Genetic mosaics and clonal analysis

How can you assess the function of a gene that is essential for embryonic development during the later larval or pupal or adult stages?????
Temperature Sensitive mutations

Regulatory mutations

Drusophila: wildtype on left. Right is antennapedia mutant with fully developed legs in place of antennae. Photo by FR Turner, Indiana Univ.
Mitosis and mitotic recombination

Problems:

1) Low Frequency clone generation (want high frequency)
2) Recombination occurs randomly across chromosome (want it to occur centromere proximal in defined arm and ideally in defined tissues)
3) How identify mutant clone? (want unambiguous identification of mutant clone in adult/larva).
FLP-FRT-mediated Mitotic Recombination (Golic and Lundquist, 1989; Xu and Rubin, 1993)
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hs-flp; m FRT40a/P[w+ GFP] FRT40a

m/+ 1xGFP; 1xP[w+]

2xGFP; 2x P[w+]

WT twin spot

m/m No GFP Clone

Mutant No w+
FLP-FRT-mediated Mitotic Recombination (Golic and Lundquist, 1989; Xu and Rubin, 1993)

hs-flp; m FRT40a/P[w+ GFP]

What if mutation has no effect on cell proliferation? What do you expect for clone sizes?

What if mutation decreases cell proliferation? What do you expect for relative clone sizes?

What if mutation increases cell proliferation? What do you expect for relative clone sizes?
FLP-FRT-mediated F1 Mosaic Screen

$$w/Y; \text{FRT40A} \times \text{yw Ey-flp; P[w+ GFP] FRT40A}$$

Screen eyes of F1 flies for increased ratio of w- to w+ tissue

Key points:
- mitotic recombination restricted to eye
- all cells undergo recombination
  - $\sim50\%$ cells w-; $\sim50\%$ cells 2xP[w+]
- screen by chromosome arm
- screen in F1 generation
  - screen thousands of flies easily
Results of similar screens by many labs

23 genes identified that when mutated
Lead to excess w- tissue versus WT twin spot

Two phenotypic classes:
1) Mutant clone – larger cells
2) Mutant clone – more cells

Four genes exhibited identical mutant phenotype
More cell proliferation
Less cell death

Salvador: 608 amino acid WW motif protein
Warts: 1009 amino acid NDR Kinase
Hippo: 669 amino acid STE-20 Kinase
Mob/Mats: 219 amino acid novel protein
expanded and merlin act in a partially redundant manner to repress tissue growth

Literature search for tumor suppressor genes -> obtain alleles of fly orthologs -> assess phenotype in eye

Expanded and merlin – member of 4.1 superfamily of adaptor proteins
Merlin = fly ortholog of NF-1 tumor suppressor
Merlin and expanded each show mild overgrowth phenotype

What is the double mutant phenotype? How to make double mutant?
Have six genes -> all with same phenotype. Two are kinases: likely signaling pathway. How do you figure out their order of action?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression</th>
<th>Phenotype</th>
<th>Gain of function phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>merlin</td>
<td>UAS</td>
<td>Wild-type wing</td>
<td></td>
</tr>
<tr>
<td>expanded</td>
<td>UAS</td>
<td>Shriveled wing</td>
<td>Decreased proliferation</td>
</tr>
<tr>
<td>hippo</td>
<td>UAS</td>
<td>Shriveled wing</td>
<td>Increased cell death</td>
</tr>
<tr>
<td>warts</td>
<td>UAS</td>
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</tr>
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<td>sal Salvador</td>
<td>UAS</td>
<td>Wild-type wing</td>
<td></td>
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<td>UAS</td>
<td>Wild-type wing</td>
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</tr>
</tbody>
</table>

Now, have opposing phenotypes. How order the actions of the genes?
The loss of function phenotype of a gene is epistatic to the gain of function phenotype of any gene upstream of it.

Loss of function in E is epistatic to gain of function in A -> D.

The gain of function phenotype of a gene is epistatic to the loss of function phenotype of any gene upstream of it.

Loss of function = too much proliferation; too little cell death
Gain of function = too little proliferation; too much cell death
Epistasis test between over-expression of expanded and loss of function in hippo

Ey-flp → hpo^- → P[w+ UAS-Ex] → GMR-GAL4

cell lethal mutation

wt → GMR-ex → hpo^- → GMR-ex; hpo^- → GMR-ex; hpo^-
Epistasis test between over-expression of hippo and loss of function in ex/mer

- y w mer^4
- ex-
- hs-flp GMR-GAL4

P[w+ GFP mer^+]
Rescuing transgene

P[w+ UAS-hpo]

wt
mer; ex mutant clone
GMR-hpo; ex-/mer- clones
Positively-marked mutant clones: MARCM system

GAL4 = transcriptional activator

GAL80 = co-repressor of GAL4

hs/Ey-flp

Tub-GAL80

UAS-GFP

Can be any promoter; often Tubulin

Ey-Flp

UAS-GFP

GAL4

Mutation of interest

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Positively-marked mutant clones: MARCM system

- DNA
- AD
- Gene X
- GAL80 = corepressor of GAL4
- UAS
- GAL4 = transcriptional activator
- Mutation of interest
- Tub-GAL80

Can be any promoter; often Tubulin

Ey-Flp
UAS-GFP
GAL4

Mutation of interest