Two classes of genetic pathways

I) Developmental/synthetic pathways
II) Regulatory/binary switch pathways

I) Developmental pathways – Synthesis and/or assembly of molecule(s), subcellular, cellular or multicellular structures. These are essentially substrates/end-products. The pathway usually reflects temporal and morphological aspects of the process.

- Examples include biosynthetic pathways, phage morphogenesis, the cell cycle and development.
- Mutations in developmental/synthetic pathway genes usually block or arrest the pathway.
- The temporal and morphological nature of the arrest phenotype in the mutant can help position the gene in the pathway.
- Regulatory/binary switch pathways are embedded within developmental pathways to control their action.

II) Regulatory/binary switch pathways – define two alternate regulatory states in a biological system. The regulatory switch pathway responds to external cues. Regulatory switches are embedded in developmental pathways to regulate and integrate the environment and/or parallel pathway.

- Examples include Gal induction, the decision to progress through the cell cycle, signal transduction, sex determination and cell fate determination.
- Regulatory switch pathways usually do not have temporal or morphological phenotypes associated with the step/gene product.
- The pathway may be active only briefly or active continuously.
- Mutant phenotypes usually reflect one or the other alternate state of the binary switch and thus usually do not help directly to position the gene in the pathway.
T4 bacterial phage 
“Self” assembly 
developmental pathway

Somatic Sex Determination

Binary Switch Pathway

\[
\begin{align*}
R \times A & \quad \text{her-1} \quad \text{tra-2} \quad \text{fem-1} \quad \text{tra-1} \\
1.0 & \quad \text{OFF} \quad \text{ON} \quad \text{OFF} \quad \text{ON} \\
0.5 & \quad \text{ON} \quad \text{OFF} \quad \text{ON} \quad \text{OFF}
\end{align*}
\]
Yeast cell cycle stages - landmark events (phenotypes)

Figure 1 Major landmark events of the S. cerevisiae cell cycle. The diagram attempts to indicate the temporal order of events, but distances between events in the diagram are not necessarily proportional to the time intervals between these events; notably, the intervals from SPBSF to BE and from IND to CK may be substantially exaggerated in the diagram. In addition, there is some uncertainty about the temporal order of some events (see text). Abbreviations: SPBSF, spindle-pole-body satellite formation; SPBD, spindle-pole-body duplication; CRF, formation of the chitin ring (shown in the diagram as a heavy line at the mother-bud junction); MRF, formation of the microfilament ring (not shown in the diagram, but found adjacent to the cell membrane in the region of the mother-bud junction); BE, bud emergence; IDN, initiation of chromosomal DNA synthesis; IS, chromosomal DNA synthesis; SPBS, spindle-pole-body separation (and formation of a complete spindle); NM, nuclear migration; mND, medial stage of nuclear division; SE, spindle elongation (as indicated in the diagram, prior to mND, the spindle microtubules do not normally stretch the length of the nucleus; it is not clear whether mND and SE should be regarded as distinct events); IND, late stage of nuclear division; CK, cytokinesis; CS, cell separation (usually monitored after mild sonication, since the time interval from cytokinesis to cell separation is highly variable [Pringle and Nor 1975]). Landmark events not shown in the diagram include replication of the 2-micron plasmid DNA, which occurs during the S phase [Zakian et al. 1979]; the periodic variations in vacuole structure and cell density (Hartwell 1970; Wiesken et al. 1971); and the various stages of cell separation (septum formation) as defined by Cash and his colleagues (Molano et al. 1980, and references cited therein).

Yeast cell cycle - Developmental pathway

From Pringle and Hartwell
Yeast cell cycle - Developmental pathway

Functional relationships between gene mediate steps.
Developmental pathway/Biosynthetic pathway

1) Dependent
   
   \[ A \rightarrow B \]

2) Dependent
   
   \[ B \rightarrow A \]

3) Independent
   
   \[ A \]

4) Independent
   
   \[ A, B \]

Functional relationships in Regulatory/Binary switch pathway

1) Negative
   
   \[- A \rightarrow B \] or \[ B \rightarrow - A \]

2) Positive
   
   \[ A \rightarrow + B \] or \[ B \rightarrow + A \]
The order of gene function in a pathway can be deduced genetically by Epistasis Analysis – the interaction of alleles of different genes involved in the same process.

\[ \text{gene-}a(\text{lf}) \text{- } P \text{ phenotype} \quad \quad \text{gene-}b(\text{lf}) \text{- } Q \text{ phenotype} \quad \rightarrow \quad \text{Distinct phenotypes} \]

For \( \text{lf} \equiv \text{Null Allele} \), the question is — what process is executed in the absence of both \( \text{gene-}a \) and \( \text{gene-}b \) activity?

Double Mutant

\[ \frac{\text{gene-}a(\text{lf})}{\text{gene-}a(\text{lf})}, \frac{\text{gene-}b(\text{lf})}{\text{gene-}b(\text{lf})} \rightarrow Q \text{ phenotype} \]

Then \( \text{gene-}b(\text{lf}) \) is epistatic to \( \text{gene-}a(\text{lf}) \) or \( \text{gene-}b(\text{lf}) > \text{gene-}a(\text{lf}) \)

For developmental pathways, epistasis analysis allows one to determine if temporal/morphological order is equivalent to causal order.

Synthesis or assembly of molecules, subcellular, cellular or multicellular structures.

Examples

**Synthetic pathway:** \[ \alpha \rightarrow \beta \rightarrow \gamma \rightarrow \delta \]

\( A, B, \) and \( C \equiv \text{gene products (enzymes) which catalyze the synthesis of a product.} \)
\( \alpha \equiv \text{starting molecule(s),} \beta \text{ & } \gamma \equiv \text{intermediates,} \delta \equiv \text{end product} \)

**Assembly pathway:**

\[ A \rightarrow [AB] \rightarrow [ABC] \rightarrow [ABCD] \]

\[ B \rightarrow [AB] \rightarrow [ABC] \rightarrow [ABCD] \]

\( A, B, C \) and \( D \equiv \text{gene products} \)
\( [AB], [ABC] \equiv \text{intermediates} \)
\( [A] \equiv \text{starting component} \)
\( [ABCD] \equiv \text{assembled end product} \)
**Regulatory/Binary switch pathway**

A, B, C - gene products regulators (at any level) of other gene products.

Gene product C (ON) specifies the cell fate “P”
- P differentiation genes ARE expressed
- Q differentiation genes are NOT expressed

When there is no active C product [C is in the OFF state or a C(lf) mutation], a default state exists → “Q” cell fate
- P differentiation genes are NOT expressed
- Q differentiation genes ARE expressed

Input Signal (e.g. X/A)

<table>
<thead>
<tr>
<th>Input Signal</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P or Q cell fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>State 1</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>P cell</td>
</tr>
<tr>
<td>State 2</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>Q cell fate</td>
</tr>
</tbody>
</table>

**Developmental pathway vs. Regulatory switch pathway**

gene-b(lf) is epistatic (>) to gene-a(lf)

I) Developmental pathway - synthesis or assembly of molecule(s), subcellular, cellular or multicellular structures. For gene-b(lf) > gene-a(lf)
- B acts before A in a dependent pathway.
- Arrest because of b(lf), function of downstream components depends on the presence of b(+).

II) Regulatory/binary switch pathway
For gene-b(lf) > gene-a(lf)
- A acts before B in the pathway
The state of the binary switch is set by the activity of the last gene in the pathway.
Genetically If=OFF
Developmental pathway – synthesis example

\[ \alpha \to \beta \to \gamma \to \delta \]

In A(If), alpha accumulates (an alpha phenotype)
In B(If), beta accumulates
In C(If), gamma accumulates

Is “temporal” order equivalent to causal order?
A(If):B(If) double mutant = A(If) alone: alpha accumulates.
A(If):C(If) double mutant = A(If) alone: alpha accumulates.
B(If):C(If) double mutant = B(If) alone: beta accumulates.

In a synthetic or assembly pathway there is a dependent sequence of events. Later events depend on prior execution of earlier events. Downstream activity is irrelevant when assayed with If mutants.

Regulatory/Binary switch pathway have a default state

- Genetically, this default state is represented by the loss of function/null phenotype of the last gene in the pathway.
- Molecularly, the default state depends on the biological process that is controlled.
How do we know genotype?

1. Propagate a homozygous strain where genotype is known by:
   a) phenotype, and/or b) complementation testing, and/or c) molecular marker/sequence analysis.
2. When doing a cross or segregating from a heterozygote need to determine genotype.
   Possibilities:
   a) Phenotype (won’t work in epistasis analysis)
   b) Knocked in marker (but co-dominant)
   c) Determine genotype by PCR/sequence (ok for epistasis analysis, not feasible in screens/large number of samples)
   d) Balancer chromosomes marked with GFP (co-dominant, by exclusion)
   \[
   \begin{align*}
   \frac{m}{GFP \text{ balancer}} & \equiv \text{Green} \\
   \frac{m}{m} & \equiv \text{nonGreen}
   \end{align*}
   
   e) Tightly linked morphological marker mutant (e.g. dpy-20 for \textit{fem-3} mutant) that segregate with the gene mutation of interest.

Regulatory/binary switch pathways (\textit{C. elegans} somatic sex example)

- Remember the wild-type function of each gene because some gene orders may not make sense
  \[\text{tra} \rightarrow^+ \text{fem}\]
  \[\text{fem-A} \rightarrow^- \text{fem-B}\]
- \textit{lf(null)} mutation is analogous to the regulatory gene or gene product being inactive (OFF or Low)
- The pathway is in two states
  - state 1 - last gene in pathway is Active (ON or High)
  - state 2 - last gene in pathway is Inactive (OFF or Low)
- It is the ON/OFF state of the last gene/gene product that determines phenotype.
- Thus when \textit{b(lf)} is epistatic to \textit{a(lf)}, \textit{b(+)} functions after \textit{a(+)}.
What sex is specified in the absence of both *fem-1* and *tra-1* products?

*tra-1(lf); fem-1(lf) is ♂(soma)*

∴ *tra-1(lf) is epistatic to fem-1(lf)*.

Thus male somatic development can occur even in the absence of the masculinizing product *fem-1(+)*

Therefore *fem-1(+) is not essential for specifying male development.*

But female development requires *tra-1(+)"

---

**How can *fem-1(+) promote male development but not be essential for it?**

<table>
<thead>
<tr>
<th>Model</th>
<th>fem-1</th>
<th>♀</th>
<th>tra-1</th>
<th>♂</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>OFF</td>
<td>ON → ♂</td>
<td>ON → ♀</td>
<td>OFF → ♀</td>
<td>♂ Development</td>
</tr>
<tr>
<td>Mutants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fem-1</td>
<td>ON</td>
<td></td>
<td></td>
<td>OFF → ♀</td>
<td>♀ Development</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tra-1</td>
<td>OFF → ♂</td>
<td>♂ Default</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ON</td>
<td>like <em>tra-1(M)</em></td>
</tr>
<tr>
<td>fem-1</td>
<td>OFF</td>
<td>♀</td>
<td></td>
<td>OFF → ♂</td>
<td>♂ Development</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tra-1</td>
<td>OFF → ♂</td>
<td>♂ Default</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ON</td>
<td>like <em>tra-1(M)</em></td>
</tr>
</tbody>
</table>

*Double Mutant* | fem-1 | OFF | tra-1 | OFF | like *tra-1(M)* above |
|                | (♂)   |     |       |     |                         |
Can the opposite model -- *tra-1* acting as a negative regulator of *fem-1* explain the phenotypic data?

<table>
<thead>
<tr>
<th>Model</th>
<th><em>tra-1</em></th>
<th><em>fem-1</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>OFF</td>
<td>ON</td>
<td>♀ default</td>
</tr>
<tr>
<td>ON</td>
<td>OFF</td>
<td>♀ development</td>
</tr>
</tbody>
</table>

**Single Mutants**

| *tra-1* (–) | *fem-1* (–) | ♀ Default |
| ON           | OFF         | like *fem-1* (±) |

| *tra-1* (–) | *fem-1* (+) | ♀ Development |
| OFF (±)      | ON          | like *tra-1* (±) |

**Double Mutant**

| *tra-1* (–) | *fem-1* (–) | ♀ Predict Default |
| OFF (±)      | OFF (±)     | Develop |

Since *tra-1*(–) is epistatic to *fem-1*(–). *tra-1*(+) must act after *fem-1*(+).

What is the epistatic relationship of *tra-1* with *fem-2* & *fem-3*?

*tra-1*(–) is epistatic to *fem-2*(–)
*tra-1*(–) is epistatic to *fem-3*(–)

:. The three *fem* genes act at the same level in the pathway.
What sex is specified in the absence of both tra-2 and fem-1 products?

tra-2(lf); fem-1(lf) is ♀
∴ fem-1(lf) is epistatic to tra-2(lf).

Thus female somatic development can occur even in the absence of the feminizing product tra-2(+).

Therefore tra-2(+) is not essential for specifying female development.

But male development, in this double mutant, needs fem-1(+)
What sex is specified in the absence of both her-1 and tra-2 products?

tra-2(lf): her-1(lf) is ♀

tra-2(lf) is epistatic to her-1(lf).

Combining all of the epistasis data
Double mutant: \( \text{her-1}(lf); \text{tra-1}(lf) \) is \( \sigma \) phenotype

Triple mutants: \( \text{tra-2}(lf); \text{fem}(lf); \text{tra-1}(lf) \) is \( \sigma \) phenotype
\( \text{her-1}(lf); \text{tra-2}(lf); \text{fem}(lf) \) is \( \varphi \) phenotype, etc.

Quadruple mutant: \( \text{her-1}(lf); \text{tra-2}(lf); \text{fem}(lf); \text{tra-1}(lf) \) is \( \sigma \) phenotype, etc.

Will a synthetic/assembly pathway explain somatic sex determination?

1) Epistatic gene order is reversed.
2) A dependent sequence of event.
   \( \Rightarrow \) Arrest phenotypes - intermediates accumulate.

- \( \text{tra-1}(lf) \) has a \( \sigma \) phenotype
- \( \text{M1} \) masculinizing starting substrate
- \( \text{fem-1}, 2, 3(lf) \) a \( \varphi \) phenotype
- \( \text{F1} \) feminizing intermediate accumulates
- \( \text{tra-2}, 3(lf) \) a \( \sigma \) phenotype
- \( \text{M2} \) masculinizing intermediate accumulates
- \( \text{her-1}(lf) \) a \( \varphi \) phenotype
- \( \text{F2} \) feminizing intermediate accumulates
- \( \text{M3} \) masculinizing final substrate
Does this synthetic/assembly sex determination pathway make biological sense?

1) Starting substrate and each intermediate specifies sexual fate.
2) What happens to the pathway in XX and XO animals? M1 and M3 specify \( \alpha \) fate.
3) How are gf mutations explained?
4) Predicts that dosage compensation should be dependent on sex determination pathway. It is not.
5) Not consistent with molecular data.

Genetic pathway for somatic sex determination & dosage compensation in *C. elegans*
Molecular Characterization of the pathway

XX    ON    OFF    ON    OFF    ON    OFF
XO    OFF    ON    ON    OFF    ON    ON

RNA Levels in Wild Type

XX    +    +    +    +    +    +
XO    +    +    +    +    +    +

X/A  Xol-1  Novel Protein
     SMC Protein
     Side-1 + 2  Zn Finger DNA Binding Proteins

tra-2 Transmembrane Receptor

tra-3 Protein

Fem-1 Ankyrin Repeat

Fem-2 Ser/Thr Protein Phosphatase

Fem-3 Novel

tra-1 Zn Finger DNA Binding Protein
**tra-1** is a sexual fate regulator in many cell types/tissues

<table>
<thead>
<tr>
<th>Gene</th>
<th>Male Fate</th>
<th>Female Fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>mab-3</td>
<td>Yolk synthesis</td>
<td>♂ sensory neuron fate</td>
</tr>
<tr>
<td>mab-23 (OFF)</td>
<td>Yolk synthesis</td>
<td>♂ sensory neurons not specified</td>
</tr>
<tr>
<td>egl-1 (OFF)</td>
<td>HSN apoptosis</td>
<td>HSN survival</td>
</tr>
<tr>
<td>ceh-20 (OFF)</td>
<td>♂ neuron survival</td>
<td>♂ neurons die</td>
</tr>
</tbody>
</table>

Default state, at the molecular level

---

**Hermaphrodite Specific Neuron (HSN) Developmental Pathway**

<table>
<thead>
<tr>
<th>Switch</th>
<th>Male Fate</th>
<th>Female Fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal vs. non-neuronal Fates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSN vs. PHB Fates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSN</td>
<td>♂ vs. ♀</td>
<td>Apoptosis</td>
</tr>
</tbody>
</table>

Successive binary decisions that display a dependent relationship
How to determine gene order when the genes have the same wild-type functions – the same (or similar) lf phenotype.

- *her-1, fem-1, fem-2*, and *fem-3* are needed for ♂ development. If there are genes that act in between. For example, *her-1* vs. *fem-1, 2*, and 3. The *tra-2* and *tra-3* genes act in between.

- Different temperature sensitive periods.
- Different maternal/zygotic activity.
- Molecular probes for the gene activity - Is the activity of one gene altered in mutants of a second (or third) gene?
- If there are gf alleles, they may be useful.

**Epistasis with gf mutations: two examples**

\[ \begin{align*}
\text{fem-3(gf) & $\uparrow$ germline} \\
\text{fem-1(lf) & $\Omega$ germline}
\end{align*} \]

**Case 1**

\[ \begin{align*}
\text{fem-3(gf): fem-1(lf) - $\uparrow$ phenotype} \\
\text{fem-3(gf) is epistatic to fem-1(lf)}
\end{align*} \]

\[ \therefore \text{fem-3(gf) can bypass the need for fem-1(lf) in directing $\uparrow$ germline development.} \]

\[ \begin{align*}
\text{fem-1} & \xrightarrow{(+)} \text{fem-3} \\
\text{ON} & \quad \text{ON} \quad \Rightarrow \text{♂} \\
\text{OFF} & \quad \text{OFF} \quad \Rightarrow \text{♀ (default)}
\end{align*} \]

\[ \begin{align*}
\text{fem-1} & \quad \text{fem-3(gf)} \\
\text{OFF} & \quad \text{ON} \quad \Rightarrow \text{♂} \\
\text{(lf)} & \quad \text{Because of gf abnormal activity}
\end{align*} \]
Case 2 \hspace{1cm} fem-1(lf):fem-3(gf) - $\varphi$-phenotype  
fem-1(lf) is epistatic to fem-3(gf) 

.: fem-3(gf) can not bypass the need for fem-1(+) in directing $\varphi$ germline development.  

Two possible orders.  
a) fem-1 $\stackrel{(+)}{\longrightarrow}$ fem-3  
\hspace{1cm} fem-1 is an obligated (+) regulator of fem-3. gf mutation does not compensate for the need of fem-1 action on fem-3.  

b) fem-3 $\stackrel{(+)}{\longrightarrow}$ fem-1  
\hspace{1cm} fem-3 is a positive regulator of fem-1. Without the downstream fem-1 product in fem-1(lf) mutants, the activity of fem-3(gf) is irrelevant.  

Redundancy uncovered by (also see Thomas 1993 TIGs 9:395)  

1) Reverse genetics, from genome sequence information  
2) Dominant mutation (e.g. unc-93 antimorphic mutations)  
3) Synthetic phenotypes - 2 examples:  
\hspace{1cm} i) gene-a null --- Phenotype A 
\hspace{1cm} gene-b null --- Phenotype B 
\hspace{1cm} gene-a; gene-b double null - New phenotype C  
\hspace{1cm} ii) gene-a null - Incompletely penetrant phenotype A 
\hspace{1cm} gene-b null - Incompletely penetrant phenotype A 
\hspace{1cm} gene-a; gene-b double null - highly penetrant phenotype A
Synthetic phenotypes with non-null alleles does not necessarily imply redundancy

1) fem-4 null --- 5% female  
   fem-5 null --- 10% female  
   fem-4; fem-5 double null --- 100% female (synergistic interaction)

| fem-4 | fem-5 | fate
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Redundant function</td>
</tr>
</tbody>
</table>

Conclude that

2) fem-4 hypomorph - 5% female  
   fem-5 hypomorph - 10% female  
   fem-4; fem-5 double hypomorph - 100% female  

Can not distinguish

\[
\begin{align*}
&\text{fem-4} \rightarrow \text{fem-5} \quad \text{or} \quad \text{fem-5} \rightarrow \text{fem-4} \\
\end{align*}
\]

Sequential function

FROM

<table>
<thead>
<tr>
<th>fem-4</th>
<th>fem-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional information is needed to understand the mechanistic basis of redundancy

1) If fem-4 and fem-5 are paralogous genes then they perform the same the function by the same mechanism.

2) fem-4 and fem-5 encode distinct gene products that:
   a) Have the same enzymatic function  
      e.g. single subunit polyA polymerase  
      vs. multisubunit polyA polymerase
   b) Have distinct functions
      i) that eventually coverage on a common process

| fem-4 | fem-5 | Process
|-------|-------|-----|
|       |       | Parallel pathway

i) Non-essential parts of a multi-component complex/process (often called fidelity/efficiency factors).  
For example, certain splicing factors
Caveats about epistasis analysis

• Need to believe that genes the under test are involved in the same biological process.
  
  * let-60 (Let) double with fem-1 (Fem) ⇒ Lethal
  
  But what does this mean?

• Hypomorphic alleles may give you different results than null alleles.

• Can not deduce gene order when gf mutations of both genes are used-no end point

• For binary switch pathways (2 phenotypic outcomes) branches and feedback is difficult to unravel.
  
  - Molecular biology and biochemistry

• Possible that a signal transduction pathway will have a graded (quantitative) outcome
  
  - Unclear how this might be related to phenotype.

• The relationship between a dependent pathway and a regulatory switch pathway may be unclear. Thus, the more one knows about the biology and phenotypes (morphological and molecular) the better.

Epistasis - ordering genes in a pathway in model organisms

• Inbred, homogeneous background

• Genotype of interacting genes known

• Null alleles or gain-of-function alleles of known behavior used to model “ON” and “OFF” states.

Epistasis - gene interactions in population/quantitative/human genetics

• Statistical deviation from additive combination of two loci on their affect on phenotype (R.A. Fisher)

• Outbred. Number of segregating loci acting on phenotype often not known.

• Alleles usually not null. Nature of allele often not known.

• Alleles may be heterozygous or homozygous.