by JNK signaling and required for JNK-mediated stress and starvation tolerance.

In summary, Aβ-42 and tau overexpression or ApoD deficiency increases oxidative stress, and reduction of this stress ameliorates the neurodegenerative phenotypes. This suggests that oxidative stress is a major component of AD toxicity and provides evidence for how *Drosophila* could be used to test AD gene functions and ROS. Further research in *Drosophila* should help uncover underlying genetic causes of AD and provide a useful context for drug screens.

Amyotrophic Lateral Sclerosis

ALS, also known as Lou Gehrig's disease, is characterized by the progressive degeneration of upper (cortical) and lower (spinal cord) motor neurons muscle dysfunction, and eventually paralysis (19). ALS is a fatal disease with no cure. 90% of ALS cases have no known family history of the disease and no known genetic cause (19). A somewhat better understanding of ALS pathogenesis has emerged from the identification and study of disease-causing mutations. These include, but are not restricted to, mutations in the Cu/Zn SOD (152), transactivating response element DNA binding protein-43 (TDP-43) (172), and vesicle-associated membrane protein (VAMP)/synaptobrevin-associated membrane protein B (VAPB/ALS8) (119). Although all these genes have been studied in *Drosophila*, we focus on SOD and VAPB in the context of mitochondrial dysfunction and oxidative stress.

Insights into ALS pathology initially came from the discovery that SOD mutations were associated with ALS, followed by the study of SOD transgenes and loss-of-function alleles (19). It quickly became apparent through animal studies that the enzymatic dismutase and catalytic copper-loading functions of SOD do not play an important pathogenic role, but that other functions or issues associated with SOD are critical (19, 147). *Drosophila* expression of human SOD1 specifically in adult motor neurons extends normal life span and rescues the life spans of short-lived *Sod*-null mutants (132). Their resistance to oxidative stress suggests that this life span extension is due to enhanced ROS metabolism (132). Surprisingly, overexpression of human-mutated SOD in wild-type *Drosophila* motor neurons results in no observable neurodegenerative or life span phenotypes (50). Interestingly, it extends the life span, augments resistance to oxidative stress, and partially rescues SOD-null mutants in a manner similar to wild-type human SOD expression (50). However, loss of fly *Sod2* increases ROS levels, accelerates neurodegeneration, and shortens life span and reduces DNA strand breakage (135). Together, the data suggest that neurons are highly sensitive to SOD and SOD2 levels, but the precise mechanism by which SOD mutations cause disease is still unknown.

The *VAPB* gene was first proposed to play a role in synaptic transmission by binding to VAMP/synaptobrevin, a vesicular protein required for synaptic vesicle fusion (168). However, loss-of-function mutations in the fly showed that the gene plays a role in neuromuscular junction (NMJ) modeling and affects synaptic microtubules (136). The discovery that ALS8 was due to a P56S mutation in *VAPB* (119) led to several studies in which it was shown that the mutant form leads to aggregate formation (32, 144) and that the mutant protein recruits the full-length VAPB protein to endoplasmic reticulum (ER)-associated inclusions (181). Although the wild-type VapB protein is ER-associated, its N-terminal major sperm protein (MSP) domain is cleaved and secreted and functions as a hormone. Mutant VAPB protein (P58S), however, fails to be secreted and instead induces an ER-associated unfolded-protein response (181).

It was recently discovered that *VAPB* mutants in flies and worms exhibit enlarged filamentous mitochondria in muscle (62). Interestingly, this mitochondrial morphology is modulated by MSP, which upon neuronal secretion binds to the muscle-based growth cone guidance receptors Roundabout and Dlar to control Arp2/3-dependent actin remodeling. As the mitochondria are docked on an actin network in muscles, polymerization and depolymerization of actin via these guidance receptors modulate muscle mitochondrial dynamics. The subsequent mitochondrial dynamics defects in *VapB* and growth cone guidance mutants reduce mitochondrial function (62).

VAPB studies in flies and worms point to a disease mechanism involving aberrant MSP hormone secretion by neurons (181), which in turn affects muscle mitochondrial function (62). Eventually, the mitochondria cannot properly buffer Ca^{2+} (206), leading to fast consecutive Ca^{2+} spikes and spontaneous muscle contractions, as observed in ALS patients (60, 166). Abnormal muscle metabolism may then lead to a decreased secretion of a BMP-like muscle hormone, which in the case of flies corresponds to glass bottom boat (Gbb) (202). Lack of Gbb secretion, in turn, leads to reduced pMAD in the presynaptic terminal (144), which may be associated with a gradual loss of NMJ maintenance (136), similar to what is observed in SOD mice and ALS patients (9, 49). This eventually may lead to synaptic retraction and loss of motor neurons in sporadic ALS (4) and SOD (177) andALS8 patients (111). Hence, an MSP-based hormone therapy may be beneficial to ALS patients. This therapy may benefit not only ALS8 patients, but also patients with *SOD* mutations and sporadic cases, as *VAPB* is also decreased in sporadic patients (4).

Polyglutamine Diseases

The nine known polyglutamine (polyQ) diseases, as a group, are common hereditary NDs. They are the X-linked recessive disease spinobulbar muscular atrophy as well as the dominantly inherited diseases dentatorubrupallidoluysian atrophy, HD, and SCA types 1, 2, 3, 6, 7, and 17 (123). In each disease, patients carry an expanded set of CAG repeats in the translated regions of otherwise unrelated genes. The exact cause of the repeat expansions remains unclear, and the instability manifests itself both somatically and in the germ line. Each disease has characteristic features, but a number of characteristics are shared and probably relate to the expression patterns of the genes involved. Besides their mostly dominant inheritance pattern, they typically manifest in adulthood and progress over the following 10 to 20 years. The more repeats the patient has, the earlier and more severe the symptoms generally are. Frequently, there is anticipation, inwhich theCAG repeats expand with each generation and the phenotypes worsen.

The polyQ diseases are widely believed to be due to a toxic gain-of-function phenotype, primarily at the protein level, although there is some evidence that they act at the RNA level too. Overexpression of polyQ-expanded proteins in the fly retina results in a rough, depigmented eye. This has proven very useful in investigations of genetic and pharmaceutical modifiers. The research on polyQ disease genes in flies has recently been exhaustively reviewed (102).

HD is an autosomal dominant neurodegenerative disorder caused by polyQ expansion of the *huntingtin* (*htt*) gene. PolyQ expansion confers a toxic gain of function to the huntingtin protein, enhances mitochondrial dysfunction, and increases oxidative damage and neuronal excitotoxicity (27, 129). *Drosophila* models of HD were developed by pan-neuronal or photoreceptor expression of the first 548 amino acids of the human HD gene with a pathogenic polyQ tract of 120 repeats (htt-Q120); these flies experience a progressive loss of motor function along with formation of huntingtin aggregates (76, 90, 200). However, expanded full-length htt [htt-Q128(FL)] leads to more subtle behavioral, neurodegenerative, and electrophysiological phenotypes prior to import of the expanded htt into the nucleus (151). htt-Q128(FL) expression in fly neurons results in impaired Ca^{2+} buffering and excitotoxicity, which could be a primary cause of neurodegeneration (151). However, data are needed to test the impact of htt-Q128(FL) expression on Ca^{2+} buffering with respect to the ER and mitochondria, key Ca^{2+} regulators.

Interestingly, in *Drosophila*, neurons and glia may be linked to the pathogenesis of polyQ proteins. Both cell types are vulnerable to oxidative stress, possibly owing to their high-energy requirements. Neither overexpression of SOD in neurons nor dietary antioxidant supplementation can, however, rescue the lethality associated with htt-Q128 expression (7). Neuronal expression of HSP27, however, improves life span, increases resistance to oxidative stress, and attenuates mild polyQ-induced toxicity (93). Moreover, HSP27 can increase glucose-6- phosphate dehydrogenase activity (142), which regulates NADPH levels and whose overexpression in *Drosophila* enhances life span and increases tolerance to oxidative stress (91). In a genetic screen to find stress-resistance genes, life span–extension mutants were characterized by their resistance to paraquat and hyperoxiainduced stress, leading to the identification of *ribose-5-phosphate isomerase* (*rpi*). Knockdown of *rpi* leads to higher NADPH levels and ameliorates the eye phenotypes caused by polyQ toxicity similar toHSP27 overexpression (196). This suggests that reducing oxidative stress does not necessarily benefit polyQ diseases, but enhancement of NADPH levels may alleviate polyQ-induced toxicity.

In a large *Drosophila* cell culture–based screen of more than 1,000 compounds to identify suppressors of huntingtin-induced degeneration, many mitochondrial respiration/ glycolysis

inhibitors including rotenone, sodium fluoride (inhibitor of glycolysis), oligomycin (ATP synthase), and 2,4-dinitrophenol (mitochondrial coupling) were identified (186). Similarly, glial expression of uncoupling protein 5, a protein involved in mitochondrial inhibition, ameliorated several polyQ-related phenotypes including life expectancy, motor performance, and bang sensitivity, although it did not rescue neuronal loss (14). It remains a mystery as to why mitochondrial inhibitors suppress neurodegenerative phenotypes of htt-Q120 flies, considering that these very same inhibitors cause neurodegeneration, as discussed for PD (185). A clue may come from an initially seemingly unrelated study of autophagy in which the antioxidants N-acetylcysteine, cystamine, and glutathione prevented the induction of both basal and induced autophagy by trehalose (182). Thus, high doses of antioxidants as well as overexpression of the ROS-lowering enzymes SOD and SOD2 impair autophagic clearance of α-synuclein and htt-Q120 aggregates. Such studies suggest that some mitochondrial inhibitors enhance the autophagic process, which in turn suppresses protein aggregate formation and neurodegeneration through induction of ROS (182). This and other forms of cellular quality control mechanisms are discussed below.

PROTEIN QUALITY CONTROL SYSTEM AND NEURODEGENERATIVE DISEASES

The quality control of proteins and the presence of functional organelles are essential for long-lived neurons. The quality control system repairs cell damage, reduces oxidative stress, controls energy homeostasis, and eliminates toxic/damaged organelles and proteins. A defect in pathways or components regulating defective protein and organelle turnover is often associated with NDs (154). Here we discuss recent work in *Drosophila* on autophagy, the ubiquitin-proteasome system (UPS) and chaperones that constitute the essential components of neuronal quality control and survival.

Autophagy and Lysosomal Degradation Prevent Neurodegeneration

In neurons, autophagy is one of the key defense mechanisms to prevent age-dependent neurodegeneration. Autophagy is a catabolic process in which cellular proteins and organelles are packaged into double-membrane structures called autophagosomes, which eventually fuse with lysosomes, where they are degraded. Autophagy occurs at basal levels in healthy cells but can be upregulated by starvation, cellular stress, high temperature, and growth factor deprivation. Turnover of defective organelles and proteins by autophagy is essential for cell quality control, renewal, and survival from cellular insults. Over the past decade, several reports have shown that autophagy plays a protective role in neurons. Several components of autophagy have been identified in yeast and are conserved across phylogeny. In recent years, *Drosophila* studies have advanced our understanding of the regulation of these processes in the context of neuronal quality control, with implications for NDs (105).

Autophagy plays an important role in quality control and survival of neurons. In flies, overexpression of htt-Q120 results in neurodegeneration. This neurodegeneration is suppressed when autophagy is increased by feeding rapamycin, a negative regulator of TOR (146). Furthermore, overexpression of rab5 attenuates toxicity in flies overexpressing htt-Q120. rab5 acts at an early stage of autophagosome formation in a complex containing beclin1 and vps34 (145). Feeding rapamycin also decreases phenotypes associated with overexpression of wild-type or mutant tau (12). Paradoxically, in a fly model of SCA3 (another polyQ disease), flies expressing pathogenic SCA3 (SCA3trQ78) display increased autophagy and neurodegenerative phenotypes, indicating that increased autophagy may promote the disease. However, reducing autophagy enhances SCA3trQ78-associated phenotypes and increases aggregate formation, suggesting that increased autophagy is a

protective response (17). These studies suggest that autophagy prevents the accumulation of toxic proteins and thereby protects neurons from degeneration.

Basal autophagy is part of the neuronal surveillance machine. A gradual loss of basal autophagy function occurs with age in wild-type flies, as evidenced by reduction of expression of autophagy-related genes such as *Atg2, Atg8A*, and *Atg18* and by accumulation of insoluble ubiquitinated proteins. Moreover, *Atg8* overexpression increases life span and reduces ubiquitinated proteins, suggesting that basal autophagy is necessary for the removal of aberrant proteins (167). In addition, flies with compromised autophagy due to mutations in *Atg7* exhibit an accumulation of inclusion bodies leading to neurodegeneration (78). Autophagy is also required for retinal maintenance during phototransduction. Removal of the key autophagy proteins Atg7 and Atg8 or overactivation of TOR (which suppresses autophagy) results in photoreceptor cell death in an age- and light-dependent manner. Moreover, genetically inhibiting TOR or inducing autophagy suppresses retinal degeneration caused by defects in the phototransduction cascade, presumably by increasing the turnover of rhodopsin (107, 197). Together these observations provide strong support for a role for basal autophagy in neuronal quality control.

Increasing autophagy is not always protective, however. Aberrant autophagy has been demonstrated in mouse models of AD. In these models, Amyloid-β accumulates in autophagic vacuoles in neurons (121). Aβ1-42, but not Aβ1-40, expression in flies also causes extensive accumulation of autophagic vesicles, age-dependent autophagic dysfunction, and neurodegeneration. Aβ1-42 impairs the degradative functions of the autophagosome but not its maturation (96). Interestingly, although autophagy is known for its protective role, a reduction of autophagy reduced Aβ1- 42-mediated neurotoxicity, whereas increased autophagy enhanced Aβ1–42-mediated neurotoxicity. It has therefore been proposed that Aβ1-42-mediated neurotoxicity is caused by leakage of damaged catabolic components from the autophagosomes into the cytoplasm (96).

Impaired autophagy is also implicated in mucolipidosis type IV (MLIV) (187), which is caused by loss of the transient receptor potential (Trp) superfamily member, TRPML1. MLIV is an early-childhood-onset lysosomal storage disease. Symptoms include severe motor impairment, mental retardation, and retinal degeneration (170). TRPML1 is widely expressed and localizes to the late endosomal and lysosomal compartments. The *Drosophila* TRPML1 homolog localizes to the same subcellular compartments as the vertebrate protein. Fly *trpml* mutants display an accumulation of aggregated macromolecules and defective mitochondria as a result of defective autophagy and impaired lysosomal function. This, in turn, is thought to result in oxidative stress and impaired synaptic transmission leading to neurodegeneration (187). Moreover, loss of *trpml* enhances htt-Q120-mediated toxicity, whereas overexpression of *trpml* in hemocytes or glia can prevent widespread neurodegeneration, suggesting a non-cell-autonomous effect for *trpml.* Given these findings, it has been hypothesized that *trpml* loss in glia could lead to membrane disintegration and the dispersion of neuroinflammatory substances and widespread degeneration (187).

Trafficking to the lysosome is required for the turnover of membrane proteins and this pathway has also been implicated in several NDs. The ESCRT complex, first isolated in yeast, plays an essential role in the formation of multivesicular bodies from endosomes (72). Their loss impairs the subsequent trafficking of proteins to lysosomes. Indeed, work done in *Drosophila* has revealed roles for ESCRT complexes during development (157), neuronal morphogenesis (82, 150), synaptic transmission (183), and neuronal quality control (155). The importance of ESCRT complexes for neuronal maintenance has been underscored by the discovery of mutations in the ESCRT-III complex gene *CHMP2B* in frontotemporal dementia (169) and ALS (133). These diseases are characterized by accumulation of

ubiquitinated protein aggregates in neurons, and ESCRT complexes play a critical role in the turnover of ubiquitinated proteins. Recent work in *Drosophila* and mammalian cells has also uncovered a role for the ESCRT complex in autophagy (155). The *Drosophila* homolog of ESCRT-III subunit snf7, *shrub*, is required for dendrite morphogenesis (173). Reduction of ESCRT-III or overexpression of a truncated CHMP2B in *Drosophila* eyes results in autophagosome accumulation and neurodegeneration (89, 156), and reduction of ESCRT complex levels in *Drosophila* also aggravates the toxic effect of htt-Q120 (156). Thus, these studies suggest that a lack of effective autophagy and functional multivesicular bodies can promote neurodegeneration.

The Role of the Ubiquitin Proteasome System in Neurodegeneration

The ubiquitin proteasome system (UPS) plays an important role in protein quality control and degradation in conjunction with autophagy, and the UPS has been implicated in various NDs (154). Genetic studies in *Drosophila* have shown that the UPS's role in protein turnover is essential for axon guidance, synaptic function and growth, axon pruning, and neuronal maintenance (46). To provide a better understanding of the molecular players involved in polyQ-induced degeneration, a number of modifier screens in *Drosophila* have uncovered several UPS-related proteins (17, 55, 102). Moreover, compromising UPS function enhances the pathogenesis of spinobulbar muscular atrophy caused by polyQ expansion of the androgen receptor gene (33), and loss of ubiquitin also enhances SCA1-Q82-induced toxicity (55), suggesting that ubiquitinmediated degradation is neuroprotective.

Another interesting link between the UPS and neurodegeneration was established in a study of SCA3 (199). Expression of wild-type SCA3, which on its own has no discernible eye phenotype, suppresses the degenerative phenotypes associated with overexpression of SCA3-Q78 as well as htt-Q120 and SCA1- Q82, indicating that SCA3 has a protective role. This suppression was also associated with a delay in nuclear inclusion formation and requires the UPS. Indeed, SCA3 possesses a ubiquitininteracting motif that is essential for SCA3 to suppress the above-mentioned phenotypes by the UPS-mediated degradation pathway (199). These data are consistent with the observations that proteasome activity and abundance decrease progressively with age in flies and that enhancing proteasome activity increases average life span, whereas suppression of proteasome function shortens life span (179). These studies suggest that the basal UPS machinery acts in parallel to the autophagy and lysosomal degradation pathways by removing toxic and accumulated proteins and promoting neuronal survival.

Crosstalk Between Autophagy and the Ubiquitin-Proteasome System Pathways

The autophagy-lysosomal pathway was originally believed to operate in parallel to but independently of the ubiquitin-proteasome pathway. However, several lines of evidence suggest crosstalk between these pathways as well as compensatory regulation of these pathways. In degenerating neurons, proteins marked by ubiquitin for proteasomal degradation often form aggregates resulting in increased autophagy. In addition, *Drosophila* autophagy mutants exhibit increased ubiquitinated protein aggregation, indicating an attempt to compensate for impaired autophagy via the UPS pathway (35). Conversely, *Drosophila* proteasome mutants, e.g., *DTS7* (β-subunit of proteasome), show increased compensatory autophagy. Part of this compensatory response may be related to the microtubule-associated deacetylase HDAC6, which interacts with polyubiquitinated proteins and is required for autophagy activation upon impaired proteasome function. This is also supported by work on a fly model of the ND for spinobulbar muscular atrophy showing that, with a compromised UPS, autophagy is activated in an HDAC6-dependent manner (128). These findings indicate the presence of an intricate machinery in neurons that triggers and coordinates autophagy and UPS to maintain cellular integrity in aging neurons.

Suppression of Neurodegeneration by Chaperones

A number of studies in *Drosophila* have identified chaperones required for neuronal quality control and survival. Cysteine-string protein (CSP), a member of the DnaJ/Hsp40 family of chaperones, is critical for neurotransmission and neuroprotection in *Drosophila* and mice. CSP's role in neurons may stem from its regulation of assembly and activity of SNARE and other synaptic proteins (212). Moreover, in a large-scale screen designed to identify suppressors of polyQ toxicity in *Drosophila*, it was discovered that overexpression of the DnaJ-1/Hsp40 and Tpr2 chaperones strongly suppresses neurodegenerative phenotypes (81). Both DNAJ-1 and Tpr2 have a J domain that stimulates Hsp70 activity. The idea that chaperones may be important for polyQ phenotypic suppression was confirmed upon overexpression of the androgen receptor 52Q with Hsp40 (174). In addition, the Hsp70, Hsc70, and Hsp40 chaperones physically interact with SCA1-Q82 in mammalian cells (36); similar findings were made regarding the coexpression of human HSPA1L (Hsp70) with SCA3-Q78. Furthermore, in the Hspb family of chaperones, Hspb7 seems to be one of the most potent polyQ aggregation suppressors in *Drosophila* (194). In addition, overexpression of nicotinamide nucleotide adenylyltransferase (NMNAT), a critical enzyme in the NAD synthetic pathway and a chaperone in *Drosophila*, suppresses aggregate formation observed in flies expressing SCA1-Q82 (208) and tau (3). These and other data suggest that chaperones prevent protein clumping, thus reducing cytotoxic stress. During the aging process of adult flies, the expression level of certain endogenous heat-shock chaperones, including Hsp40 and Hsp70, drops over the first two weeks (179). Thus, pharmacologically inducing chaperone expression in some ND fly and mouse models may suppress ND-associated phenotypes.

FORWARD GENETICS TO DECIPHER NEURODEGENERATION

Use of systematic forward genetic screens to identify suppressors/enhancers of degenerative phenotypes caused by overexpression of genes that cause degeneration has not been uncommon (17, 54), as mentioned in previous sections. However, extensive forward genetic screens designed to isolate mutations that cause neurodegenerative phenotypes have not been performed systematically. Hence, in contrast to its use in other areas of fly biology, the contribution of forward genetics to our understanding of neuroprotection/neurodegeneration has, so far, been rather limited, although some screens in the past 40 years have led to the isolation of mutations in genes that cause neurodegenerative phenotypes (92).

The first screens were not designed per se to isolate these mutants. They used the countercurrent assay to identify viable mutants with defects in phototaxis (11) or electroretinograms (ERGs) to screen viable mutants for defects in phototransduction (68, 125). Some of the isolated mutants showed obvious behavioral defects. For example, *knockdown* (*kdn*) mutants are easily knocked down (57) whereas others such as triosephosphate isomerase (*tpi*) (also known as *wasted away* and *sugarkill*) had temperature-sensitive paralytic phenotypes (127). Similarly, Heisenberg and colleagues (67) performed a phototaxis screen followed by secondary morphological screens of the brain. They identified some mutants displaying morphological defects in neurons that worsened with age, such as *swiss cheese* (*sws*). Some phototactic mutants also displayed reduced longevity and neurodegeneration, as in *drop dead* (*drd*) (70) and *bubblegum* (*bgm*) (110). Here we briefly review the salient features of evolutionarily conserved genes as well as *drd* (Table 1).

Phototaxis Mutants

The first neurodegenerative mutant, *drd*, was identified serendipitously by Benzer and coworkers (70) in a phototaxis screen. *drd* mutant flies initially behaved normally, but problems with their gait manifested with age. *drd* mutant flies have shortened life spans, and

most die within 10 days of eclosion (70). Mutant brain sections showed central brain lesions, and the optic lobes degenerated with time (28, 70). Subsequent work revealed that the cause of neurodegeneration in *drd* mutants was cell non-autonomous, as the glia display defects prior to symptom onset, whereas the neurons are largely normal (28). *drd* encodes a putative integral membrane protein with unknown function (18). The N-terminal domain is homologous to an RNA-binding protein, and the C-terminal domain is homologous to a predicted acyltransferase.

Heisenberg and colleagues (67) also performed a small phototaxis screen and isolated several mutants that display neurodegenerative phenotypes, including *vacuolar medulla* (*vam*) (38), *sws* (85), and *vacuolar peduncle* (*vap*) (42). Young flies display normal or near-normal brain structure, whereas older flies develop vacuoles in the central brain or optic system that worsen with age (reviewed in Reference 84). In *sws* and *vam* mutants, the neurons undergo apoptosis, whereas *vap* mutants display signs of autophagic cell death (24, 84). Glial cell defects in *sws* mutants appear prior to the neuronal defects, indicating a cell-nonautonomous contribution to neurodegeneration (85). Glial cell changes have been reported in many NDs, and their contribution to pathogenesis is attracting increased attention (108).

sws encodes an evolutionarily conserved phospholipase (101). Mutations in the human homolog, neuropathy target esterase (NTE), were recently found to cause an ND termed spastic paraplegia type 39 (SPG39). SPG39 patients develop childhood-onset and progressive motor neuron degeneration (143). Mice with a neuronal-specific loss of NTE are viable but display severe neurodegenerative phenotypes, including progressive vacuolization in the brain, similar to *sws* flies (2). In addition, both sws and NTE are localized to the ER (2, 117). Ectopic expression of mouse NTE in *sws* mutant flies rescues the phenotypes associated with loss of sws (117). sws binds and inhibits cAMP-activated protein kinase (PKA), whose overexpression leads to neurodegeneration in flies. This suggests that sws affects neuronal integrity through regulation of the PKA pathway (16).

Bang-Sensitive Mutants

The *technical knockout* (*tko*) (153) mutation that causes bang sensitivity was also isolated serendipitously (77, 163). *tko* causes semilethality, and survivors exhibit temporary paralysis upon mechanical stimulation, such as banging the culture tubes against a hard surface (77, 163). Additional bang-sensitive mutants, including *kdn* (52) and *stress-sensitive B* (*sesB*) (52, 57), have been isolated. These mutants display a shortened life span and exhibit abnormal spontaneous activity in their dorsal longitudinal muscles (52, 57). Histological sections of mutant brains revealed age-progressive vacuolation, indicating neurodegeneration (53).

Several bang-sensitive mutations affect mitochondrial metabolism. *tko* encodes a mitochondrial ribosomal protein required for the translation of mitochondrial-encoded genes (153). *kdn* encodes citrate synthase, a mitochondrial enzyme that catalyzes the first step of the citric acid cycle (52). *sesB* encodes a mitochondrial translocase of adenine nucleotides (210). The underlying mechanism for bang sensitivity in several mitochondrial mutants remains to be elucidated. It will be interesting to determine whether this bang-sensitivity phenotype is due to an ATP deficiency or if another mitochondrial function is responsible. Defects in mitochondrial functions and quality control systems are now considered to play a role in numerous NDs (see above sections).

Life Span Screens

To investigate the use of flies to isolate genes related to human ND, Min and Benzer screened for viable mutant flies with a shortened life span, followed by histological analyses of their brains (109, 110). One of the genes they identified in this screen was *bgm. bgm* mutants exhibit age-dependent dilation of photoreceptor axons (110). *bgm* encodes a fly homolog of the mammalian Very Long Chain Fatty Acid (VLCFA) acyl-CoA synthetase, and *bgm* mutants exhibit elevated levels of VLCFAs, as seen in human adrenoleukodystrophy (ALD) (110). Dietary treatment used for ALD patients successfully reduced VLCFA levels and suppressed the neurodegenerative phenotypes in flies (110), suggesting that *bgm* flies can be used for drug screening.

One of the early phototaxis screens identified mutant flies that were paralyzed at 37°C upon rescreening (165, 204). Ganetzky and colleagues screened this collection, together with other temperature-sensitive paralytic mutants, for mutants with shortened life spans and degenerative brain defects (127). They identified 15 mutations affecting at least 9 different loci that exhibit neurodegenerative phenotypes. Two, *Atpα* and *Tpi*, are directly related to human diseases (58, 127). Flies with *Atpα* mutations have a shortened life span. At 37°C, they display neuronal hyperexcitability in the flight motor pathway and are paralyzed 10–30 seconds after the temperature shift. The mutant flies are also bang-sensitive and display progressive vacuolization of their brains (126). *Atpα* encodes the α-subunit of the Na+/K+ ATPase. Mutations in the human homolog of *Atpα*, Atp1a3, were later reported to cause rapid-onset dystonia parkinsonism, or Dystonia 12 (43). Dystonia 12 patients display bulbar and limb dystonia (abnormal muscle movement) and parkinsonism (48).

Tpi mutants have shortened life spans temperature-sensitive paralysis and exhibit progressive vacuolization in brains, but loss of *Tpi* does not affect ATP levels (31, 58). *Tpi* encodes triosephosphate isomerase, an enzyme in the glycolytic pathway. Mutations in its human homolog, *Tpi1*, have been identified in patients with TPI deficiency, a metabolic and neurodegenerative disease (122).

Electroretinograms and the Identification of Neurodegenerative Mutants

Genetic screens to identify genes required for phototransduction by ERG in *Drosophila* have also led to identification of numerous mutations that cause retinal degeneration (68, 125): the retinal degeneration (rdg) mutants (198). Most rdg mutants exhibit ERG defects and morphological defects of the retina that worsen with light exposure and age. *rdgB* and *rdgC* encode highly conserved proteins, and ectopic expression of mammalian *rdgB*, *nir2*, significantly rescues the neurodegenerative phenotypes of *Drosophila rdgB* mutants (34). *rdgB/nir2* encodes a transmembrane protein that transfers phosphatidylinositol between the ER and the Golgi apparatus, suggesting a role for lipid metabolism in neurodegeneration. These mutants have been studied extensively and reviewed elsewhere (198).

A mosaic ERG screen of essential genes designed to isolate genes that affect synaptic transmission (103) led to the identification of *nmnat* (207). Loss of *nmnat* causes a severe photoreceptor degeneration. Interestingly, overexpression of enzymatically inactive NMNAT in flies expressing atx-1[82Q] protects against the neurodegenerative phenotypes associated with aggregated atx-1[82Q]. NMNAT functions as a chaperone protein in a variety of contexts, similar to Hsp70 and other chaperones (207, 208). Additional studies indicate that NMNAT also suppresses a tau-induced neurodegeneration in *Drosophila* (3). In vertebrates, NMNAT protects against Wallerian degeneration (6), suggesting that NMNAT function is conserved.

An ERG screen designed to isolate mutations that affect synaptic transmission and synapse formation (103) also led to the isolation of mutations with a progressive age-dependent

decline in the ERG response. The mutations were mapped to the mitochondrial *methionyl-tRNA synthetase* (*aats-met*) (10), and electron micrographs revealed that the photoreceptors degenerate as the flies age. Mutant flies have elevated ROS, exhibit a mitochondrial unfolded-protein response, and have a defective Complex I. The identification of the *aats-met* gene led to the observation that *MARS2*, the human homolog maps to a genomic region previously shown to harbor mutations for autosomal recessive spastic ataxia with leukoencephalopathy (ARSAL/SPAX3) (178). A search for mutations in *MARS2* in ARSAL patients confirmed that ARSAL was caused by a complex set of rearrangements of the locus, probably caused by a DNA replication error because of the presence of numerous line-transposable elements surrounding the locus (10). This is a rare example of a fly gene study directly leading to the identification of the mutation associated with a novel human ND.

CONCLUSION

Use of *Drosophila* to study gene function has provided interesting insight into the role of oxidative stress in a number of NDs. *Drosophila* serves as an in vivo model for testing of oxidative stress levels and mitochondrial function using readouts such as life span, motor abilities, neuronal maintenance, and mitochondrial function and morphology. Interestingly, reduction of oxidative stress improves toxicity in AD and PD *Drosophila* gene models but has little to no effect on polyQ diseases. Furthermore, SOD enzymatic activity does not play a role in ALS toxicity. This raises an important point: Although oxidative stress is generally harmful to cells, it may also serve as a protective mechanism by inducing autophagy in a context-dependent manner. Moreover, the cell has developed a number of ROS-regulatory mechanisms: (*a*) removal of ROS by scavengers, (*b*) regulation of mitochondrial redox reactions, (*c*) regulation of mitochondrial energy reactions, and (*d*) mitophagy. Furthermore, studies confirm that the quality control system is crucial to neurons, as they are long-lived and high-energy-demanding cells. Thus, it is not surprising that impairment in the essential quality control system leads to neurodegeneration in humans and in flies. Moreover, neurodegenerative phenotypes caused by expression of several toxic proteins in flymodels are suppressed by upregulating various quality control systems, including the UPS, autophagy, and lysosomal degradation. Hence, the identification of drugs that stimulate quality control systems may be a critical step in alleviating several NDs. Accordingly, a better understanding of the quality control systems and their regulation is needed to find specific drug targets. The fly offers a suitable in vivo model to prescreen numerous potential drugs (1). By using large scale forward genetic screens, researchers can also use this model to find new players in the quality control systems.

To date, unbiased genetic screens have identified several new loci, some of which are now known to cause human diseases. However, none of the screens that led to the isolation of these mutants were primarily designed to isolate neurodegenerative mutations. Yet, ERGs provide for a very sensitive and direct readout of the neuronal function of the photoreceptors. Screening for progressively worsening ERGs with age is simple and can be done in mutant eye clones if the mutations cause lethality during development (10, 207). Such screens have been carried out in our lab and have led to the isolation of mutations in numerous loci that cause neurodegenerative phenotypes (M. Jaiswal, V. Bayat, B. Xiong, K. Zhang, H. Sandoval, S. Yamamoto, H.J. Bellen, unpublished data). These studies should facilitate the identification and characterization of novel genes, and thereby cellular mechanisms, required for neuroprotection. Forward genetic studies will also provide us with mutations in numerous genes whose human homologs are linked to neurodegeneration but whose in vivo roles remain ill defined. Defining the precise role of those genes in vivo will also facilitate the design of therapies.

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Table 1

Genes identified from forward genetic screens whose loss of function causes neurodegeneration

Gene	Defect	Protein function/human homolog	Related disease	Reference
drd	Phototaxis	Unknown		(70)
sws	Brain histology	NTE	SPA39	(67)
kdn	Bang-sensitive	Citrate synthase		(57)
tko	Bang-sensitive	Mitochondrial ribosomal protein S12		(77)
bgm	Short life span	VLCFA acyl-CoA synthetase	ALD	(110)
Tpi	Short life span	Triosephosphate isomerase	TPI deficiency	(58)
Atpα	Short life span	Na+/K+ ATPase	Dystonia 12	(127)
rdgB	ERG	Nir2		(68)
nmnat	ERG	Nmnat	Wallerian degeneration	(207)