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Functional genomics approaches to neurodegenerative diseases

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Abstract Many of the neurodegenerative diseases that afflict humans are characterised by the protein aggregation in neurons. These include complex diseases like Alzheimer's disease and Parkinson's disease, and Mendelian diseases caused by polyglutamine expansion mutations [like Huntington's disease (HD) and various spinocerebellar ataxias (SCAs), like SCA3]. A range of functional genomic strategies have been used to try to elucidate pathways involved in these diseases. In this minireview, I focus on how modifier screens in organisms from yeast to mice may be of value in helping to elucidate pathogenic pathways.

Overview of diseases

Intracellular protein misfolding/aggregation are features of many late-onset neurodegenerative diseases, called proteinopathies. These include complex diseases like Alzheimer's disease and Parkinson's disease, and Mendelian diseases caused by polyglutamine expansion mutations [like Huntington's disease (HD) and various spinocerebellar ataxias (SCAs), like SCA3]. Currently, there are no effective strategies to slow or prevent the neurodegeneration resulting from these diseases in humans (reviewed in Rubinsztein 2006). A major aim of the neurodegenerative disease field is to try to identify the pathogenic processes underlying these diseases as these may provide the bases for therapeutic strategies. For complex diseases, a major challenge is to identify causal variants. The mutations underlying many of the more common monogenic diseases in this category have been elucidated, and the challenge is to characterise how they perturb neuronal and sometimes glial functions and cause disease.

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Modifier genes

One way to characterise disease pathways is to identify genes which modulate the phenotypes of the primary mutation causing a monogenic disease. This approach is likely to be tractable, if one considers the situation in HD, for example. About 70% of the variance in the age at onset of HD can be accounted for by polyglutamine repeat number in the disease-causing allele (reviewed in Imarisio et al. 2008). The larger the repeat, the earlier onset tends to occur. The residual variance in age at onset unaccounted for by the polyglutamine repeat numbers is likely to be partly due to genetic factors, because for a given polyglutamine repeat number, age at onset may be more similar between siblings than between unrelated individuals (Rosenblatt et al. 2001; Squitieri et al. 2000; Telenius et al. 1993; Wexler et al. 2004). Thus, even in a Mendelian disease that is associated with a high-penetrance "deterministic" mutation, there are likely to be other genetic modifiers. This situation is likely to apply to most of the Mendelian neuronal proteinopathies. One of the key motivations for finding such modifiers is that they may be associated with druggable pathways.

Data from genome projects has provided a foundation for functional genomic screens in a wide range of organisms from yeast to man that have already provided important insights into the biology of many of these diseases. I review selected examples in the context of neurodegenerative proeinopathies.

Yeast (*S. cerevisiae*) as a tool

Yeast have proved a powerful tool for generating hypotheses relating to huntingtin pathogenesis. Mutant huntingtin forms aggregates and has toxicity in yeast, and the availability of deletion libraries allows one to rapidly screen for genes that modify these phenotypes. Muchowski and colleagues screened such a library for suppressors of polyglutamine toxicity and identified modifiers that may regulate vesicle transport, vacuolar (lysosomal) degradation, transcription, and prion-like aggregation (Giorgini et al. 2005). One of the strongest suppressors was Bna4 (kynurenine 3-monooxygenase), an enzyme in the kynurenine pathway of tryptophan degradation. Their data and follow-up work suggested that the HD mutation may lead to accumulation of the kynurenine pathway metabolites 3-hydroxykynurenine and quinolinic acid, leading to generation of reactive oxygen species. Since most brain kynurenines are synthesized in astrocytes and microglia, and microglia are activated in individuals with Huntington's disease and by intrastriatal injection of quinolinic acid in rats, they speculated that some of the effects on the HD mutation may not be autonomous to neurons but instead may be mediated by glia (Giorgini et al. 2005).

Lindquist and colleagues have used this yeast approach with success in models of Parkinson's disease (PD) (Cooper et al. 2006). PD is associated with the accumulation of α-synuclein in susceptible neurons. Point mutations in this protein (e.g, A53T) or duplications of this gene that increase expression of the wild-type protein cause rare familial forms of PD. Lindquist's group showed that the toxicity of wild-type or A53T α-synuclein in yeast was associated with early changes in ER-to-Golgi trafficking. They performed a genome-wide screen to identify modifiers of this toxicity in yeast and the largest group of modifiers were those functioning at this step in the secretory pathway, including the Rab guanosine triphosphatase Ypt1p, which associated with cytoplasmic α-synuclein inclusions. Importantly, they showed that this modifier was relevant in *Drosophila*, *C. elegans*, and human dopaminergic neuron models expressing α-synuclein (Cooper et al. 2006). This study, like the Muchowski paper (Giorgini et al. 2005), elegantly shows how one can use yeast as a tool to generate testable hypotheses relating to human disease pathogenesis.

C. elegans as a tool

One of the proteins that accumulates in neurons in sporadic AD is the microtubule-associated protein tau. Mutations in tau cause a rare form of frontotemporal dementia. Schellenberg and colleagues generated a *C. elegans* model overexpressing such a tau mutant that developed insoluble tau accumulation, neurodegeneration, and uncoordinated movement. They then screened this model with a library of RNAi clones that target about 85% of *C. elegans* genes. This study yielded many possible modifiers of tau toxicity (Kraemer et al. 2006). Similar types of screens in *C. elegans* have been reported in other diseases like HD (Nollen et al. 2004).

Drosophila as a tool

Drosophila are one of the classical tools used for unbiased genetic screens. Accordingly, fly models have been generated for a wide range of neurodegenerative diseases, including polyglutamine diseases (e.g., HD, spinocerebellar ataxias types 1 and 3), Mendelian forms of PD, and tauopathies. Genetic screens have been performed in a range of models to identify modifiers (reviewed in Bilen and Bononi 2005). Two recent studies have highlighted that genetic modifiers may be enriched if one starts off by selecting genes whose protein products interact with the disease protein. Indeed, up to 45% of such interactors may be genetic modifiers compared to 1–4% typically seen in unbiased genetic screens (Kaltenbach et al. 2007). Another important finding coming from such screens is that modifiers are not necessarily shared between different polyglutamine diseases and, indeed, may have opposite effects in different diseases (Branco et al. 2008).

Mammals

It is difficult to identify modifier genes for diseases like HD in humans because of the complexity of their genetic backgrounds, the limited number of naturally occurring variants expected to have major effects on the phenotype, differences in polyglutamine repeat number between patients, and unreliable age-at-onset data for a disease with an insidious onset in humans. However, these problems can be circumvented by performing screens of crosses of HD mice and mice mutagenised with N-ethyl-N-nitrosourea (ENU). ENU can transfer its ethyl group to oxygen or nitrogen radicals in DNA, causing mispairing and base-pair substitution if not repaired. Thus, ENU causes point mutations. The highest mutation rates occur in premeiotic spermatagonial stem cells, with single-locus mutation rates of 6-1.5 $\times$ 10^{-3}, equivalent to obtaining a mutation in a single gene of choice in 1/175 to 1/655 gametes screened (Hitotsumachi et al. 1985; Justice et al. 1999; Shedlovsky et al. 1993). ENU screens are both relatively cheap and feasible (Brown and Nolan 1998). This approach will be much closer to saturating for the possible relevant genes than studies relying on naturally occurring variants in

humans. If there are genetic pathways that modify the progression of polyglutamine diseases, then one ought to be able to identify these in mice. To date, no genetic modifier screen data have been published for neurodegenerative disease in mice, but this may be possible with ENU-based approaches. However, ENU mutagenesis already has been used as a tool to identify novel genes that result in defined phenotypes. Davies and colleagues have demonstrated the power of this approach by identifying new mutations that cause spinocerebellar ataxia—they have described this phenotype with missense mutations in SNAP-25, a SNARE protein essential for exocytosis (Jeans et al. 2007), and in Af4, a transcription factor (Isaacs et al. 2003).

The ability to link neurodegenerative phenotypes with different genetic lesions will be enhanced in the future by the growth of libraries of embryonic stem (ES) cells with knockout alleles. However, ENU mutagenesis has the ability to create missense mutations and hypomorphs, thus allowing the understanding of genotype-phenotype correlations that may be different to what is seen with complete nulls.

General issues

The increasing ability to undertake systematic modifier screens will likely translate into a growth of valuable data about possible pathways involved in diseases. There are a number of issues that need to be considered when interpreting such studies. First, in RNAi-based screens, sensitivity and specificity may be imperfect. On the one hand, one can get off-target effects that may mislead unless the validation approaches are robust to this. On the other hand, the knockdowns with certain RNAs may be ineffective either because the actual knockdown is weak or because the knockdown may have only phenotypic consequences in the specific assay at very low levels. So, negative results may not always be interpretable. Second, it is frequently difficult to move from a genetic target to a specific mechanism. Grouping modifier genes by function can be misleading or uninformative; for instance, certain ubiquitin ligases may actually be operating to regulate transcription of other relevant targets. Ultimately, we need to be able to identify the mechanisms for such modifying effects. This objective is important for many of the functional genomic screens because it provides an understanding of the biological pathway that is contributing to disease and removes effects that may be trivial. For instance, if one does a screen in higher eukaryotes that uses an external phenotype or behaviour as a readout in a model that has significant apoptosis, then some of the modifiers may be genes that are involved in apoptosis execution. This event may be quite late in the disease pathway in a specific cell and may yield limited information when one is striving to identify early consequences of the mutation. Hopefully, the greater understanding of genes and their functions coming from other functional genomics projects will provide the basis for better hypothesis generation for detailed functional studies.

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