Deciphering Neurodegeneration: Models and Methods

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The challenge of neurodegeneration

The brain is a complicated organ—billions of cells, vast numbers of connections

Understanding how it works is a challenge, as is understanding what happens when it goes wrong

Huge diversity in cell types

Thousands of different neuronal types

Glial cells (astrocytes, microglia, oligodendrocytes) add a further layer of complexity
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Different diseases, brain regions and cells
Further complicated by regional and cellular specificity

<table>
<thead>
<tr>
<th>Disease</th>
<th>Primary region</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s</td>
<td>Cortex</td>
<td>Plaques and tangles</td>
</tr>
<tr>
<td>Parkinson’s</td>
<td>Midbrain</td>
<td>Lewy bodies</td>
</tr>
<tr>
<td>ALS</td>
<td>Motor neurons</td>
<td>TDP-43</td>
</tr>
<tr>
<td>Prion</td>
<td>---</td>
<td>Spongiform, PrP deposition</td>
</tr>
</tbody>
</table>

Nature versus nurture
Strong genetic component to neurodegeneration
Also clear environmental insults directly linked to degeneration, as well as risk factors identified through epidemiology

Reductionism versus systems biology
Whole brain function
Regional structures
Neuronal networks
Cells
Molecular biology
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Models and methods

Cellular models:
- Yeast
- Primary cells
- Cell lines
- Stem cell derived cells

Model organisms:
- Drosophila melanogaster
- Caenorhabditis elegans
- Danio rerio
- Mus musculus
- Rattus rattus

Cellular models

- Reduce the complexity of the model system to be examined
- Very powerful method to understand cellular function and dysfunction
- Results need to be evaluated in the context of frequently highly complex neurological disorders involved multiple neuronal systems
- Different cellular systems have their own benefits and drawbacks

Yeast

Saccharomyces cerevisiae – baker’s yeast

- Extremely easy to grow, very simple genetics (12MB, just over 6000 genes)
- Has been used as a screening system for understanding a range of neurological disorders
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Yeast (2)

Vol 442|3 August 2006| Nature
The physical basis of how prion conformations determine strain phenotypes

Primary cells
- Derived for the most part from rodent models, either embryonic or post natal
- Are the closest model cellular system to the in vivo situation
- Limited by their sensitivity, difficulty in manipulation and genetic background – rodent cells may not express human genes with the same cellular or regional pattern as found in the equivalent human cell types

Cell lines
Immortalized mammalian cells
- HeLa cells – derived from human cervical cancer tumour
- Easy to grow, easy to manipulate – but differ from neuronal cells in a number of ways
Stem cells
• Pluripotent self renewing cells, capable of differentiating into any cell type
• Can be derived from embryos (ES cells) or induced from terminally differentiated cells (iPS cells)
• Potentially very powerful tool for modeling neurological disease, especially genetic forms of disease
• Protocols for differentiating into several types of neurons and glial cells exist

Stem cells (2)
• Both iPS and ES derived cell models becoming more common as systems to model neuronal function – combine neuronal functionality with human genetic background

Model organisms
• Allow much more complex analysis of genetic impact on neuronal function in the context of a complete organism
• Many aspects of biology need to be taken into consideration when selecting a model organism:
  – Do they possess the same functional neuronal networks as found in humans?
  – Do they have orthologs of the human genes under consideration?
  – How easy is it to manipulate their genetics and to measure phenotypes?
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Worms
* Caenorhabditis elegans
  * Nematode worm, 1mm long

  - Simple genetics, easy to examine phenotypes
  - Rapid breeding and derivation
  - Simple nervous system - 300 neurons

Flies
* Fruit fly - Drosophila melanogaster

  - Simple genetics, highly amenable to manipulation
  - Simple nervous system
  - Very well documented phenotypes

Flies (2)
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Fish

Zebrafish – Danio rerio
Similar to Drosophila, highly characterized genetics and phenotyping

Translucent embryos and transgenic manipulation allow detailed visualization of nervous system

Rodents

Mus musculus
Rattus norvegicus

• Much more complicated nervous system, closer to humans
• Allows analysis of more complex phenotypes, including movement disorders and simple cognition/memory
• Battery of techniques allow manipulation of genetics

Methods

• Huge array of research methods available
• New techniques are being developed all the time
• Specific approaches must be tailored to the research question or hypothesis under consideration
• Most investigations use a combination of several different techniques to address a question from a number of angles at the same time
Molecular biology approaches

- Sequencing and recombinant DNA technology allow (relatively) easy manipulation of DNA and RNA
- Directly relevant to the modeling of genetic forms of neurodegenerative disorders
- Individual genes can be removed, overexpressed or altered base pair by base pair
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Genomic editing
Recent development of sequence specific genomic editing, potentially specific down to single nucleotides

Zinc finger nucleases (shown above), CRISPR technology, Transcription activator-like effector nucleases (TALENs)

RNAi
Uses cellular RISC machinery to knockdown gene expression

Both siRNA and shRNA approaches can be used to generate acute or long term knockdown

Overexpression
• Can be achieved through a range of different expression systems, manipulated by recombinant DNA technology
  – Bacterial plasmid mediated, often with CMV gene promoter
  – Viral mediated, often inserting gene of interest and promoter into host genome
• Both can utilize epitope or fluorescent tags
• Both techniques co-opt cellular machinery to allow increased levels of mRNA for a given gene
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**Gene expression**  
- Can be measured using a wide variety of approaches, from gene specific through to genome wide.
  
  ![Gene expression diagram](image1)

- Relies on detection of mRNA transcripts.
- Resolution can be at the transcript or exon level.
- Quality of RNA purified is critical for accurate determination of message level.

**rtPCR**  
Amplifies RNA message by converting to cDNA and then amplifying signal by polymerase chain reaction.

If carefully calibrated and normalized to house keeping genes, can be used in a quantitative fashion (qPCR).

**Microarrays**  
- Alternative to rtPCR uses microchips spotted with oligonucleotide probes.
- Provides quantitative readout at gene or exon level.

![Microarrays diagram](image2)
RNAseq
- Latest approach to assessing gene expression
- Uses next generation sequencing to generate quantitative transcript level information across the genome
- Potentially very powerful approach, however is (currently) expensive and very, very data intensive

Protein analysis
- Several different approaches can be used for analyzing individual proteins
- Most utilize protein specific antibodies to detect and quantify
- Very reliant on how specific the antibody in question is
- Phosphospecific antibodies allow analysis of phosphoproteome

Immunoblot
- Immunoblot then used to detect specific proteins
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ELISA
Enzyme linked immunosorbent assay

- Allows quantitative analysis of isolated proteins
- Has been used to great effect with Abeta peptide and alpha synuclein

Mass spec and proteomics

- Used for many years as a means to analyze small molecules and individual proteins
- Very accurate estimation of molecular mass
- Now applied to whole cells/entire proteome
- Can be used to measure post-translational modification

Microscopy

- Comes in many shapes and sizes including:
  - Brightfield
  - Fluorescence
  - Confocal
  - Electron
- Allows subcellular analysis of cells and tissues
**Immunocytochemistry**

Uses specific antibodies to label proteins in cells prior to detection of fluorescent tags.

**Live cell imaging**

- Advances in fluorescent microscopy over the last decade or so mean that cellular behavior can be measured in real time using fluorescent probes or tags.
- Very powerful technique for getting an insight into how a process changes over time, most microscopy and protein analysis tends to be carried out as static measurements.

**Electrophysiology**

Patch clamping with glass micropipettes to measure electrochemical gradients across membranes and across time.

Critically important for assessing whether neurons are functional, and can yield key insights into alterations in neuronal behavior.
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Tool compounds

Using a chemical approach to induce a desired phenotype or to modulate the activity of a specific protein

MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

The future

• Increasing focus on genome/proteome wide analysis of human derived cellular models
• Combinations of multiple model organisms used to tease out gene function
• Genomic editing will allow high level of resolution at a cellular and organism level as to the function of specific amino acids in any given protein

Any questions?

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