Virus engineering for neuroscience

Ian Wickersham
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10/16/2014
Why viruses?

- “Cell-type-specific” expression -> targeting based on gene expression
- “Circuit-specific” expression -> targeting based on synaptic connections
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AAV: workhorse for transgene delivery

- high (but slow) expression levels, nontoxic
- specialist cores make high quality preps
- packaged as different “serotypes” (strains); serotype determines “tropism” (which cells it infects)
- small packaging capacity.
- expression of two genes from same virus is not high
- nonenveloped virus so can’t be easily recoated with other viruses’ envelope proteins
- DNA genome -> can be made Cre (or Flp, etc.) dependent

AAV: workhorse for transgene delivery

• almost no viral sequences left in vector genome

• components of vector genome: ITRs, promoter, (kozak sequence), transgene, woodchuck posttranscriptional regulatory element, polyadenylation signal

• genome must be <4.7 kB including ITRs
Targeting using promoters unsuccessful for many neuron types

- Interneuron subtypes in particular
- Cre lines method of choice
CURRENT STANDARD:
CRE MICE + AAV-FLEX

<table>
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<th>ITR</th>
<th>Prom</th>
<th>lox1</th>
<th>lox2</th>
<th>GFP</th>
<th>lox1</th>
<th>lox2</th>
<th>WPRE</th>
<th>pA</th>
<th>ITR</th>
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</table>
Design and characterization of a FLEX switch for ChR2mCherry. A, FLEX switch recombination sequence for stable inversion proceeds in two steps: (1) inversion followed by (2) excision. loxP and lox2272 are orthogonal recombination sites. B, Construct design for FLEX-for-ChR2mCherry and FLEX-rev-ChR2mCherry. CAG, CMV enhancer/β-globin chimeric promoter; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element; ITR, inverted terminal repeat. C, Images showing mCherry fluorescence in HEK 293 cells for FLEX-for-ChR2mCherry and FLEX-rev-ChR2mCherry in the presence and absence of Cre. D, Colocalization of EGFP and mCherry fluorescence (yellow arrowheads) in HEK 293 cells cotransfected with FLEX-rev-ChR2mCherry and Cre-IRES-EGFP.

Figure 2.

Cre-dependent ChR2mCherry expression in transgenic mice using rAAV-FLEX-rev-ChR2mCherry. A, Distribution of fluorescent neurons resulting from a large coinjection (150 nl) of rAAV-ChR2-EGFP and rAAV-FLEX-rev-ChR2mCherry into the hypothalamus of wild-type mice. Extensive fluorescence from EGFP (left) but no fluorescence from mCherry in brain slices (right) shows the absence of background expression with rAAV-FLEX-rev-ChR2mCherry. The background image of the slice was obtained from 4′,6′-diamidino-2-phenylindole fluorescence. B, Top, Schematic showing location of the imaged area in caudal arcuate nucleus. Bottom, mCherry fluorescence only in the arcuate nucleus after a large injection of rAAV-FLEX-rev-ChR2mCherry into the hypothalamus of pomc-cre;rosa26-loxSTOPlox-eyfp mice. Compare distribution of fluorescence with A. C, Colocalization of mCherry and EYFP fluorescence in arcuate nucleus. D, E, Similar to B and C; in this case, agrp-cre;rosa26-loxSTOPlox-eyfp mice were used with rAAV-FLEX-rev-ChR2mCherry virus injections. F, Top, Image showing neuron morphology from the arcuate nucleus of labeled POMC neurons. Bottom, Higher-magnification image of boxed area. G, H, Axonal projections of AGRP neurons infected with rAAV-FLEX-rev-ChR2mCherry. Strong axonal labeling was observed in the paraventricular nucleus of the hypothalamus (G) and paraventricular thalamus (H). 3V, Third ventricle; D3V, dorsal third ventricle.
Figure 3.
Photostimulation of AGRP and POMC neurons in the hypothalamus. A, B. Whole-cell voltage-clamp recordings from mCherry positive neurons in hypothalamic slices from agrp-cre or pomc-cre mice infected with rAAV-FLEX-rev-ChR2mCherry. Light pulses (500 ms) of varying power elicited ChR2mCherry-mediated inward currents. C, D, The peak current is plotted as a function of laser power for AGRP (C) and POMC (D) neurons. E, F, Perisomatic repetitive stimulation with 1 ms light pulses at 10 Hz in AGRP (E) and POMC (F) neurons. Blue dashes mark timing of light flashes.
Channelrhodopsin-assisted circuit mapping for hypothalamic neuronal circuits: AGRP→PVN and POMC→PVN. A, Diagram of a sagittal hypothalamic section depicting anatomy of connections between ARC and PVN. The pink box denotes the plane of the coronal slice. B, Coronal slices containing PVN, but not arcuate nucleus, were used for whole-cell voltage-clamp recordings from PVN neurons. C, Fluorescence image showing POMC axonal projections to PVN. Blue box outlines region of laser stimulation in E, PVN boundary is outlined in red, location of recorded cell body is marked by a star, and recording pipette is outlined in yellow. D, Overlay of POMC→PVN IPSCs resulting from three photostimulation trials at a site perisomatic to a voltage-clamped PVN neuron. E, Synaptic input map shows mean current responses over 100 ms time window as a color map in voltage-clamped PVN neuron resulting from LSPS of axons originating from POMC neurons. The position of the soma is marked with a star. F–H, Similar to C–E, but in this case, projections arise from AGRP neurons.
Cre mice/rats: effective but limiting

• Only practical for targeting one or two cell types at a time

• Precludes use in most other species

• Mouse lines expensive to create and maintain, crossing takes time
How to achieve highly multiplexed investigation?

- Opsins: ChR2, NpHR, Arch, ArchT, Chrimeon, Chronos, iC1C2, JAWS…

- Indicators: GCaMP6, ArcLight, ASAP1, B-GECO1, R-GECO1, R-CaMP1.07…

- Not to mention Cas9, dominant negative mutants, GRASP…

- But crossing mouse lines to achieve progeny expressing n recombinases (Cre, FlpO, KD, B2, B3…) does not scale well.
MULTIPLEXED OPTOGENETICS
Goal: cell-type-specific transgene expression in wild-type animals of any species
MULTIPLEXED OPTOGENETICS
CRISPR/Cas9: potential for “somatic knock-ins”?


In progress: system for selective expression in cortical interneuronal subtypes

- targeting major categories of cortical & hippocampal interneurons

- NSF grant
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“Circuit-specific” targeting: selective transgene expression in neurons based on their connectivity

- Retrograde

- “Anterograde” (monitoring/manipulating axons)

- Transsynaptic
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RETROGRADE TARGETING: DELIVERY VIA AXONS
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DELIVERY VIA AXONS
RABIES VIRUS

Wickersham et al. 2007a
RABIES VIRUS

Wickersham et al. 2007a
RABIES VIRUS

Wickersham et al. 2007a
RABIES VIRUS

- carries own polymerase; can NOT use exogenous promoters, but strong expression in all cell types

Wickersham et al. 2007a
RABIES VIRUS

Wickersham et al. 2007a
RETROGRADE TARGETING WITH A RABIES VIRAL VECTOR

Wickersham et al. 2007a
RETROGRADE TRANSDUCTION WITH RV-CHR2(RVG)

Praneeth Namburi, Tye lab, 2014
Retrograde delivery of ChR2 using RV for patch confirmation of connectivity

Kiritani et al. 2012
Apicella et al. 2012
Kress et al. 2013

Courtesy of the Society for Neuroscience.
LENTIVIRUS
LENTIVIRUS
WITH RV ENVELOPE, RETROGRADELY INFECTIOUS

Mazarakis et al. 2001
Wickersham et al. 2007a
Kato et al. 2011
Figure removed due to copyright restrictions.

RETROGRADE INFECTION WITH LV-CRE(RVG)
Wickersham et al. in press
“T-LOOP” LENTIS:
HIGH, FAST, TET-REPRESSIBLE EXPRESSION
FROM SINGLE COMPACT CASSETTE

Cetin & Callaway ’14

Figure removed due to copyright restrictions.
LV-TTE(RVG)
RETROGRADE INFECTION WITH LV-TTE(RVG)

Praneeth Namburi, Tye lab, 2014

Courtesy of Praneeth Namburi. Used with permission.
“Circuit-specific” targeting: selective transgene expression in neurons based on their connectivity

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ANTEROGRADE TARGETING: DELIVERY TO AXONS VIA SOMATA
• RNA genome -> can NOT be made Cre (or Flp, etc.) dependent
• enveloped virus -> can be easily recoated with other viruses’ envelope proteins

Wickersham et al. 2013
Wickersham et al. 2013
Wickersham et al. 2013
VSV G

Wickersham et al. 2013
Wickersham et al. 2013
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RABIES VIRUS
MONOSYNAPTIC TRACING

Wickersham et al. 2007b
MONOSYNAPTIC TRACING USING RETROGRADE COINFECTION
TARGETING INFECTION WITH ENVA/TVA

Wickersham et al. 2007b
TARGETING INFECTION WITH ENVA/TVA

Wickersham et al. 2007b
TARGETING CELL TYPES
WITH AAV-FLEX-TVA-G + RV(ENVA)

Wall et al. ’10
4.7 kb packaging limit

deposited with
Addgene
UNC vector core
UPenn vector core

Source: Kohara, Keigo, Michele Pignatelli, et al. “Cell Type-specific Genetic and Optogenetic Tools

Kohara et al. 2014
Watabe-Uchida... & Uchida ‘12
Hitti & Siegelbaum ’14
Miyamichi...& Mizrahi ’14
Krashes...& Lowell ’14

Betley...& Sternson ‘13


Miyamichi...& Luo ’11
Fu...& Stryker ’14

Stepien...& Arber ‘10
Tripodi...& Arber ‘11
Pivetta...& Arber ’14
Levine...& Pfaff ‘14

Yonehara...& Roska ’11


Cruz-Martín... & Huberman ‘14


Lammel, Lim... & Malenka ‘13

Takatoh...& Wang ‘13

Sun...& Xu ‘14
Yonehara, Farrow... & Roska ‘13

Figure removed due to copyright restrictions. Please see Figure 1 from Garcia, Isabella, Cynthia Kim, et al. "Genetic Strategies to Investigate Neuronal Circuit Properties using Stem Cell-derived Neurons." *Frontiers in Cellular Neuroscience* 6 (2012).


All are using first-generation system
Vector evolution: LV vs RV

RV:

LV:

Figure removed due to copyright restrictions. Please see Figure 1 from Cockrell, Adam S., and Tal Kafri. "Gene Delivery by Lentivirus Vectors." Molecular Biotechnology 36, no. 3 (2007): 184–204.
Major limitations of first-generation monosynaptic tracing

1) Only retrograde

2) Typically labels only a fraction of presumed inputs

3) Double labeling of inputs to two populations not effective

4) **Cytotoxic** - doesn’t allow long-term studies (imaging, gene knockout, cognitive and behavioral paradigms….)
RV TOXICITY

In progress: system for **nontoxic** monosynaptic tracing

- for long-term monitoring & manipulation of identified synaptically connected neurons
  - RV based
  - progressing well:
  - NIMH grant
Enter Plasmids

**Instructions:** Enter plasmid name, plasmid type and a one sentence description of plasmid use and then click Add button. Click the "Enter Data" or "Finished/Update" button to enter or modify data. Please include only those plasmids that have been constructed in your lab. Your progress is currently saved to your Addgene account and you can return at anytime to edit or complete your deposit. Be aware that you will no longer be able to modify plasmid information once you have requested a deposit kit.

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<td>Expresses GCaMP6s</td>
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<td>Expresses mTagBFP2 and postsynaptic mGRASP component</td>
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<td>Expresses EGFP-P2A-B19G</td>
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Resources

- “A Plasmid Editor”
- addgene.org
- NCBI BLAST
- neb.com
- epochlifescience.com
- UPenn vector core (AAV)
- UNC vector core (AAV)
- MIT vector core (HSV)
- Salk vector core (RV)
- Duke vector core (RV)
- jaxmice.jax.org
Thanks to:

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Fall 2014

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