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News and Commentary

'Functional DNA array' in the fly: implication for neuronal degeneration

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From the beginning of the previous century, the *Drosophila* genetic system has provided a powerful approach for elucidating basic cellular functions in the context of a developing and functioning multi-cellular system. Recently, *Drosophila* has been used to elucidate mechanisms of human neurodegenerative disease, including Alzheimer's, Parkinson's, and Huntington's disease.[1] These studies have led to new insights into the normal functions of disease-causing proteins, and have provided *Drosophila* models for investigating genetic approaches to prevent or delay the toxic effects of disease proteins. Here we describe a recently developed technique for genetic screening in *Drosophila*, the gain-of-function screen (GOF screen),[2,3] and its application for identifying gene products that can reduce neurodegeneration in *Drosophila* models.

Previous screens for novel or functional genes in *Drosophila* were designed to isolate mutants exhibiting some kind of unusual phenotype or dominant modifiers of abnormal phenotypes. These screens used chromosome-deficient or mutagenized flies to identify genes that, when mutated in one or two copies, could enhance or suppress a mutant phenotype. In the cell death field, these types of screens have been extremely successful and efficient for genetically dissecting the cell death-signaling pathways, including the identification of Reaper (Rpr), Head Involution Defective (Hid), Grim, and DIAP1.[4] However, because more than half of all genes in the genome do not mutate to any visible phenotype, it is likely that these screens did not fully identify all the relevant interacting genes. Recently, a gain-of-function/misexpression screen (GOF screen) was developed to identify such genes[2,3] (Figure 1). In the GOF screen, the expectation is the same as with those described above, i.e., that a change in the dosage of a molecule will affect the phenotype. This screen is based on the GAL4/UAS system, which has been used extensively to force the ectopic expression of genes in flies.[5] In the GOF screen, the EP-element[2] or GS vector[3] are used. These transposon vectors contain one or two copies of the upstream activating sequence (UAS) enhancer at the end of the transgene, which drive the expression of the closest downstream endogenous gene when combined with a transgene expressing GAL4 under the control of a tissue-specific promoter. Thus, in the GOF screen, when EP or GS lines are crossed to various GAL4 driver lines, a huge number of unknown genes in the genomes of specific tissues are overexpressed (Figure 1).[2,3]

To identify novel components affecting the degenerative changes in neurons frequently observed in human neural diseases, the *Drosophila* GOF screen can be more useful than any other genetic screen, because it enables the rapid identification of overexpressed gene products. If some groups of overexpressed genes can improve the pathogenic phenotypes in fly neurodegenerative models, such gene products may have the potential to be directly applicable to the prophylactic or therapeutic treatment of human diseases, for example, via gene therapy. Because the GOF screen also depends on the transposase-based EP-element or GS vector insertion into the *Drosophila* genome, standard plasmid-rescue methods can be used to identify candidate genes, making use of the complete *Drosophila* genome information.[2,3] Based on these properties, the GOF screening method in *Drosophila* can be used as a 'functional DNA array *in vivo*'.

Gain-of-function studies with the GOF screen were successfully performed to generate a *Drosophila* model of human neurodegenerative diseases (Table 1). This approach was first used to study human polyglutamine repeat disease. The polyglutamine diseases are characterized by the late-onset progressive neuronal loss of specific brain regions. Expansion of a polyglutamine repeat within respective disease proteins confers a dominant toxicity on the proteins. Two different human genes for polyglutamine diseases, Huntington's disease and spinocerebellar ataxia (SCA)/Machado-Joseph disease (MJD), have been introduced into *Drosophila*.[6,7] In both cases, expression of a truncated form of the protein containing the expanded polyglutamine region in the fly compound eye results in adult-onset progressive degeneration of pigment cells and photoreceptor neurons. As in the diseased neural tissues from humans, where nuclear inclusions are often seen, abnormal protein aggregation is observed in the nuclei of the eye imaginal disc cells, suggesting that *Drosophila* may use common molecular pathways to execute the pathogenic changes and neurodegeneration that characterize these diseases.[6,7] The general caspase inhibitor p35 has little or no effect on these disease phenotypes, suggesting that polyglutamine-induced cell death might be caspase-independent in *Drosophila*. Use of the GOF screen, has led to the identification of molecules that can modify the progression of neurodegeneration.[8,9] Expression of Hsp70 (a major stress-induced molecular chaperone) or dHdj1, the

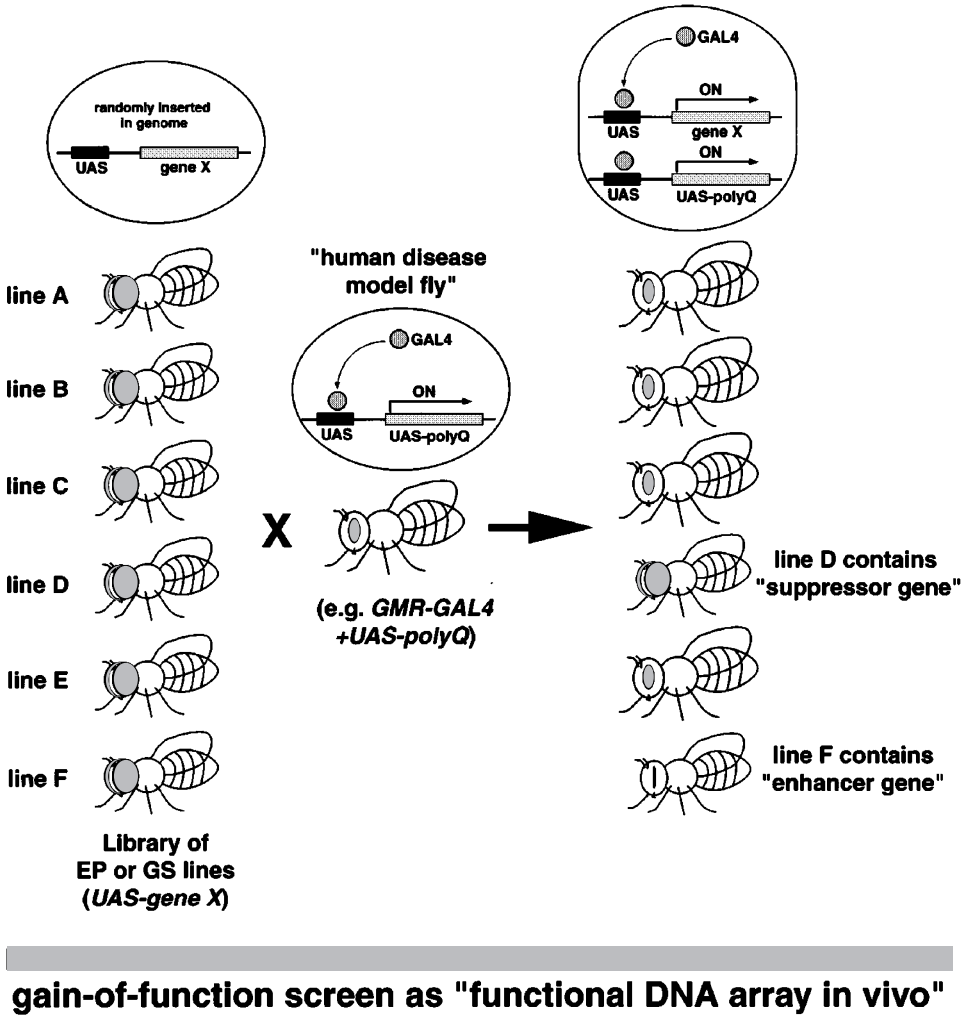

Figure 1 The gain-of-function screen in *Drosophila*. Screening method for the identification of genes that affect neural degeneration in fly models for human diseases is shown in a case of polyglutamine disease model. For this screen, females of the genotype *GMR-GAL4+UAS-polyQ* (these flies always exhibit 'rough eye' phenotype) are crossed to males from each individual line from the collection of EP or GS lines. The EP or GS lines have one UAS-vector randomly inserted in the genome. Each F1 progeny will contain both overexpressed polyglutamine and product of unknown gene X in *Drosophila* compound eye. If the product of gene X can inhibit the polyglutamine-mediated toxicity in neural tissues, the rough eye phenotype will be improved. Strains producing either suppressed or enhanced effect against rough eye phenotype of *GMR-GAL4+UAS-polyQ* fly are then scored and analyzed

fly orthologue of Hsp40, significantly suppresses the polyglutamine-induced eye degeneration without affecting the formation of nuclear inclusions.[8] Another polyglutamine disease model was generated by expressing the full-length human spinocerebellar ataxia type 1 (SCA1) gene, ataxin-1, in *Drosophila*. Expression of ataxin-1 containing the expanded polyglutamine region in the fly eye leads to eye degeneration as well as the formation of nuclear inclusions in photoreceptor neurons. In this model, the GOF screen also identified several dominant modifiers, including a molecular chaperone (DnaJ1) and a cellular detoxification enzyme (GST-θ).[9] These findings may be relevant to the treatment of human polyglutamine diseases and perhaps to other neurodegenerative diseases that may be caused by toxic inclusions or aggregation, such as Parkinson's and Alzheimer's disease.

To reveal the precise mechanisms of neural degeneration using the GOF screen, other kinds of neurodegenerative models in *Drosophila* are needed, and many researchers are working to develop new fly models for human neuronal diseases (Table 1).[10,11] Parkinson's disease is a common neurodegenerative syndrome characterized by the loss of dopaminergic neurons in the substantia nigra, formation of filamentous intraneuronal inclusions (Lewy bodies), and an extrapyramidal movement disorder. Mutations in the α-synuclein gene are linked to familial Parkinson's disease, and α-synuclein accumulates in Lewy bodies and Lewy neurites.[12] Normal and mutant forms of α-synuclein were expressed in *Drosophila* neurons and adult-onset loss of dopaminergic neurons, filamentous intraneuronal inclusions containing α-synuclein, and locomotor dysfunction were observed.[10]

Table 1 *Drosophila* models for human neurodegenerative diseases

Human disease	Gene product	Promotor or GAL4	Phenotypes
Huntington's disease[6]	Huntingtin	*GMR*	Photoreceptor degeneration
SCA3/MJD[7,8]	Polyglutamine	*GMR-GAL4*	Photoreceptor degeneration Rough eye and loss of pigment
SCA1[9]	Ataxin-1	*GMR-GAL4*	Photoreceptor degeneration Rough eye and loss of pigment
		ap-GAL4	Degeneration of interneuron
Parkinson's disease[10]	α-Synuclein	*elav-GAL4*	Loss of dopaminergic neuron Reduction of locomotor activity
		GMR-GAL4	Retinal degeneration
Alzheimer's disease[11]	Tau	*elav-GAL4*	Reduction of viability Neuronal degeneration
		Cha-GAL4	Loss of cholinergic neuron
Alzheimer's disease[17]	Presenilin	*GMR-GAL4*	Rough eye and loss of pigment
Alzheimer's disease[16]	APP	*69B-GAL4*	Wing blister

Alzheimer's disease is characterized by senile plaques, which are composed of extracellular deposits of Aβ peptide, and neurofibrillary tangles (NFTs), which are composed of intracellular filamentous aggregates of hyperphosphorylated tau proteins. Mutations in the gene encoding tau protein cause frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Transgenic mice expressing mutant tau (P301L) mimic the features of human tauopathies, including the development of NFTs, amyotrophy, and progressive motor disturbance.[13] As done for the mouse model, the fly model of tauopathies was generated by expressing mutant tau (R406W or V337M).[11] Pan-neuronal expression of mutant tau in flies resulted in late-onset neurodegeneration, early death, and the accumulation of abnormal tau but not NFTs.[11] These neuropathological phenotypes were observed preferentially but not absolutely in cholinergic neurons. In the mouse model of tauopathies, the NFTs formed by the expression of mutant tau were enhanced by the co-expression of a mutant form of APP (APP695-Swedish, K670N and M671L) or by the injection of Aβ42.[14,15] Given that an APP695-Swedish fly has been generated,[16] it would be interesting to examine the neuropathology of a mutant tau/APP695-Swedish double transgenic fly. In these fly models for Parkinson's and Alzheimer's diseases, the ectopic expression of a mutant form of human tau or α-synuclein can cause a rough-eye phenotype or photoreceptor cell degeneration,[10,11] indicating their practical application for a large-scale GOF screen.

In conclusion, several *Drosophila* neurodegenerative models have been made that recapitulate the essential features of the human disorder they represent, and make possible a powerful genetic approach to understanding human diseases, for example, Parkinson's and Alzheimer's disease. Future studies using GOF screening in these fly models should elucidate the pathogenic mechanisms of the diseases and develop opportunities for the prophylactic or therapeutic treatment of neurodegeneration.

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