Transgenesis: Insert exogenous DNA into the germline.

Three requirements of Transgenesis:

1) DNA must insert into the genome of the germline.  
   - How do you get the DNA in?

2) You need to know the DNA got into the germline?  
   - How do you know if it gets in?  
   - How do you track it across generations?

3) Exogenous DNA must stay in the genome.  
   - How do you keep it in?

If you can get exogenous DNA into the genome and keep it there, then what can you do with it?

Drosophila = model organism in which transgenesis has been leveraged to greatest degree.

Key aspects of power of transgenesis => transfer tools to other model systems (mice/fish).
A case study in transgenesis → DNA Transposons
- P elements (fly-specific)

1. What is a P element?
   a. DNA transposon: jumps as a DNA intermediate
      A. Other DNA transposons: Pgy, bae, Tol2, Mines (Mi4)

B. P element structure
   1. Autonomous P element = can jump on its own
      - LTRs required in cis for transposition
      - LTRs encode a single polypeptide = Transposase
      - ORF1-92-13: Trans element of trans P element Ty

      - Intact LTRs
      - Internally deleted = non-essential transposase.

How can you use P elements for transgenesis?

C. Germline Transposition: Two Component System
   - DNA you want to integrate
   - Helper plasmid
   - Non-essential LTRs
   - Essential Transposase

   Plasmid backbone
   - Co-inject
   - DNA in the germline?

A. W-/w-
   - Cad cells
   - F1 progeny w- or w-
Can get DNA, can follow DNA, + it is stable. What can you do w/it? => A lot, but need easier way to drive new genes.

1) Genetic Control of Transposition
   a) X-chromosome – one P[wt] element: 
   b) Chromosomally stable source of transposase: CyO(Δ 2-3)

How do these help?

2) What happens to P element w/o Transposase => total P[wt]
   a) w/o transposase:
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Uses of P element

1) Insectional mutagen:
   a) gene is tagged by P element → access to DNA
   b) some Most Tpos = insert non-randomly in the genome.
   c) Mouse - Piggybace → K.O. collect

2) Enhancer Trap or Gene Trap: identify genes based on + localization.
   Enhancer Trap: Enhancer → Gene X → Exon

Gene Trap:

3) Gal4/UAS system (Brand + Perrimon '93)
   a) Yeast Gal4/UAS system: UAS → GAL4-inducible gene
   b) Enhancer Trap
   c) Can use characterized enhancers: GAL4 + characterized enhancer
   d) UAS-linked transgene:

2) Genomic Tiling of Regulatory regions

3) Split-GAL4 Systems: Intersectional -
   a) Gal4/Gal80
   b) split-GAL4
FLP-FRT System

1) Site-specific recombinase system analogous to Cre-Lox
   A) FLP = site-specific recombinase
   B) FRT = FLP recombinase target - 34 bp minimal sequence

2) Outcome of FLP/FRT mediated recombination depends on orientation of FRT sites

A. Direct repeats - same molecule

B. Inverted repeats - same molecule

C. Different molecules

3) FLP-FRT mediated deletions

A. Two Types of Deletions

B. Generate + map thousands of inserts of each type of P element

C. How to create deletions:

\[
\begin{align*}
\text{P[w+ FRT] hs-flp or...} & \quad \text{or...} \\
\text{P[w+ FRT] +} & \quad \text{P[w+ FRT] +}
\end{align*}
\]

1. Library of molecularly defined deletions that aggregate tile across the genome, uncovering >95% of all genes.
Phi-C31 Integrate System

A) C31 integrate mediates site-specific and irreversible recombinat b/w two different attachment sites
- att (285 bp) - core/minimal sequence ~ 34 bps
- attP (221 bp) - any DNA > 100kb

B) Example:

1. Advantages:
   A. Can insert any piece of DNA > Landing Pad > 100kb
   B. PElemenst themselves - size limit is ~ 30kb
   C. Removes problem of position effect - (PElements)

Example: Have a protein what is phosphorylated?

Question: What is effect of PElem on prot

Experiment: Make three transgenes:

UAS - Wild-Type (S)  UAS - Phosphomimetic (S= E)  UAS - Yatable

Q: C31 based libraries - all elements inserted into same loc

1. RNAi libraries
   - UAS-linked RNAi transgenes for essentially all genes

2. ORFeome
   - most genes

3. Regulatory regions
   - Gal4

4. Genomic rescue regions
Recombination Mediated Exchange

- can swap out cassettes using CRISPR system in genes

Gene X

- Linker
- Minos insert MiMIC system
- comes in three reading frames

Gene X

- Libraries exist of different exchange cassettes in all 3' frame reading

E.g.

- Diao et al. Cell Reports vol. 10, p1410
- Nagarkar - Jaiswal et al. ELIFE 2015, e05338
- Venken et al. Nature Methods vol. 8, p737
Genetic Modifiers: hooded *φ*<sub>type</sub> - recessive trait (hypoactive allele)

- black hood on otherwise white rat
- black stripe down back

*Castile* 1964

*Wild-type* = all black/brown

**Question:** Does the nature of a gene/gene mutant change?

"Normal" hooded *φ*<sub>type</sub>

- think of as a disease *φ*<sub>type</sub>
- sib-mating

Select for rats w/ more black

- for + mate together

All black except for a little white under the belly + @ times the flank

- All white except a short hood lying anterior to the shoulders

- Outcross to *wt* + rescue

- *Hooded* *φ*<sub>type</sub>

- *Hooded* *φ*<sub>type</sub>

- *Hooded* *φ*<sub>type</sub>

- Expected:

- Model 1?

- Model 2?

Converge on the same *φ*<sub>type</sub>

How identify the enhancers/sups? of *k*<sub>2</sub> = *k*<sub>1</sub> = *wt*
Genetic Modifier Screen: Simon + Rubin 1991, Cell

**Question:** What genes act downstream of the sevenless RTK?

**Why case?**

A) sev = RTK; EGFR = RTK + one of oncogenes identified.

B) Don't know how RTKs signal to the nucleus?

C) Model: all RTKs transduce signal via same or similar downstream pathway.

\[
\begin{array}{c}
\text{Ysev} \\
A \\
B \\
C \\
\text{YEGFR}
\end{array}
\]

**What was known?**

A) sev = RTK required for R7 cell fate

specific in flies; homozygous viable -

\[
\text{WT} \quad \text{sev-}
\]

B) Saturation mutagenesis identified eight genes that when mutated lack R7

boss (bridges of sev: ligand)

? sin a: seven nucleus in absence of nuclear protein

unclear how signal is transduced from cell membrane to nucleus.

C) Model: Genes that act downstream of sev likely act downstream of many other RTKs and are thus essential for embryonic, or larval life

D) How identify genes downstream of Sev? → Create a sensitized genetic background? in which

- 100% 50% reduction in for of gene
- d-stream of sev 7R7

\[
\begin{array}{c|c|c}
\text{sev for} & \text{R7} \\
\hline
100% & 50% & \text{NO R7}
\end{array}
\]

Amount of sev for required for R7.

- want background w/ this much gene from just barely enough = R7 if all else @ 100%.