Genetic Modifiers

Hooded Rats: hooded q*type = recessive trait (hypo or hypomorphic mutant in mice)
- black hood on otherwise white rat, w/ black strip down back

- q*type = All black
- Castle, 1919

Question: Does the nature of a gene q*mutat change?

"normal" hooded q*type

(soscease q*type)

1. sib mating

2. sib mating

select for more black by
selecting for rats with
the most black+

- mating together

All black except for a little white
on belly + sometimes the flanks.

- Models:
  1) The nature of the gene
     mutant changed.
    2) Nature of gene mutant = same
       selected for genetic modifiers
       in each direction.

Test: Cross each inbred line to a third
and wild-type line + select for the
hooded q*type in the F2 gen.

Expectation: Model 1?
Model 2?

Converge on the same q*type. ✗
How you can assess the function of a gene that is essential for embryonic development during larval development?

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<tr>
<td>1</td>
<td>embryo</td>
<td>Larva</td>
<td>Adult</td>
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<td>2</td>
<td>gene essential but want to access for here or here</td>
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1) Conditional alleles (classical)
   a) Temperature sensitive alleles
   b) regulatory mutants - wings - disrupt of adult specific regulatory - Antp - ectopic expression in antennae

2) Genetic Mosaic analysis: create genotypically mutant cells in otherwise wt background
   - embryo
   - heterozygous for mutation of interest
   - create cells a few homozygous mutant clones |

   * 1) Cre-Lox in mice
   * 2) FLP-FRT in flies

3) Genetic Modifier screens: Simon + Rubin, 1991
   a) Requires a sensitized background, what is a sensitized background?
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<tr>
<td>Specific Question: What genes act downstream of the sevenless (sev) receptor tyrosine kinase?</td>
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2. Why care?
   - sev = RTK = EGFR = RTK = one of first oncogenes identified
   - Don't know how RTK's signal to the nucleus.
   - Model: all RTKs transduce signal via same mech.

3. What was known?
   a. sev = RTK required for RT cell-fate specification, homotypic viable.

<table>
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<th>WT</th>
<th>sev-</th>
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<td>CO</td>
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<td>FE</td>
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   b. saturated mutagenesis identified only two other genes that when mutated result in loss of RT
      - boss - bride of sevenless (legend)
      - seven in absencia: a nuclear protein

4. Model: Genes that act downstream of sev likely act downstream of many other RTKs + are thus essential for embryogenesis/larval life

4. How identify genes downstream of sev?
   a. Create a sensitized background:
      But, how?

         |amt of sev gene for sensitized background
         | 100%  
         | sev  
         | fez  
         | sob  
         | +/-  

      Create a background in which a 50% reduction in the ffs of genes downstream of sev cause a loss of RT.
How do you create a sensitized genetic background?

1) Start with a sev null background: sev^{dr} \rightarrow WT, sev^{dr}, sev^{dr}. \text{p(sev)}

2) Make and add a sev genomic construct. 
   - WT
   - non WT
   - WT

3) Engineer potential temp. sens. mutations in sev.
   A) How?
   - Use known T.s. mutations in Src, a tyrosine kinase, as a guide

   \[ \text{Generate potential T.s. mutations in sev} \]
   \[ \text{Assay} \quad \text{WT sev} \rightarrow \text{mutant} \]
   \[ \text{at} \quad 22.7^\circ C \quad \text{and} \quad 24.3^\circ C \]

   1. Experiment
   \[ \text{WT sev construct} \rightarrow \text{sev}^{dr} \cdot \text{p(sev)} \rightarrow \]
   \[ \text{at} \quad 22.7^\circ C \rightarrow 24.3^\circ C \]
   \[ \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{No Lt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.} \quad \text{Rt.}
   \]
   \[ \text{100% of ommatidia} \quad \text{100% of ommatidia} \]

   6 T.s. mutations
   \[ \text{sev}^{ts} \cdot \text{p(sev)} \rightarrow \text{sev}^{ts} \cdot \text{p(sev)} \rightarrow \]
   \[ \text{at} \quad 22.7^\circ C \rightarrow 24.3^\circ C \]

   Will a 50% decrease in gene product of genes downstream of sev result in the loss of Rt?
Screen:

\[ \frac{\text{Sev}^{d2}}{\text{Sev}^{d2} \times \frac{\text{TH3} \text{ SB P(Sev)}^{b4}}{5 \text{ Sev}^{d2} + \text{TH3} \text{ SB P(Sev)}^{b4}}} \]

22.7°C

\( \frac{3}{5} \) screen 30,000 E1 flies for loss of WT

20 Dominant Enhancers (\( \text{WT} \rightarrow \text{OE} \))

↓

7 complementation groups (most mutants)

Now what?

1) Do they act downstream of other RTKs?

- Ellipse = dominant allele of the EGF receptor

2) What is their phenotype in the eye?

- 4/7 genes required for the dev. of all photoreceptors

- E(Sev)sc, E(Sev)v1a, E(Sev)v2a, E(Sev)v2b

3) How identify the gene that when mutated yields the relevant phenotype?

- Map genetically or 2 deficiencies to a defined genetic locat

+ Gene rescue

- Allele sequencing

- Evolve 1 generation of additional alleles

Type of genetic interaction provides information on direct of gene for...

- Enhance a loss of for mutant: genes act in same direction

- Suppress a gain of for mutant: genes act in opposite direction

- Enhance a gain of for mutant: genes act in same direction

- Suppress a loss of for mutant: genes act in opposite direction
$E^{(sev3C)} = case\ study$

1) Two alleles - $E^{(sev3C)\, elb^+}$ and $E^{(sev3C)\, elb^F}$
   
   a) Recessive lethal mutations: $E^{(sev3C)\, elb^+}/E^{(sev3C)\, elb^F} \rightarrow inviable$

2) Genetic mapping places $E^{(sev3C)}$ on 3R, next to Curled.

3) Deficiency mapping localizes $E^{(sev3C)}$ to limited DNA region
   
   a) $E^{(sev3C)\, elb^F}$ fails to complement $Df(3R)\, by10$
      
      $E^{(sev3B)\, elb^F}$
      
      \[ \frac{TM\, 5\, 5\, b}{TM\, 3\, 5\, b} \]

   b) Complements $Df(3R)\, by62$

4) Gene rescue: Real has been mapped to this region
   
   Ras "" implicated in RTK signaling
      
   a) Exp - create a ras transgene
      
      - ask can it rescue $E^{(sev3C)\, elb^+}$? $E^{(sev3C)\, elb^+}\rightarrow Ras$ regulatory regions

\[ \frac{CyO}{TM\, 3\, 5\, b} \]

5) Allele sequencing: both $E^{(sev3C)}$ alleles $\rightarrow$ missense mutations in Ras
Genetic Mosaics

1. What is a genetic mosaic?
   - an organism composed of two or more genetically distinct cells.

2. How do you make genetic mosaics?
   - CRISPR mice
   - mitotic recombination
   - FLI1/FTO flies

Mitotic Recombination

1. Normal mitosis: cell heterozygous for 3 mutations.

2. X-ray induced mitotic recombination: x-rays induce mitotic recombination at low frequency at random points along chrom.

Result: cells can become homozygous or heterozygous for the DNA distal to the breakpoint.

Limitations:
1. Low frequency of clone generation: want high frequency
2. Recombination occurs randomly along chromosome: want recombination to occur near a marker (why?)
3. How to identify mutant clone (WT): ability to identify mutant clone + twin input unambiguous
4. Can't control in which cells clones occur or in which chromosomal arm in a specific tissue/cell type
FLP-FRT-mediated mitotic recombination

2. Xu + Nellen, 1993: Created a set of centromere proximal, viable 
FR + FRT-bearing P inserts in each chromosome arm.

Outcome -
1) hi-level recomb
2) centromere proximal

+ hsf1P

w- hsf1P/; w- j m- FRT40A
PE[w P, GFP FRT40A

1) Mutant clone: m-/m- - what else?
2) Twin-spot: +/- - what else?
3) All other cells: +/- - what else?

What if mutant had no effect on cell proliferation? How can you distinguish these 
post by looking @ the dif. clones?
23) **FLP-FRT mediated F1 Mosaic Screen**

**Key Points:**
1. Meiotic recombination is restricted to the eye.
2. Essentially all cells undergo recombination in F1 generation.
3. Screen by each chrom. arm.
4. Screen in F1 generation and recover mutations in the affected fly.

- Can screen thousands of flies.

Many groups used the above screen to identify mutations that affect cell growth.

**Results:**
1. 23 genes identified.
   a) 2 phi-type classes
      i. Much larger cells than wild-type.
      ii. Many more cells.
   b) Four genes exhibited the same phenotype.
      i. More cell proliferation (t cells).
      ii. Less cell death (t cells).
2. 4 genes
   a) Salvador - 608-kDa WW domain protein
   b) Warts - 105-kDa NDR kinase, phosphorylation
   c) Hippo - 68-kDa STE20 family kinase
   d) Mdm/Mdm - 219 novel protein
How identify the gene that when mutated yields the observed phenotype with excess prolifertation?

1) Salvador
   a) Fine-scale genetic mapping localized sau to a 20-kb region.
      1. 5 genes in the region.
      2. sequenced all 5 genes in all 4 alleles.
      3. 4 of 5 genes: no Δ in any background.
      1 of 5 Δ: premature stop codon in each background.

2) Hippo
   a) Fine-scale genetic mapping localized hippo to a 40-kb.
      b) sequenced all genes in the region in all alleles in each allele.
      c) cDNA of this gene rescues the hippo mutant phenotype when driven
         under the control of a ubiquitous G418.

   
   Kinase Domain   Actin-binding Domain   Dimerization Domain

3) Mek/Moa: 219x Novel protein

4) Warts: 1009x kinase
   5-PPXY motifs
   PPXY motifs interact with WW domains