The use of model organisms in studying cancer
I. Why animal models?
   What makes a good model organism?
   Historical perspective

II. Reverse genetics vs Forward genetics
   example of reverse genetics -- studying an oncogene in the fly

III. Examples of studying cancer relevant pathways in model systems using specific developmental phenotypes

IV. Genome-wide RNAi screens

V. Forward genetic screens

VI. Metastasis models in flies
What is cancer?

What processes are involved in cancer?
I. Why animal models?

What makes a good model organism?

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Why animal models?

What makes a good animal model?
Size

Numbers

Generation time

Ability to house and maintain easily

Ability to do experiments that are either impractical, impossible, or unethical in humans

Genome sequenced?

Conservation of growth pathways
Drosophila in cancer research. An expanding role.
Potter CJ, Turenchalk GS, Xu T.
Which model organism is “the best”?

**Species specific advantages**

- egg size
- external development
- close relationship to humans
- ability to perform genetic experiments
- ability to perform microsurgeries
- ability to get large quantities of eggs at any time
Early insights into Cell division: some historical perspective

Starfish and sea urchins
- discovery of cyclins (sea urchins)
- purification / identification of MPF (starfish)

Surf clam (spisula solidissma)
- hundreds of millions of eggs
- rapid synchronous divisions
- allowed better study of cyclins and
discovery that cyclins interact with cdks

Xenopus -- cell cycle extracts, cycling extracts
- DNA replication
- Chromosome segregation
- Cell cycle progression

http://www.bio.indiana.edu/facultyresearch/faculty/Pomerening.html
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Reverse Genetics and Forward Genetics

Reverse genetics:
* biased, candidate approach
* start with a gene, then examine the phenotype of its mutation or over-expression
* can be used to study known tumor suppressors, oncogenes, etc.

Forward genetics:
* unbiased, phenotype-driven approach
* start with a phenotype, work to identify the gene
* can identify genes whose role in a process was unanticipated

In the age of sequenced genomes:
Genome-wide RNAi based screens are a little bit of both
Multiple Endocrine Neoplasia Type 2 (Men2)

- monogenic (caused by dominant, gain-of-function mutations in Ret, an RTK)

- multiple endocrine system tumors -- medullary thyroid carcinoma (MTC)

- variable age of onset, severity and type/number of other tumors
Read RD, Goodfellow PJ, Mardis ER, Novak N, Armstrong JR, Cagan RL.

A Drosophila model of multiple endocrine neoplasia type 2.
Reverse genetics/forward genetics meet:

Reverse genetics -- examine the phenotype(s) of mutation in or over-expression of a gene.

You can take THAT model, and do a genetic screen for modifiers. The modifier screen is a forward genetic approach looking for modifiers in an unbiased way, but starting with a phenotype from the reverse genetics approach.

Forward genetic screens -- perform a screen to identify mutations that give a certain phenotype.
Read RD, Goodfellow PJ, Mardis ER, Novak N, Armstrong JR, Cagan RL.


<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene function</th>
<th>Allele</th>
<th>GMR-dRet\textsuperscript{MEN2}</th>
<th>GMR-dRet\textsuperscript{MEN2A}</th>
<th>GMR-dRet\textsuperscript{MEN2B}</th>
</tr>
</thead>
<tbody>
<tr>
<td>spiiz</td>
<td>dEGF receptor ligand</td>
<td>spiiz\textsuperscript{3547}</td>
<td>WS(65)</td>
<td>WS(86)</td>
<td>WS(46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>spiiz\textsuperscript{1056}</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>spiiz\textsuperscript{4}</td>
<td>WS(46)</td>
<td>WS(56)</td>
<td>WS(66)</td>
</tr>
<tr>
<td>drk</td>
<td>Ortholog of Grb2 protein</td>
<td>drk\textsuperscript{320451}</td>
<td>N</td>
<td>N</td>
<td>SS(95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>drk\textsuperscript{13809}</td>
<td>WS(28)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>drk\textsuperscript{1626}</td>
<td>MS(100)</td>
<td>WS(24)</td>
<td>SS(100)</td>
</tr>
<tr>
<td>Sos</td>
<td>RasGTP-exchange factor</td>
<td>Sos\textsuperscript{35846}</td>
<td>ME(86)</td>
<td>SE(100)</td>
<td>SE(97)</td>
</tr>
<tr>
<td>Gap1</td>
<td>RasGTPase activating factor</td>
<td>Gap1\textsuperscript{82}</td>
<td>ME(86)</td>
<td>SE(100)</td>
<td>SE(97)</td>
</tr>
<tr>
<td>Ras85D</td>
<td>Ras ortholog</td>
<td>Ras85D\textsuperscript{6677}</td>
<td>MS(100)</td>
<td>WS(76)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ras85D\textsuperscript{406}</td>
<td>WS(67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ksr</td>
<td>Kinase suppressor of Ras</td>
<td>ksr\textsuperscript{627}</td>
<td>N</td>
<td>MS(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ksr\textsuperscript{622}</td>
<td>WS(18)</td>
<td>WS(84)</td>
<td>WS(67)</td>
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<td>phl</td>
<td>Raf kinase</td>
<td>phl\textsuperscript{L.29}</td>
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<td>N</td>
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<td></td>
<td></td>
<td>phl\textsuperscript{R.26}</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dsh</td>
<td>MAP kinase kinase</td>
<td>Dsh\textsuperscript{3}</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Src64R</td>
<td>Src kinase ortholog</td>
<td>Src64R\textsuperscript{21}</td>
<td>WS(86)</td>
<td>WS(85)</td>
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<td>Src42A</td>
<td>Src kinase ortholog</td>
<td>Src42A\textsuperscript{101068}</td>
<td>WS(56)</td>
<td>WS(74)</td>
<td></td>
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<tr>
<td>bsk</td>
<td>c-jun kinase (JNK)</td>
<td>bsk\textsuperscript{1}</td>
<td>WS(47)</td>
<td>WS(44)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>bsk\textsuperscript{2}</td>
<td>WS(43)</td>
<td>WS(86)</td>
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<tr>
<td>Fra</td>
<td>c-jun transcription factor</td>
<td>Fra\textsuperscript{A1965}</td>
<td>WS(46)</td>
<td>WS(56)</td>
<td>WS(61)</td>
</tr>
<tr>
<td></td>
<td>dock</td>
<td>Ortholog of Nck adaptor</td>
<td>dock\textsuperscript{13421}</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>dsh</td>
<td>Wnt, JNK signaling</td>
<td>dsh\textsuperscript{4}</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Genetic interactions are indicated according to strength of the phenotype: W, weak; M, moderate; and S, strong; and the type of interaction: S, suppressor; E, enhancer; N, no interaction. WE, for example, indicates a weak enhancer. Parentheses indicate penetrance of the interaction as a percentage.
How can identifying genetic modifiers help cancer patients?
Genetic modifiers in the fly may serve as genetic modifiers in human disease:

Why does the same mutation lead to different course of disease in different patients?

Risk factors affecting prognosis/disease progression?
Can model organisms help us identify cancer drugs directly?

Can model organisms identify potential environmental hazards?
ZD6474 Suppresses Oncogenic RET Isoforms in a Drosophila Model for Type 2 Multiple Endocrine Neoplasia Syndromes and Papillary Thyroid Carcinoma

Marcos Vidal, Samuel Wells, Anderson Ryan and Ross Cagan
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Wild type hermaphrodite nematode

aMuv nematode


But why would we care about the C. elegans vulva?
Vulva development in C. Elegans involves the Ras pathway.

We can use this system to learn more about Ras and other signaling pathways.

Photoreceptor specification in flies

“sevenless” (sev)
“son of sevenless” (sos)

Rogge RD, Karlovich CA, Banerjee U.
Genetic dissection of a neurodevelopmental pathway:
Son of sevenless functions downstream of the sevenless and EGF receptor tyrosine kinases.
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Genome wide RNAi in C.elegans

Can look for a variety of phenotypes:
- cell cycle phenotypes
- growth
- development

Reviewed in:
Genome Biol. 2001; 2(2): reviews1005.1–reviews1005.3.
High-throughput reverse genetics:
RNAi screens in Caenorhabditis elegans
Cornelia I Bargmann
Planaria RNAi screen


Table 1. Summary of S. mediterranea RNAi Screen Results

<table>
<thead>
<tr>
<th>Screen and Phenotype Details</th>
<th>No. Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total screened</td>
<td>1065</td>
</tr>
<tr>
<td>Total with phenotype</td>
<td>240</td>
</tr>
<tr>
<td>No regeneration</td>
<td>69</td>
</tr>
<tr>
<td>Limited regeneration</td>
<td>35</td>
</tr>
<tr>
<td>Reduced regeneration</td>
<td>36</td>
</tr>
<tr>
<td>Caudal blastema</td>
<td>6</td>
</tr>
<tr>
<td>Regression</td>
<td>23</td>
</tr>
<tr>
<td>Curling</td>
<td>48</td>
</tr>
<tr>
<td>Blastema morphology</td>
<td>43</td>
</tr>
<tr>
<td>Photoreceptors</td>
<td>79</td>
</tr>
<tr>
<td>Lesions</td>
<td>20</td>
</tr>
<tr>
<td>Lysis</td>
<td>76</td>
</tr>
<tr>
<td>Blisters and/or Bloating</td>
<td>8</td>
</tr>
<tr>
<td>Behavior</td>
<td>44</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>8</td>
</tr>
<tr>
<td>Antibody only</td>
<td>21</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
</tr>
</tbody>
</table>
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Zebrafish screen - Analysis of heterozygous progeny of recessive lethal genes in zebrafish:

Many tumor suppressor mutations may be recessive lethal

-> isolate embryonic lethal mutations and examined het progeny for increases in incidence of tumors


### Table 1. Tumor Incidence in Zebrafish RP-Heterozygous Lines and in the Colony

<table>
<thead>
<tr>
<th>Gene</th>
<th>Line</th>
<th>Age Range</th>
<th>Initial Number of Fish</th>
<th>Number Lost</th>
<th>Number of Grossly-Apparent Tumors/Number of Fish/Bearing Number of Fish Examined</th>
<th>Number of zMPNST/Number of Fish Examined</th>
<th>Tumor Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L35</td>
<td>hi258</td>
<td>up to 21.5 mo</td>
<td>13</td>
<td>7</td>
<td>5/6</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>S15a</td>
<td>hi2649</td>
<td>up to 18 mo</td>
<td>9</td>
<td>2</td>
<td>4/7</td>
<td>6*7/7</td>
</tr>
<tr>
<td></td>
<td>S8</td>
<td>hi1974</td>
<td>up to 22 mo</td>
<td>19</td>
<td>6</td>
<td>6/13</td>
<td>9/13</td>
</tr>
<tr>
<td></td>
<td>L36a</td>
<td>hi10</td>
<td>up to 22 mo</td>
<td>14</td>
<td>6</td>
<td>4/8</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>L36</td>
<td>hi1807</td>
<td>up to 21.5 mo</td>
<td>14</td>
<td>6</td>
<td>4/8</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>L7</td>
<td>hi1061</td>
<td>up to 22 mo</td>
<td>14</td>
<td>4</td>
<td>6/10</td>
<td>6*7/10</td>
</tr>
<tr>
<td>B</td>
<td>S7</td>
<td>hi10348</td>
<td>up to 22 mo</td>
<td>19</td>
<td>4</td>
<td>3/15</td>
<td>7/15</td>
</tr>
<tr>
<td></td>
<td>L13</td>
<td>hi1016</td>
<td>up to 23 mo</td>
<td>15</td>
<td>4</td>
<td>2/11</td>
<td>5/11</td>
</tr>
<tr>
<td></td>
<td>S18</td>
<td>hi1026</td>
<td>up to 24 mo</td>
<td>23</td>
<td>9</td>
<td>1/14</td>
<td>6/14</td>
</tr>
<tr>
<td></td>
<td>S29</td>
<td>hi2903</td>
<td>up to 22 mo</td>
<td>18</td>
<td>3</td>
<td>4/15</td>
<td>4/15</td>
</tr>
<tr>
<td></td>
<td>L23A</td>
<td>hi2582</td>
<td>up to 22 mo</td>
<td>40</td>
<td>5</td>
<td>3/35</td>
<td>5/35</td>
</tr>
<tr>
<td>C</td>
<td>S12</td>
<td>hi1227</td>
<td>all at 22 mo</td>
<td>14</td>
<td>1</td>
<td>0/13</td>
<td>1/13</td>
</tr>
<tr>
<td></td>
<td>acidicLP1</td>
<td>hi1444</td>
<td>up to 22.5 mo</td>
<td>18</td>
<td>0</td>
<td>1/18</td>
<td>1/18</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>hi2437</td>
<td>all at 23 mo</td>
<td>19</td>
<td>2</td>
<td>0/17</td>
<td>1/17</td>
</tr>
<tr>
<td></td>
<td>L24</td>
<td>hi1284</td>
<td>all at 26 mo</td>
<td>18</td>
<td>0</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td></td>
<td>S15</td>
<td>hi2430</td>
<td>all at 23 mo</td>
<td>16</td>
<td>0</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>D</td>
<td>NA</td>
<td>Colony</td>
<td>20-26 mo</td>
<td>152</td>
<td>ND</td>
<td>4/152</td>
<td>17/152</td>
</tr>
</tbody>
</table>
Comparing the relative proliferation of wild-type and mutant cells.

- Neutral
- Mutant tissue underrepresented (common)
- Mutant tissue overrepresented (rare)
mutagenize

The Screen

mutagenize

males

mutant/+ 

+/+ females

For additional review, see:

FLP/FRT-induced mitotic recombination

A

UCLA Undergraduate Research
Consortium in Functional Genomics
http://www.bruinfly.ucla.edu/methods.php
Newly hatched larva

eye imaginal disc, ~20 cells

10 rounds of cell division

Adult eye
(20,000 cells)
Screening for negative regulators of cell growth and proliferation
Negative Regulators on Chromosome 2R

Wild-type
Analysis of mosaic tissue

Wild-type = GFP
Newly hatched larva
~ 20 cells give rise to eye disc

Third instar larva
early larva

proliferation
proliferation and differentiation

Pupal lattice

apoptosis

Adult eye
Increased tissue in clones in the eye

Wild-type
Control

hippo (hpo)

Extra inter-ommatidial cells in mutant tissue

During development, to control/restrict growth:

(a) cells stop dividing          (b) cells die (apoptosis)

When a mutation results in extra cells, it can be because

(a) cells keep dividing    and/or (b) cells don't die
Newly hatched larva
~ 20 cells give rise to eye disc

Third instar larva

Pupal lattice

Adult eye

early larva

proliferation

proliferation and differentiation

apoptosis
hpo mutant tissue shows decreased cell death
And suppresses GMR-grim induced death

Quantifying division & growth

create homozygous clones

+/-

Time

-/-

Flow Cytometric Analysis

Dissociate cells

Count cells in mutant clone and wild-type clone to measure proliferation rate (doubling time)

<table>
<thead>
<tr>
<th>Cell Counts</th>
<th>wt clones</th>
<th>hpo mutant clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>%G1</td>
<td>28.6</td>
<td>24.8</td>
</tr>
<tr>
<td>%S</td>
<td>11.1</td>
<td>10.7</td>
</tr>
<tr>
<td>%G2</td>
<td>61.2</td>
<td>64.5</td>
</tr>
</tbody>
</table>


**Sav-Hpo-Wts complex linked to human cancer**

*Human sav (hWW45) is mutated in cancer cell lines*


*Lats1 (wts) KO mice develop soft tissue sarcomas*

Mosaic screens can identify non-autonomous phenotypes

wild-type  Uba1
Non-autonomous tumor suppressor phenotypes include components of the ubiquitin pathway and endocytic trafficking:


Both non-autonomous growth and neoplastic growth
Suppression of cell death screens:

* Resistance to cell death is important in cancer
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VI. Metastasis models in flies
UCLA Undergraduate Research
Consortium in Functional Genomics

http://www.bruinfly.ucla.edu/methods.php
(from review)

**a Ectopic expression**

Tissue-specific promoter-GAL4; UAS-GFP; UAS-Gene X

Gene X-expressing cells are marked by GFP

**d MARCM**

ey-FLP; UAS-GFP; UAS-Gene X; Tubulin-GAL4 FRT Tubulin-GAL80 FRT mutant

Mutant clones are positively marked by GFP
A Genetic Screen in Drosophila for Metastatic Behavior

Raymond A. Pagliarini and Tian Xu
Degradation of basement membrane/invasion into ECM

Box 2 | Implanting tissue in adult hosts to assay for tumour growth

From the following article:

Spindle orientation, asymmetric division and tumour suppression in Drosophila stem cells.

Cayetano Gonzalez

Nature Reviews Genetics 8, 462-472 (June 2007)

Other areas of investigation in model systems:

Epigenetics (in fact, Polycomb was first discovered in flies)

micro RNAs (C. elegans and flies are very useful)

Asymmetric cell division (initial divisions in C. elegans, etc.)

Regeneration (planaria, axolotls/salamander, zebrafish, Xenopus, etc.)
Summary:

Model organisms are useful in analysis of basic processes relevant to cancer (e.g., growth, cell division).

Reverse genetics -- start with an oncogene or tumor suppressor and make an animal model (ret, MEN2).

Start with a pathway implicated in cancer and study its role in developmental processes in a specific model organism (Ras pathway in vulva development or photoreceptor specification).

Genome-wide RNAi screens/forward genetic screens can identify genes involved in growth and proliferation, *in vivo* analysis of these genes.

Modeling metastasis in model organisms.