OPINION

Modelling neurodegenerative diseases in *Drosophila*: a fruitful approach?

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Human neurodegenerative diseases are characterized by the progressive loss of specific neuronal populations, resulting in substantial disability and early death. The identification of causative single-gene mutations in families with inherited neurodegenerative disorders has facilitated the modelling of these diseases in experimental organisms, including the fruitfly *Drosophila melanogaster*. Many neurodegenerative diseases have now been successfully modelled in *Drosophila*, and genetic analysis is under way in each of these models. Using fruitfly genetics to define the molecular pathways that underlie the neurodegenerative process is likely to improve substantially our understanding of the pathogenesis of the human diseases, and to provide new therapeutic targets.

In the early twentieth century, two fields of science were blossoming on either side of the Atlantic. In the United States, Thomas Hunt Morgan was using the fruitfly *Drosophila melanogaster* to investigate and expand the Mendelian theory of heredity[1], while in Europe, the neuropathological features of major neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, were being defined by figures such as Alois Alzheimer[2] and Friederich Lewy[3]. Over the subsequent century, the foundation was laid to unite these seemingly disparate fields of study. First, *Drosophila* genetics emerged as a powerful tool with which to discover the basic genetic and cell-biological pathways that underlie complex biological processes[4]. Second, comparative genetic analyses showed that these basic cell-biological pathways are remarkably conserved between invertebrates and mammals. And finally, sequencing of the human and fly genomes showed unequivocally the high degree of interrelatedness of the two species[5].

Our understanding of neurodegenerative disease has also progressed steadily over the past century. We now know that neurodegenerative disorders represent a clinically, neuropathologically and, possibly, pathogenically related subgroup of human diseases. Disorders such as Alzheimer's, Parkinson's and Huntington's diseases generally occur late in life, involve the progressive loss of specific neuronal populations and follow an inexorable course that results in the death of the patient. The treatments for these diseases remain solely symptomatic, at least in part because many of the fundamental mechanisms that underlie neuronal death in these disorders remain unknown. Most cases of common neurodegenerative diseases occur apparently sporadically, but studies of rare familial forms have identified causative single-gene mutations. With the identification of these human gene mutations, it has become possible to create useful models of human neurodegenerative disease in the fruitfly.

Making the model

The principal goal of developing disease models in fruitflies is the eventual use of genetic techniques to define molecules and pathways that mediate neuronal death in the human disorders. First, however, a relevant model must be created. There are three main approaches to modelling human diseases, including neurodegenerative disorders, in *Drosophila*. Traditionally, forward-genetic approaches have been used (BOX 1). Mutations are selected on the basis of a neurodegenerative phenotype, and human homologues of the identified *Drosophila* gene products are plausible candidates for involvement in neurodegenerative diseases. Alternatively, 'reverse genetics' can be used. In this case, the *Drosophila* homologue of a specific gene that is implicated in a human disease is targeted, and phenotypes that result from altered expression of the gene are studied. Useful phenotypes can emerge by reducing or eliminating (knocking out) gene expression, or by overexpressing the gene product.

An even more direct path from human disease to invertebrate model is possible with certain human disorders — those caused by a toxic dominant gain-of-function mechanism. If disease is produced in humans by the action of a toxic protein, it might not be necessary, or even desirable, to manipulate the invertebrate homologue of the human disease-related gene. Instead, simple expression of the toxic human protein in the model organism might accurately model the disease. Toxic dominant mechanisms almost certainly operate in neurodegenerative disorders such as Huntington's disease and amyotrophic lateral sclerosis (ALS), and similar mechanisms have been proposed in more common neurodegenerative diseases. The formation of insoluble protein aggregates (inclusion bodies) characterizes virtually all neurodegenerative diseases, leading many investigators to speculate that certain proteins can become toxic after abnormal folding and aggregation events (FIG. 1).

Our laboratory and others have used the GAL4/UAS (upstream activating sequence) system to express human proteins in *Drosophila*[6] (FIG. 2). In this system, a human disease-related transgene is placed under the control of the yeast transcriptional activator GAL4. In the absence of GAL4, the transgene

Box 1 | Classical *Drosophila* genetics applied to neurodegeneration

The amenability of *Drosophila* to large-scale forward-genetic screens has provided a powerful method with which to investigate a wide range of biological processes. Mutants are selected on the basis of a desired phenotype, often shortened lifespan in the case of neurodegeneration mutants, and are then analysed to determine the identity of the mutated gene. Forward-genetic screens have identified a variety of mutations in endogenous fly genes that produce degeneration of the nervous system.

Substantial death of neurons in flies often produces many holes, or vacuoles, in central nervous system tissue. So, *Drosophila* mutants that show neurodegeneration have traditionally been dubbed descriptively: *swiss cheese*[53], *bubblegum*[51], *spongecake*, *eggroll*[54]. Sometimes, gene names reflect the abnormal behaviour of mutant flies: *drop dead*[55] and *pirouette*[56], for example.

The *bubblegum* mutant provides the most direct connection between a fly neurodegeneration mutant and a human disease[51]. Both *bubblegum* flies and patients with the metabolic disorder adrenoleukodystrophy (ALD) accumulate abnormal amounts of very long chain fatty acids (VLCFAs). The *bubblegum* mutant flies have a mutation in the VLCFA acyl coenzyme A synthetase gene. This enzyme has reduced activity in patients with ALD, but the VLCFA acyl coenzyme A synthetase gene is not mutated in the human disease.

Primary defects in glial cells have been implicated as an important mechanism of neurodegeneration in *Drosophila*. The *drop dead* and *swiss cheese* mutants show glial abnormalities before neurons degenerate[53,55]. Intriguingly, primary glial cell defects apparently underlie neurodegeneration in some forms of human hereditary peripheral nerve degeneration, such as Charcot–Marie–Tooth disease[57].

is inactive. When flies that carry the human transgene are crossed to flies that express GAL4 in a specific tissue or cell type, the human protein is made only in the tissues that have GAL4. Many cell-type and developmentally regulated *GAL4* ('driver') lines exist at present, and are readily available from public stock centres. So, the effect of expressing a human transgene in many different tissues and at various developmental times can be assayed without creating many independent transgenic fly strains. This system provides a particular advantage for studying neurodegenerative disease, because the issue of cell-type specificity can be readily addressed.

A model of Parkinson's disease. Parkinson's disease is a movement disorder of late adult onset with three main clinical features: resting tremor, rigidity and bradykinesia. The neuropathological hallmarks of this condition are progressive degeneration of dopamine neurons in the substantia nigra pars compacta, and the presence of cytoplasmic neuronal inclusions known as Lewy bodies (FIG. 1). Most cases are sporadic, but missense mutations (A53T and A30P) in the α-synuclein gene have been found in families with autosomal-dominant Parkinson's disease[7,8]. Although these mutations are a rare cause of familial Parkinson's disease, α-synuclein is an important component of Lewy bodies in the brains of patients with both familial and sporadic Parkinson's disease[9], indicating that the protein might have a central role in the pathogenesis of both forms of the disorder. A *Drosophila* model of Parkinson's disease has been produced by expressing wild-type and mutant forms of human α-synuclein in flies[10]. The expression of human α-synuclein in flies recapitulates key neuropathological features of Parkinson's disease (BOX 2). Transgenic flies show age-dependent, progressive degeneration of specific dopamine neurons, associated with the development of α-synuclein-immunoreactive cytoplasmic aggregates (FIG. 1). Under the electron microscope, these aggregates appear fibrillar, as are authentic Lewy bodies. In addition, the flies have progressive motor deficits. The development of a faithful model of Parkinson's disease, the most common movement disorder and the second most common neurodegenerative disease, will allow a genetic analysis of this important disorder.

Modelling Alzheimer's disease. Alzheimer's disease is the leading cause of dementia worldwide, and is defined pathologically by extracellular amyloid plaques and intracellular neurofibrillary tangles (FIG. 1), accompanied by neuronal loss. Neurofibrillary tangles are composed of aggregated, hyperphosphorylated forms of the microtubule-associated protein TAU. The distribution and extent of neurofibrillary pathology correlates with clinical symptomatology and neuronal loss[11]. Neurofibrillary tangles are also prominent in other neurodegenerative diseases, including the dominantly inherited frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). The recent discovery of mutations in the *TAU* gene in families with FTDP-17 provides compelling evidence that TAU dysfunction is directly involved in neurodegeneration[12]. A *Drosophila* model that is relevant to Alzheimer's disease and frontotemporal dementia has recently been created by expressing wild-type and mutant forms of human TAU[13] in fruitflies. The model reproduces several features of the human disease: age-dependent, progressive neurodegeneration, relative specificity of neuronal loss, premature death, and neuronal accumulation of abnormally phosphorylated forms of TAU.

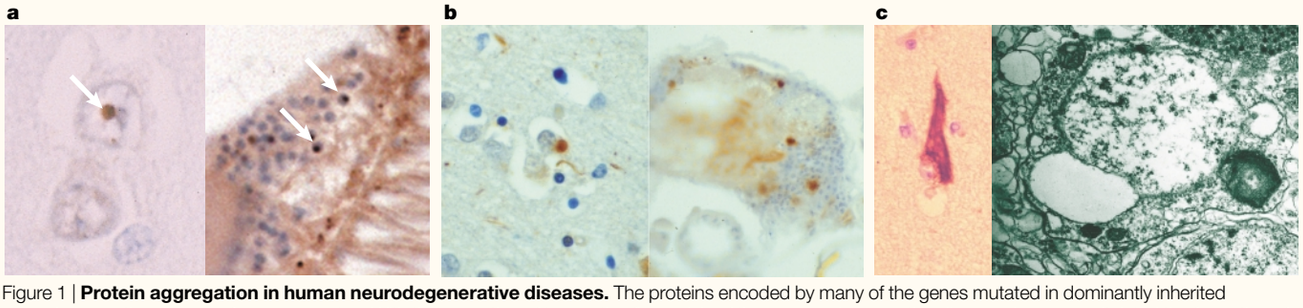

Figure 1 | **Protein aggregation in human neurodegenerative diseases.** The proteins encoded by many of the genes mutated in dominantly inherited neurodegenerative diseases form aggregates. With the exception of tauopathy, fly models of these neurodegenerative diseases have recapitulated the formation of these inclusions as well as neurodegeneration. **a** | The characteristic nuclear aggregates of polyglutamine diseases (arrows) are shown by immunohistochemistry in a brain section from a patient with Huntington's disease (left), and in a *Drosophila* model of Huntington's disease (right). **b** | Similarly, α-synuclein-immunoreactive cytoplasmic inclusions (Lewy bodies) are present in tissue from a patient (left) and in the *Drosophila* model (right). **c** | By contrast, TAU-rich neurofibrillary tangles form in Alzheimer's disease and related disorders (left), but not in fly models (right).

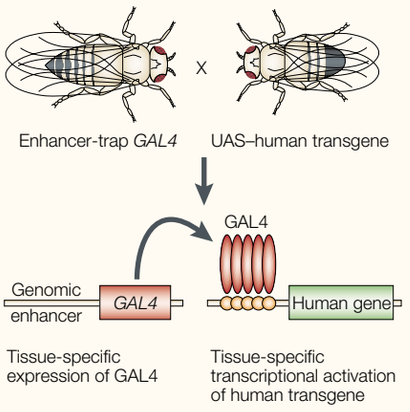

Figure 2 | **Making the fly model.** Nearly all of the current fly models of neurodegenerative diseases have been made using the GAL4/UAS (upstream activating sequence) system[6]. This system allows the ectopic expression of a human transgene in a specific tissue or cell type. Two transgenic fly lines are created. In the first (UAS–human-transgene fly), the human disease-related transgene is placed downstream of a UAS activation domain that consists of GAL4-binding sites. GAL4 is a yeast transcriptional activator; in the absence of ectopically expressed GAL4, the transgene is inactive in these transgenic flies. The transgene is activated by crossing these flies to transgenic flies that express GAL4 (enhancer-trap *GAL4* fly), also known as the 'drivers'. A wide array of 'driver' flies have been made and characterized. The *GAL4* gene is placed downstream of a cell- or tissue-specific promoter. Examples include the pan-neural promoter *elav* (*embryonic lethal, abnormal vision*) or the eye-specific promoter *GMR* (*Glass Multimer Reporter*). So, the transgene will be activated in the progeny of this cross in a specific cell or tissue type, depending on the 'driver'. This is especially important in studying neurodegenerative diseases, as questions regarding cell-type-specific death can be investigated.

Neurodegeneration is much more severe in flies that express mutant TAU than in flies that express the wild-type protein. Intriguingly, however, neurodegeneration occurs without the formation of neurofibrillary tangles (BOX 2). In *Drosophila*, at least, the neurotoxic properties of TAU might be conferred by protein alterations such as phosphorylation, rather than by tangle formation.

The other distinctive neuropathological feature of Alzheimer's disease is amyloid. Amyloid is produced by the sequential action of three enzymes — α-, β- and γ-secretase — on the amyloid precursor protein (APP). The activity of γ-secretase requires presenilin[14], and studies using putative aspartyl protease active-site inhibitors indicate that presenilin might itself be γ-secretase[15], although there is considerable disagreement about this issue. Recent studies have shown that the genes for both APP and presenilin can be mutated in familial Alzheimer's disease[16,17]. Missense mutations in presenilin 1 and 2 cause 80% of cases of familial Alzheimer's disease, and seem to increase production of the amyloidogenic Aβ42 peptide. Mutations in APP are much less common.

Studies in *Drosophila* have begun to address questions about the normal function of APP, as well as the mechanisms by which APP dysfunction might cause neurodegeneration in Alzheimer's disease. It was recently reported that APP might function as a vesicular kinesin 1 receptor in *Drosophila*[18]. Altered expression of the *Drosophila* homologue of APP, APP-like protein (Appl), and ectopic overexpression of human wild-type and mutant APP, caused axonal transport defects *in vivo*. Moreover, overexpression of human wild-type and mutant APP caused increased cell death in the larval brain. The toxicity of APP seemed to be dependent on both the possession of a β-amyloid peptide sequence and the carboxy-terminal cytoplasmic region that putatively binds kinesin 1. Although the presence of amyloid plaques was not reported in this study, a previous study showed that the β-amyloid peptide could be generated from a modified fragment of human APP in *Drosophila* both *in vitro* and *in vivo*[19].

Significant insights into the cellular pathways that govern amyloid production have been obtained by forward-genetic strategies. Studies of endogenous presenilins in *Drosophila* and *Caenorhabditis elegans* have revealed a role for these proteins in the proteolysis of another transmembrane protein, Notch[20–22]. In *Drosophila*, presenilin mediates the proteolytic cleavage of Notch, thereby allowing the intracellular domain of Notch to access the nucleus and activate transcription[23]. Interestingly, mutations in the human *NOTCH3* gene cause a distinctive neurological disorder, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)[24]. Further characterization of the endogenous fly presenilin/Notch pathway might provide insights into the biology of Alzheimer's disease. An intriguing hint of such parallel biology has been provided by recent data indicating that the carboxy-terminal fragment of APP, released by γ-secretase cleavage, might enter the nucleus and activate transcription, as does the intracellular domain of Notch[25].

Models of polyglutamine diseases. A diverse group of dominantly inherited neurodegenerative diseases is caused by expansions of polyglutamine-encoding CAG trinucleotide repeats within specific proteins[26]. These disorders have a common molecular pathogenesis: the expanded polyglutamine sequence confers a dominant gain of function on the encoded protein. There is a critical threshold for glutamine repeat number, below which the disease does not occur, and above which there is a correlation between glutamine repeat length and disease severity, with an inverse correlation between repeat length and age of onset. The neuropathological hallmarks of these diseases are strikingly selective neurodegeneration and neuronal intranuclear inclusions. Each of the polyglutamine disorders affects a distinct population of neurons. The differences in neuronal specificity are apparently conferred by the protein context of the expanded polyglutamines.

Recent work in *Drosophila* by Marsh and colleagues[27] has refined our understanding of how the expanded polyglutamine region confers a toxic gain of function on the disease-associated protein. They have shown that the polyglutamine peptide itself is neurotoxic in a length-dependent and progressive manner *in vivo*[27]. Furthermore, by expanding the polyglutamine segment of the *Drosophila* gene *dishevelled* (which is not associated with disease) they were able to show that the intrinsic toxicity of the polyglutamine segment could be altered by the protein context, which might explain the differing neuronal specificities of these diseases. In addition, they showed that the polyglutamine segment reduced, but did not abolish, the normal function of the Dishevelled protein. Interestingly, a recent report has indicated that the pathogenesis of Huntington's disease might result from a partial loss of function of the huntingtin gene[28].

Spinocerebellar ataxia type 3 (SCA-3), also known as Machado–Joseph disease, is the most common autosomal-dominant inherited ataxia. Patients often have extrapyramidal

Box 2 | **Neuropathology in fly models**

Huntington's disease
- Retinal degeneration
- Nuclear inclusions

Tauopathies
- Cholinergic neurodegeneration
- No large filamentous aggregates

Parkinson's disease
- Dopaminergic neurodegeneration
- Filamentous cytoplasmic inclusions

Spinocerebellar ataxia type 1
- Retinal abnormalities
- Nuclear inclusions

Spinocerebellar ataxia type 3
- Retinal degeneration
- Nuclear inclusions

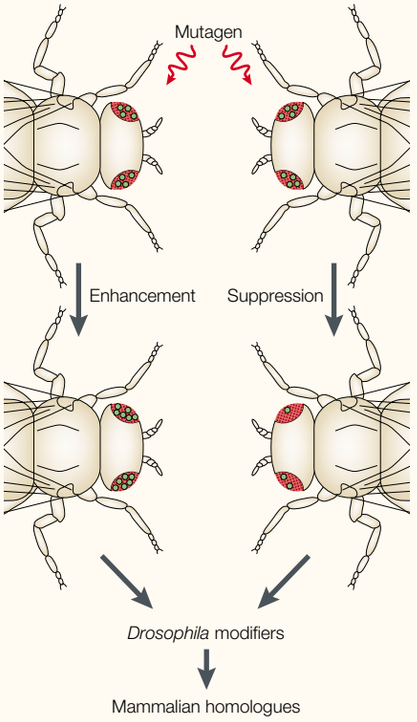

Figure 3 | **Unbiased identification of second-site modifiers of neurodegeneration in flies.** Once the fly model of a neurodegenerative disease has been characterized, genetic analysis can be carried out to identify modifier genes that enhance or suppress the phenotype. Such genes are likely to encode proteins that are involved in the cellular pathways that mediate neurodegeneration in the flies. Unbiased forward-genetic screens allow the identification of modifiers without preconceived ideas as to their function. Random mutations are induced in the flies' genome using chemicals or insertional mutagenesis techniques. These new mutations are then passed on to progeny flies. New mutations are screened for their ability to enhance or suppress the neurodegenerative phenotype (here depicted as dying cells in the retina; green circles). The interacting genes can then be mapped and cloned. Alternatively, collections of *Drosophila* stocks with known specific mutations (induced by mutagenesis) are available, and individual crosses can be carried out with the fly model. The progeny can then be analysed for enhancement or suppression of the phenotype. Mammalian homologues of these modifiers should be studied to verify their role in the human disease.

signs, such as stiffness, in addition to ataxia. Post-mortem analysis of patients reveals degeneration of cerebellar dentate neurons, and loss of neurons from the basal ganglia and brainstem[29]. Expression of truncated carboxy-terminal fragments of ataxin 3 with an expanded polyglutamine region is sufficient to cause progressive degeneration of cerebellar neurons in mice[30]. Warrick and colleagues have made a *Drosophila* model of SCA-3 that reproduces, in the *Drosophila* eye, some of the neuropathological features of the human disease[31] (BOX 2). The expression of truncated fragments of human ataxin 3 with an expanded polyglutamine repeat in the *Drosophila* retina produces late-onset progressive neurodegeneration. It is important to note that the expression of unexpanded ataxin 3 protein does not cause retinal degeneration. In addition, expanded ataxin 3 protein accumulates in neuronal intranuclear inclusions that are typical of the human disease.

Huntington's disease is an autosomal-dominant disorder. Patients present with chorea, dementia and neuropsychiatric symptoms. All patients have an expansion of greater than 35 repeats in the polyglutamine segment of the mutant huntingtin protein[32]. The neuropathology is characterized by progressive neuronal loss from the striatum and frontal cortex, in association with the presence of neuronal intranuclear inclusions[33] (FIG. 1). Expression of expanded (120 glutamines), but not unexpanded, huntingtin protein under an eye-specific promoter in the *Drosophila* retina produces age-related progressive neurodegeneration accompanied by nuclear inclusions[34] (FIG. 1; BOX 2). There is also a direct relationship between the number of expanded repeats and the severity of neurodegeneration. The degenerative nature of the pathology, the presence of characteristic intranuclear inclusions, and the graded pathology produced by increasing polyglutamine number, represent strong points of similarity between the fly models and their cognate human diseases. Another fly model of Huntington's disease that is the product of the GAL4/UAS system has recently been reported[35]. Expression of the first exon of the huntingtin gene with 93 glutamines also resulted in progressive neurodegeneration in the retina. Further pharmacological and genetic analyses that have been carried out in this model will be discussed later.

Spinocerebellar ataxia type 1 (SCA-1) is a dominantly inherited ataxia. The main neuropathology is selective degeneration of cerebellar Purkinje cells and ataxin-1-positive neuronal nuclear inclusions. *Drosophila* models of SCA-1 have been created by the expression of expanded and unexpanded ataxin 1 using the GAL4/UAS system[10,36]. As in other polyglutamine models, expression of expanded ataxin 1 produces retinal pathology and nuclear inclusions (BOX 2). Surprisingly, the expression of unexpanded ataxin 1 is also toxic. So, ectopic expression has revealed an intrinsic toxicity of the normal ataxin 1 protein that might be relevant to the human disease.

Using *Drosophila* models

Once relevant *Drosophila* models of neurodegenerative disease have been created, the genetic potential of the system can be exploited. Second-site modifier analysis identifies unlinked mutations that either suppress or enhance neurodegeneration (FIG. 3). Such modifier genes encode proteins that are involved in the pathogenesis of the neurodegenerative process in flies, and potentially in the human disease as well. One strength of genetic analysis in *Drosophila* is that the whole cellular cascade that mediates neurodegeneration, including both specific interactors and downstream elements, can be defined. In practical terms, the phenotype that is used to select genetic modifiers should be externally visible, easily scored and involve structures that are not essential for viability. Abnormalities of the *Drosophila* eye have therefore been the phenotypes of choice in modifier screens.

Modifier identification can follow both biased and non-biased strategies. In the biased 'candidate' approach, mutations are selected on the basis of pre-existing hypotheses, and these mutations are tested for their ability to suppress or enhance neurodegeneration. Candidate testing can rapidly confirm the role of suspected mediators, but is limited by preformed hypotheses. The second approach is to do an unbiased forward-genetic screen. A forward-genetic screen interrogates the genome for mutations that modify a neurodegenerative phenotype, without bias as to possible function. Random mutations are produced by chemical or insertional mutagenesis, and the ability of these mutations to suppress or enhance the phenotype of interest is tested. The unbiased approach has the potential to identify new proteins, or to implicate previously defined cellular pathways that were not suspected to be important in neurodegenerative disease.

Using the candidate approach, Warrick *et al.*[37] have shown that the chaperone system of heat-shock proteins is involved in polyglutamine toxicity. Overexpression of the molecular chaperone HSP70 suppresses the toxicity of expanded ataxin 3, whereas expression of a dominant-negative heat-shock cognate enhances toxicity. The HSP40 family members regulate the activity of HSP70 and often act as co-chaperones. Chan *et al.*[38] showed that overexpression of dHdj1 (DnaJ-1), the fly orthologue of the human HSP40 member HDJ1, suppressed the toxicity of expanded ataxin 3. Mutant forms of dHdj1 enhanced toxicity. Furthermore, they showed synergy between Hsp70 and dHdj1 in suppressing ataxin 3 toxicity. The same researchers have very

recently shown that the chaperone system might also be important in the pathogenesis of Parkinson's disease[39]. Overexpression of human HSP70 ameliorated dopaminergic cell loss in *Drosophila* that expressed α-synuclein. Furthermore, expression of a dominant-negative mutant of endogenous *Drosophila* Hsp70 enhanced the toxicity of α-synuclein in dopaminergic neurons. As in the human disease, Lewy-body-like inclusions in flies were immunoreactive for endogenous Hsp70. All these studies support the idea that misfolded proteins and/or aggregates might be toxic to neurons, and that molecular chaperones are important in combating their toxicity. Alternatively, the sequestration of chaperones into aggregates might lead to neurodegeneration. Further studies should distinguish between these two possible mechanisms.

Two other groups have implicated the heat-shock chaperone system in neurodegeneration on the basis of forward-genetic approaches. Kazemi-Esfarjani and Benzer carried out a forward-genetic screen to identify mediators of polyglutamine toxicity[40]. They found that the expression of two proteins, dHdj1 and Tpr2 (tetratricopeptide repeat protein 2), another gene homologous to a human gene that encodes a protein bearing a chaperone-related J domain (TPR2), suppress polyglutamine toxicity in the fly eye. A genetic screen for modifiers of expanded ataxin 1 toxicity identified mutations in proteins that are involved in the heat-shock chaperone response and the ubiquitin/proteasome system[36]. Consistent with other studies, overexpression of *dHdj1* suppressed the toxicity of expanded ataxin 1.

The ataxin 1 screen also identified genes and pathways that had not previously been implicated in polyglutamine-induced neurodegeneration[36]. Overexpression of the gene that encodes glutathione-*S*-transferase (GST) suppressed ataxin 1 toxicity. The human family of GSTs is involved in cellular detoxification and uses reduced glutathione to eliminate potentially damaging proteins that result from chemical and oxidative stress. Another finding was that overexpression of a *Drosophila* gene, *nucleoporin 44A*, which bears homology to a yeast nuclear-pore protein, suppressed ataxin 1 toxicity. Nuclear translocation of a cleaved fragment of the expanded protein is thought to be central to the pathogenesis of polyglutamine disorders. The mechanism by which overexpression of this gene suppresses toxicity is unclear, but it might be due to abnormal pore formation, impairing translocation of cleaved ataxin 1 to the nucleus. RNA-binding proteins were also identified as modifiers. Disturbances in RNA stability and processing might, therefore, also be involved in the pathogenesis of SCA-1. The roles of all these proteins should be assessed and verified in mammalian systems. All might hold promise as new therapeutic targets.

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Transcriptional dysregulation is increasingly being implicated in the pathogenesis of polyglutamine disorders. Proteins that contain expanded polyglutamine tracts have been shown to interact directly with and sequester transcriptional cofactors, including CREB-binding protein (CBP), thereby causing decreased transcription[41]. An alternative mechanism might work through the effects of expanded polyglutamine proteins on histone acetylation. An important class of modifiers that were identified in the ataxin 1 forward screen included a group of transcriptional cofactors[36]. Four of these, Sin3A, Rpd3, CtBP and Sir2, are known to regulate gene expression through histone acetylation. Recently, Steffan *et al.* have provided further evidence for the role of impaired histone acetylation in the pathogenesis of polyglutamine diseases[35]. *In vitro*, they showed that the expanded huntingtin peptide interacted with the acetyltransferase domains of several transcriptional cofactors, including CBP. This impaired their acetyltransferase activity, and caused reduced acetylation of histones H3 and H4. Furthermore, in a *Drosophila* model of Huntington's disease, the authors were able to ameliorate retinal neurodegeneration and early death induced by expanded huntingtin by feeding flies with histone deacetylase (HDAC) inhibitors. This was also confirmed genetically: the toxic effect of expanded huntingtin was ameliorated in a loss-of-function mutant of Sin3A (a cofactor that is a component of HDAC complexes). Surprisingly, this effect of Sin3A on neurodegeneration is opposite to that found in the ataxin 1 screen[36]. These findings need to be further validated in other systems and polyglutamine models, but indicate that different expanded polyglutamine proteins have different effects on transcriptional cofactors.

Modifier analysis has implicated apoptosis as the cell-death mechanism in some *Drosophila* models of human neurodegenerative diseases. Overexpression of the baculovirus anti-apoptotic protein p35 partially suppresses ataxin 3 toxicity[31], although similar suppression does not occur in the fly model of Huntington's disease[34]. Furthermore, in flies with retinal degeneration produced by mutations in *Drosophila* rhodopsin, cell death is blocked by co-expression of p35 (REF. 42). The human disease retinitis pigmentosa can also be caused by mutations in rhodopsin, and apoptosis has been implicated in the human disorder.

The future

Drosophila models now exist for a range of human neurodegenerative diseases, and the stage is set for a comprehensive genetic analysis of pathways that mediate neuronal degeneration. Modifier screens continue in the polyglutamine models, and are also under way in other *Drosophila* models. Identification of new genes that are involved in the pathogenesis of the human diseases will be a key goal of these studies. Mammalian homologues of *Drosophila* modifiers must be studied to verify their roles in human disease. In the long term, the products of these genes might provide valuable drug targets. In addition, homologous loci represent attractive candidates for sequencing in families with inherited forms of neurodegenerative diseases in which the underlying mutation remains unknown. Identifying mutations in familial disease in a gene that was originally isolated as a modifier in a *Drosophila* model would provide a rigorous demonstration of the relevance of the *Drosophila* system to human disease.

Although existing fly models of neurodegenerative disease show significant promise, refinements to existing models might also prove to be valuable. In mice, combining both human TAU and amyloid pathology in the same animal markedly increases the degree of neurofibrillary pathology[43,44]. Perhaps co-expressing APP or β-amyloid with human TAU would produce synergistic effects in flies. Attempts to model two prominent neurodegenerative diseases, ALS and Creutzfeldt–Jakob disease, in flies have, so far, been unsuccessful. Expression of disease-linked mutant forms of superoxide dismutase[45] or prion protein[46] failed to produce salient features of the human disorders. Further attempts to create models for these diseases, perhaps by introducing human-specific cofactors, might be productive.

There are also many opportunities to model loss-of-function diseases in *Drosophila*

by reducing or abolishing gene expression. In particular, loss-of-function mutations in the parkin gene are an important cause of familial Parkinson's disease[47]. Mutation of the *Drosophila parkin* homologue could provide a useful model of recessive familial Parkinson's disease.

Recent technical advances have made conditional expression feasible in transgenic animal models. In a conditional transgenic mouse model of Huntington's disease, disease progression can be reversed by abolishing expression of the mutant huntingtin, even after the onset of behavioural and neuropathological abnormalities[48]. Conditional transgenic systems also exist in *Drosophila*[49,50], and could be used to explore the relationship between duration of gene expression and disease onset and progression.

Drug screening in flies. Their low cost, small size (several hundred thousand flies can be kept in a single lab) and short lifespan make *Drosophila* an attractive organism for use in several drug applications. Candidate drug compounds can be tested in *Drosophila* models by assaying the same phenotypes as are used in genetic analysis. Compounds that suppress neurodegenerative phenotypes in flies could represent potential therapeutic agents for the human disease. Steffan and colleagues have recently shown that HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA), can ameliorate neurotoxicity in a *Drosophila* model of Huntington's disease[35]. This drug is approved by the US Food and Drug Administration (FDA) for use in other conditions. If the findings in *Drosophila* can be verified in mammalian models of polyglutamine disease, then this class of drug deserves serious consideration for use in clinical trials of Huntington's disease and related conditions. In another condition, the metabolic disorder adrenoleukodystrophy (ALD), patients accumulate abnormal amounts of very long chain fatty acids (VLCFAs); this phenotype is also found in *bubblegum* mutant flies[51]. Feeding developing flies with monounsaturated fatty acids ('Lorenzo's oil') causes normalization of VLCFAs and prevents neurodegeneration. Similar treatment of ALD patients also lowers VLCFA levels, but unfortunately does not arrest neurological deterioration in most cases[52].

Direct screening of drug libraries for compounds that ameliorate disease phenotypes represents another potential application of *Drosophila* models. In addition to providing insights into disease pathogenesis, drug screening in flies could represent a rapid and inexpensive way to obtain pharmacological candidates for testing in mammalian systems.

Concluding remarks

Despite extensive research in vertebrate systems, there are many outstanding questions about the pathogenesis of neurodegenerative diseases. What are the cellular pathways that mediate neurodegeneration? What underlies the remarkable tissue and cellular specificity seen in these diseases? What is the role of inclusion bodies? *Drosophila* models of neurodegenerative disease have the potential to provide substantial insights into these questions, as illustrated by the recent paper by Steffan and colleagues[35]. However, caution is warranted. Despite the conservation of important basic cell processes in *Drosophila* and mammals, there are likely to be subtle but important differences between the organisms that might result in observations in *Drosophila* that are not relevant to human disease. For example, full-length unexpanded ataxin 1 is not toxic in humans, but is toxic in *Drosophila*[36] and mice. Steffan *et al.*[35] showed *in vitro* that even unexpanded huntingtin protein fragments can inhibit acetyltransferase activity; they suggest that its deleterious effects are averted in humans by its low expression and location. Therefore, the strategies used to make transgenic flies might not model pathology comparable to that seen in humans, owing to inappropriate levels of expression that will result in the activation of non-relevant pathways. There might also be important cellular pathways that require specific molecules that are not present in the fly. This could explain the failure of researchers to make a compelling model of ALS[45]. Despite these reservations, the remarkable recapitulation of the principal features of these neurodegenerative diseases in flies does support the idea that homologous cellular pathways are involved in pathogenesis in both organisms. It is to be hoped that observations made in flies, particularly with regard to modifier genes, will be verified promptly in mammalian systems.

Drosophila models might be less useful in addressing the mechanisms of production of the behavioural and motor abnormalities that are so prominent in these diseases. For example, the extensive anatomical differences between the fly and vertebrate motor systems will probably limit the contribution of the *Drosophila* model to questions about exactly how the abnormal movements in Parkinson's disease are caused by the loss of specific populations of dopamine neurons. Reproducing the cellular biochemistry of neurodegenerative disorders is likely to be the most useful feature of fruitfly models. One hundred years after Morgan's work, researchers have found a new role for *Drosophila melanogaster* in their fight against human diseases. Would Morgan or Alzheimer have predicted this remarkable convergence?

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Online links

DATABASES

The following terms in this article are linked online to:

FlyBase: http://flybase.bio.indiana.edu/
Appl | *bubblegum* | CtBP | *dishevelled* | DnaJ-1 | *drop dead* | *eggroll* | *elav* | *GMR* | Notch | *parkin* | *pirouette* | Rpd3 | Sin3A | Sir2 | *spongecake* | *swiss cheese* | Tpr2

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
APP | ataxin 1 | ataxin 3 | CBP | HDJ1 | HSP70 | huntingtin | *NOTCH3* | parkin | presenilin 1 | presenilin 2 | prion protein | superoxide dismutase | α-synuclein | TAU | TRP2

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
ALD | ALS | Alzheimer's disease | CADASIL | Charcot–Marie–Tooth disease | Creutzfeldt–Jakob disease | FTDP-17 | Huntington's disease | Parkinson's disease | retinitis pigmentosa | SCA-1 | SCA-3

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OPINION

The problem of functional localization in the human brain

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Functional imaging gives us increasingly detailed information about the location of brain activity. To use this information, we need a clear conception of the meaning of location data. Here, we review methods for reporting location in functional imaging and discuss the problems that arise from the great variability in brain anatomy between individuals. These problems cause uncertainty in localization, which limits the effective resolution of functional imaging, especially for brain areas involved in higher cognitive function.

In the past ten years, rapid improvements in imaging technology and methodology have had an enormous impact on how we assess human cognition. Detailed anatomical images can be combined with functional images that are obtained using techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to address questions that relate to normal and abnormal brain function. A chief advantage of techniques such as fMRI and PET over methods such as electroencephalography, magnetoencephalography and neuropsychology is their ability to localize changes in brain activity with a high degree of spatial resolution. In this article, we will argue that specifying where in the brain an activation has occurred is both conceptually and technically more difficult than has been generally assumed.

Processing steps in functional imaging

A functional imaging study involves the collection of one or more functional scans for each subject, which show signal changes in regions where neuronal work has increased (FIG. 1). To compensate for subject movement, it is usual to realign the functional images to one of the images in the series. Most investigators collect a separate structural scan that has good spatial resolution to image the anatomy of the brain. The structural scan may have a different field of view, voxel size or orientation, so that it will need to be coregistered to the functional images using an automated image-matching algorithm.

There are now two possible approaches to the analysis. The first is to proceed directly to the statistical analysis. This approach maintains a very clear relationship between the subject's anatomy and activation. Many researchers prefer the second approach, which