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Of fish, flies, worms and men: powerful approaches to neuropsychiatric disease using genetic models

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Neuropsychiatric diseases are common; current therapies are only partially effective in controlling symptoms and there are no curative treatments. These diseases cause significant suffering and cost a staggering amount to society in treatment, care and lost productivity, and are consequently a high priority for research. The past two decades have witnessed an extraordinary expansion in our knowledge of the molecular basis of neurological diseases. Much of this progress has arisen through mapping gene loci in families with genetically-determined neurological diseases. The subsequent positional cloning of genes whose mutation provokes neurological disease has provided researchers with a solid foundation for investigating molecular mechanisms that might provide novel therapeutic targets. Although in some instances the relationship between common sporadic neurological diseases and their rare Mendelian phenocopies remains uncertain, the unequivocally causative connection between genetic mutation and disease etiology in hereditary diseases is important. There are few examples of sporadic neurological diseases in which the etiological factors are known in any detail, but it is expected that mechanistic commonality between genetic and sporadic forms of diseases will allow meaningful insights to be gained into the latter from understanding the former. This prediction is supported by the many clinical, pathologic and biochemical features that are shared between sporadic and hereditary forms of many diseases.

Advances in neurogenetics have provided some practical problems regarding the most effective way in which to exploit new information in order to move forward rapidly to detailed mechanistic understanding and new therapies. Although basic cell biological functions of proteins can be characterized rapidly in cultured cell lines, there are concerns that the mechanisms identified by these approaches might not be directly relevant to the abnormal

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functions of the proteins in disease. This is especially true in instances where mutation of a ubiquitously expressed gene provokes a specific neurological phenotype, suggesting that the pathogenically-relevant functions of the protein might be unique to a subpopulation of neural cells. Cultured neurons only partially address this question, since it is difficult to culture specific neuronal subpopulations of relevance in many instances, and it is becoming apparent that glial cells, the blood brain barrier, and the CNS internal environment may also play critical roles in pathogenesis of neurological disease (Boillee et al., 2006; Kordower et al., 2008; Li et al., 2008; Yamanaka et al., 2008). In addition, functional disturbances underlying psychiatric disease and some movement disorders (for example dystonia) are not accompanied by cell death or known biochemical markers of cell dysfunction (Bressman and Ozelius, 2007). In these circumstances, characterization of gene function might only be adequately accomplished in the context of neural circuits. Finally, many of the diseases show chronic evolution over a time course that could not be practicably mimicked in cultured cells. These considerations have led to the use of *in vivo* models of disease. Generation of transgenic mice is now routine and such models that recapitulate genetic human disease abnormalities have provided key insights into pathogenesis through hypothesis-driven studies. However, the generation and maintenance of transgenic mouse colonies is labor-intensive and there are practical limits on the number of experiments that can be carried out and consequently on the range of hypotheses that can be tested. This proves limiting in some situations; for example, when there are many alternative hypotheses or a number of putative modifier genes or environmental influences that might be important, an unmanageable number of experimental possibilities arises. The situation becomes more difficult when unsuspected mechanisms are important, since these might be overlooked by the traditional hypothesis-driven approaches than can be practicably deployed in murine models. These considerations have led some researchers to look to other experimental systems that might provide opportunities for higher-throughput *in vivo* approaches based on modification of phenotypic outcomes and unbiased by presumptions about mechanism. Three non-mammalian models that were well-established in other fields of biological research, *Caenorhabditis elegans*, *Drosophila melanogaster* and *Danio rerio*, have been adopted for studies of pathogenic mechanisms for this reason. Each of these model systems is represented in an emerging literature in neuropsychiatric disease research, in which the functional consequences of genetic manipulations that mimic human disease have been studied, the functions of relevant proteins elucidated *in vivo*, and in some cases unbiased phenotype-modifier screens carried out to reveal new pathogenic mechanisms. In this special issue of *Neurobiology of Disease*, we showcase some recent contributions of these model systems to our understanding of the molecular basis of neurological disease.

Worm: The nematode *C. elegans* was first employed for genetic studies by Brenner in the early 1970s who shared the 2002 Nobel Prize for discoveries in programmed cell death made using this model system (Brenner, 1974; Sulston and Brenner, 1974). The nervous system of this simple invertebrate organism is characterized by a highly uniform number of neurons that are stereotyped in terms of topology, neurochemistry and connectivity. Consequently, individual cells can be reliably identified experimentally; furthermore simple and reproducible motor behaviors can be used as assay end points. There is surprising phylogenetic conservation of genes implicated in human diseases and their homologues in *C. elegans*, allowing functional evaluation of many biochemical pathways of interest *in vivo*. Genetic techniques allow the expression of transgenes in specific neuronal populations, genes can be targeted using bacterial substrate expressing RNAi, and stable mutants can be recovered relatively easily from mutagenesis screens. *C. elegans* has a short generation time and lifespan; the model has found particular utility in studies on aging and its genetic modifiers, in addition to the identification of genetic interactions in cell death and other pathways. Reviews by Wentzell and Kretschmar, Dimitriadis and Hart, and Voisine *et al.* in this issue summarize the generation and analysis of *C. elegans* models of Parkinson's disease (PD), Huntington's disease (HD) and dementia, and

discuss the roles of molecular chaperones in the pathogenesis of neurodegenerative diseases associated with protein misfolding, elucidated using *C. elegans* models.

Fly: The common fruitfly *D. melanogaster* was first employed in laboratory genetic studies by Castle at the turn of the 20th century. The rapid generation time of flies, coupled with the induction of mutations and the ability to identify mutants by morphological changes subsequently allowed Morgan to demonstrate that chromosomes form the physical basis of heredity and to propose the concept of genetic linkage, observations for which he was awarded the Nobel Prize in 1933 (Morgan, 1910; Morgan, 1911a; Morgan, 1911b; Morgan, 1911c). Since then, the fruit fly has continued to be widely used as a genetic model, exploiting both classical phenotype-based genetic screens and techniques for genetic manipulation, including the relatively straightforward production of gene knockdowns, deletions and transgenic insertions. Consequently, *Drosophila* has found major application in the analysis of genetic interactions in neurological disease. In addition, the powerful genetic approaches possible in *Drosophila* have allowed modeling of diseases with more complicated genetics such as mitochondrial diseases. Reviews by Palladino, Wentzell and Kretzschmar, and Ambegoekar *et al.* in this issue describe the development and analysis of *Drosophila* models of PD, HD, motor neuron disease, dementia and mitochondrial disease.

Fish: Use of the zebrafish *Danio rerio* as a genetic model was first reported by Streisinger in the early 1980s (Streisinger *et al.*, 1981). Zebrafish develop externally and are transparent for the first few days of life, allowing direct observation of development. Coupled with the ability to house large numbers of fish practicably, this allowed mutagenesis screens to recover of mutants affecting specific stages of embryogenesis and elucidation of conserved molecular mechanisms underlying early vertebrate development (Haffter *et al.*, 1996; Mullins *et al.*, 1994). Techniques for genetic manipulation of zebrafish are available; transgenic animals can be generated easily, and although the generation of stable gene knockout lines is complex, transient knockdown can be achieved by straightforward means using antisense oligonucleotides. Highly conserved zebrafish orthologues of genes involved in human neurological disease have been identified almost without exception. The zebrafish CNS is organized similarly to other vertebrate species; many brain regions relevant to neuropsychiatric diseases show recognizable homology between fish and human, and zebrafish have a complex behavioral repertoire. Consequently, the zebrafish may find particular utility in modeling diseases with behavioral manifestations, and in studies aiming to determine the basis for vulnerability of particular subsets of CNS neurons to ubiquitously expressed genetic mutants. In addition, as an aquatic organism, the zebrafish presents an opportunity to screen small compounds against relevant phenotypes for drug lead discovery. Chemical libraries can be dissolved in small volumes of water and drug exposures of large numbers of model organisms readily achieved in 96-well plates, allowing phenotype based identification of chemical modifiers (Zon and Peterson, 2005). The adoption of zebrafish as a model of neurological disease is relatively recent. Articles in this issue by Panula *et al.*, Bandmann and Burton, and Mathur and Guo review the neurochemical systems of the zebrafish brain, genetic zebrafish models of PD, HD, dementia and motor neuron disease, and the application of zebrafish for studies of complex behavioral phenotypes.

In addition to review articles summarizing the properties and applications of each of these model systems for neuropsychiatry research, this issue also presents a selection of original papers reporting important novel research findings utilizing these models.

Mutations of the gene encoding LRRK2 are the most frequent genetically-determined form of Parkinsonism identified to date (Zimprich *et al.*, 2004). In this issue, Yao *et al.* report a new model of LRRK2 Parkinsonism. Transgenic *C. elegans* expressing human LRRK2 showed loss of dopaminergic neurons and motor abnormalities, in an age- and mutation-dependent

manner. This model will likely be useful for uncovering pathogenic properties and genetic interactions of LRRK2. Four other genes, whose mutation can unequivocally provoke familial PD, encode α -synuclein (Polymeropoulos et al., 1997), Parkin (Kitada et al., 1998), PINK-1 (Valente et al., 2004) and DJ-1 (Bonifati et al., 2003). Like LRRK2, their functions are incompletely understood and their role in sporadic Parkinson's disease is unclear. Since most cases of late onset sporadic PD do not appear to have an exclusively genetic basis, there is much interest in the interactions between environmental factors thought to stress relevant cellular populations and genetic alterations that affect protein expression or function, which may mediate pathogenesis of sporadic PD in combination. In this issue, several papers report gene-environment interactions potentially relevant to PD. Saini *et al.* report that Parkin-null flies show reduced lifespan, which can be rescued by antioxidants, chelation of metals, or over-expression of an antioxidant protein SOD1. This suggests that the phenotype caused by loss of Parkin in *PARK2*-linked PD may be at least partially attributable to oxidative toxins that are thought important in PD pathogenesis, and conversely that levels of Parkin expression may influence cellular vulnerability to exogenous toxins in sporadic PD. Sallinen *et al.* report that PINK1 knockdown zebrafish show loss of specific groups of dopaminergic neurons, but also enhanced vulnerability to otherwise sub-effective doses of the neurotoxin MPTP. These data suggest that PINK1 expression levels may modulate responses to exogenous agents in sporadic PD, and also that *PARK6* Parkinsonism may arise through defective cellular defenses against mitochondrial toxins. Two other papers show interesting interactions between cytosolic dopamine and neurodegeneration-provoking factors. Lawal *et al.* report that over-expression of the vesicular monoamine transporter, which contributes to the regulation of cytosolic dopamine levels in dopaminergic neurons by transporting cytosolic dopamine into synaptic vesicles, protects against the neurotoxic effects of the pesticide rotenone in *Drosophila*. Similarly, Bayersdorfer *et al.* show that inhibition of dopamine synthesis in *Drosophila* protects dopamine neurons from rotenone or α -synuclein over-expression. These findings suggest that the selective vulnerability of dopamine neurons to mitochondrial toxins and α -synuclein is mediated in part by dopamine itself, implying that composite effects of small changes in expression or localization of the multiple proteins that govern cytosolic dopamine levels may influence cellular vulnerability to PD-relevant stressors.

One potential advantage of non-mammalian models is the possibility to rapidly test therapeutic interventions in statistically meaningful samples of animals. Two reports in this issue test therapeutic hypotheses using panels of related compounds or multiple interventions that would not be feasible in mammalian models. First, Mocko *et al.* tested the activity of phenothiazine derivatives in protecting against dopaminergic neurotoxicity of rotenone and MPTP in a *C. elegans* model. By testing different chemical modifications to free phenothiazine, they were able to demonstrate that the neuroprotective activity exhibited by phenothiazine in this model was entirely dissociable from the dopamine receptor agonist or antagonist properties of modified phenothiazines, and did not require mitochondrial targeting. Second, Bortvedt *et al.* used a *Drosophila* model of HD to test two different therapeutic modalities, an intracellular antibody ('intrabody') against Huntingtin, and cystamine, which inhibits tissue transglutaminase, possibly disrupting polyglutamine crosslinking. They demonstrate an additive effect of combined treatments in this model, with unexpected dissociation of increased longevity and improved retinal photoreceptor pathology dependent upon the timing of treatment.

Finally, one of the principal advantages of non-mammalian models is that genetic modifier screens can be performed. Saja *et al.* report the results of an extensive unbiased genetic screen in a *Drosophila* model of infantile neuronal ceroid lipofuscinosis, a severe neurodegenerative disorder resulting from loss of function of lysosomal palmitoyl-protein thioesterase-1 (Vesa et al., 1995). The substrates of the enzyme are largely unknown, as are the downstream consequences of accumulation of palmitate-modified proteins. The unbiased genetic screen

was carried out in a gain-of-function *ppt-1* over-expressing model, revealing six unequivocal genetic modifiers of *ppt-1* over-expression. The products of these genes have some unexpected functions, including mRNA localization and glial cell migration, in addition to other novel modifiers consistent with previous work suggesting that *ppt-1* function is linked to cellular trafficking and synaptic growth.

What of the future? Non-mammalian models have already shown proof-of-principal that relevant pathological, cellular and biochemical abnormalities can be provoked by genetic or environmental manipulations mimicking those underlying human disease. Along with refinement of current models and generation of models of different diseases, current work is focused on the deployment of methodologies that cannot be practicably used in mammalian systems, such as genetic and chemical modifier screens, *in vivo* imaging of pathogenesis and large scale evaluations of gene-environment interactions. The next major question, to which the answer is only starting to form, will be the extent to which the findings in lower vertebrates and invertebrates are directly relevant to understanding the pathogenesis of human disease. Initial indications are highly encouraging, and it is worth reflecting that many basic cellular functions are conserved from yeast through mammals. In the context of relevant differentiated cellular populations and extracellular environment, gene and protein functions elucidated in phylogenetically lower species will likely provide worthwhile insights into disease and novel lines of enquiry for further studies. Of course, fish, fly and worm models will not replace mammalian models, or (more importantly) clinical research, but they do provide another weapon in the neurobiologist's armamentarium, enabling mechanistic dissection of these important diseases from a different angle.

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