Introduction to Genetic Tools in Neurobiology & NS201B Overview

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The Neuron Doctrine
The fundamental unit in the nervous system

Santiago Ramón y Cajal 1852-1934
1906 Nobel Prize in Physiology or Medicine (shared with Camillo Golgi)
The Polarity Hypothesis
Directional flows in dendrites, soma and axons
Introduction to Genetic Tools in Neurobiology

• Genetic approaches to investigate the complexity of the nervous system
  – Lineage tracing of neuronal origin
  – Cell biology of neuron
  – Neural circuits and connectivity

• Model organisms
  – Caenorhabditis elegans
  – Drosophila melanogaster
  – Mus musculus
  – Rattus rattus

• Technical approaches
  – Transgenic
  – Conditional gene targeting

• Functional outcomes
  – Loss of function vs. Gain of function
  – Cell type-specific vs. circuit-dependence

Take Home Message:
Genetic tools allow temporal and spatial controls to study gene and circuit functions in neurobiology
Genetic tools in neurobiology research in *Drosophila*

- The “GAL4-UAS” technique – misexpression of gene
- The “FLP/FRT” technique – germline mosaics
- The MARCM system – single cells & clonal analyses
The “FLP/FRT” technique

- **FLP**: a recombinase encoded by the yeast 2µm plasmid
- **FRT**: FLP recombination target present in the 2µm plasmid as 599bp inverted repeats

(Golic and Linquist, Cell 1989)
The “FLP/FRT” technique

Red Eye

hs-FLP

W+

White Eye

w/w
loss of eye color; ‘white’ eye

w/
hsp70-W+
One copy of eye color transgene

hsa0-W+/hsp70-W+
two copies of eye color transgene

60' @ 38.5°C
60' @ 37°C
30' @ 37°C

(Golic and Linquist, Cell 1989)
Mosaic Analysis with a Repressible Cell Marker (MARCM)

- Positively marks a small population of wild type or mutant cells
- Generate homozygous mutant cells from heterozygous precursors via mitotic recombination
- MARCM-ready flies: GAL4-UAS, GAL80 and FLP/FRT
- Applications:
  - Lineage analysis
  - Investigating gene function in single or small populations of cells
  - Neuronal circuit tracing
  - Growth cone signaling
  - Axon pruning

(Lee T, Luo L, Neuron, 1999)
Mosaic Analysis with a Repressible Cell Marker (MARCM)
Example: MARCM used to analyze morphology

- Assessment of cell autonomous gene function
  - Single cells labeled
  - Early lethal genes can be analyzed in specific cell types at later stages of development

- Cut (ct) gene
  - Transcription factor (homeobox gene)
  - Mutants are embryonic lethal
  - Expressed in Drosophila Peripheral Nervous System (PNS) sensory neurons
Drosophila da neuron clusters (from one segment)

Gal4^{109(2)}^{80}, UAS-mCD8-GFP (all da neurons)
**H**

<table>
<thead>
<tr>
<th>Cut Immunoreactivity</th>
<th>Class I</th>
<th>Class II</th>
<th>Class IV</th>
<th>Class III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendrite morphology</td>
<td>Undetectable</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Simple</td>
<td>Simple</td>
<td>Complex space-filling</td>
<td>Complex “spiked”</td>
</tr>
</tbody>
</table>
Limitations of MARCM

• **Timing of GAL80 elimination:** due to protein perdurance, MARCM can only be used to reliably label single cells 24-48 hours after the induction of mitotic recombination.

• **Maternal GAL80 contribution:** limits the efficacy of the MARCM system in studying early embryo development.

• **Gene of interest:** the high abundance and perdurance of protein may interfere with interpretations.

• **Some mutant cells may not be labeled by Gal4-UAS:** Gal4 is only expressed in subset of cells, but hs-FLP/FRT recombination is ubiquitous.
Genetic tools for neuronal functions and neural circuits in mouse brain

- Transgenic targeting of specific neuron type
  - cis-regulatory elements as drivers
  - Bacterial artificial chromosome (BAC) transgenics
  - Gene targeting (“knock-in”) w/ or w/o internal ribosomal entry site (IRES)
  - Enhancer trap – random insertion of target genes in the genome under the control of a minimal promoter
- Binary expression strategy – Cre-loxP, FLP-FRT & Tet-on/Tet-off systems
  - Cre drivers
  - Cre reporter lines
- GFP reporter and its variants
Schematic diagrams of conventional and conditional knockout in mice
Strategy for cell type-specific gene knockout

MOUSE A:

MOUSE B:

MOUSE A x B:

In type a cells: Cre+

In all other cells: Cre−

( Tsien et al., Cell 1996a)
Strategy for cell type-specific gene knockout

A. Construct (pJT-CRE) for production of Cre Mouse

B. Construct (pcAct-XstopX-LacZ) for production of Reporter Mouse

(Tsien et al., Cell 1996a)
Activity dependent modification of CA1 synapse, mediated by NMDA receptor, is required for spatial learning.
**ROSA26 & its variants**

- The gold standard in reporting Cre activity
- A “promoter trap” – similar to the concept of “enhancer trap” in Drosophila
- The ROSAβgeo26 (GtROSA26) line was initially derived from pools of ES cells infected with the retroviral gene trap vector Gen-ROSAβgeo at low multiplicity of infection
- βgeo encodes a bifunctional lacZ/neomycin phosphotransferase gene and the ROSA26 strain is one of several strains that exhibits broad lac Z staining.

![Map of the ROSA26 locus](image)
**GFP reporter and its variants**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Sequence Details</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>wtGFP</td>
<td>Phe^{64}-Ser-Tyr-Gly-Val-Gln^{69}...Ser^{75}...Tyr^{145}...Thr^{203}</td>
<td>Chalfie et al. 1994</td>
</tr>
<tr>
<td>ECFP</td>
<td>Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala blue shifted humanized codon usage</td>
<td>Heim and Tsien 1996</td>
</tr>
<tr>
<td>EGFP</td>
<td>Phe64Leu, Ser65Thr red shifted humanized codon usage</td>
<td>Cormack et al. 1996</td>
</tr>
<tr>
<td>EYFP</td>
<td>Ser65Gly, Val68Leu, Ser72Ala, Thr203Tyr red shifted humanized codon usage</td>
<td>Ormo et al. 1996</td>
</tr>
</tbody>
</table>

![Absorbance and emission spectra of ECFP, EGFP, and EYFP](image-url)
GFP reporter and its variants
Variable expression patterns of XFP in the hippocampus and cerebral cortex

- Thy1 – a member of the Ig superfamily expressed by projection neurons in many parts of the nervous system
- Characteristics of thy1-XFP transgenic mice
  - Label axons and dendrites even though not fused to peptides designed to facilitate transport
  - Stable expression of XFP up to 9 months with no discernible effect on synaptic structure
  - Remarkable variability in patterns of XFP expression in mice generated from the same construct
  - Each line exhibit unique, heritable pattern of expression
  - Double transgenic lines can be achieved

(Feng et al., Neuron 28: 41-51, 2000)
Cre reporter lines

WPRE: woodchuck hepatitis virus posttranscriptional regulatory element (to enhance RNA stability)  
Enhanced fluorescent labeling in the new Cre reporter lines

Mechanisms of temporal control of site-specific recombination

A Post-translational control

[Diagram showing molecular interactions and regulatory processes related to temporal control of site-specific recombination.]
References


Reviews

2016-2017 NS201B Overview

- Signaling I: Notch signaling & lateral inhibition (Cheyette)
- Signaling II: Shh & Wnt (Cheyette)
- Neurocircuit development (Stryker)
- Signaling III: Neurotrophic factors (Huang)
- Growth cone dynamics (Weiner)
- Dendritogenesis (YN Jan)
- Synapse formation I (Ullian)
- Problem set I (2nd students)
- Synapse formation II (Ullian)
- Synapse homeostasis (Davis)
- Cortical development I (Rubenstein)
- Cortical development II (Pleasure)
- Cortical development III (Alvarez-Buylla)
NS201B 2016-2017

- Neurodevelopment & behavior (Manoli)
- In vitro models & neuropsychiatric diseases (Willsey)
- Paper discussion – Cortical development (Pleasure)
- Neurogenetics I (Sanders)
- Neurogenetics II (Ptacek)
- Myelination/Oligodendroglia/Schwann cells (Chan)
- Astrocytes (Molosky)
- Problem set II (2nd year students)
- Glia paper discussion (Molosky)
- Final Exam (1/18/17 – 1/20/17)