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 a 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
Yeast cell cycle stages - landmark events (phenotypes)

Yeast cell cycle - Developmental pathway

From Pringle and Herskowitz
Yeast cell cycle - Developmental pathway

Regulatory switch
- Environment
- Checkpoint central
- Environment

Functional relationships between gene mediated steps.
Developmental pathway/Biosynthetic pathway
1) Dependent
   A → B
2) Dependent
   B → A
3) Independent
   A → B
4) Interdependent
   A, B

Functional relationships in Regulatory/Binary switch pathway
1) Negative
   A → B or B → A
2) Positive
   B → A or A → B

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.

gene-a(lf) → P phenotype
gene-b(lf) → Q phenotype
Distinct phenotypes

For a Null Allele, the question is — what process is executed in the absence of both gene-a and gene-b activity?

Double Mutant

gene-a(lf) gene-a(lf)
gene-b(lf) gene-b(lf)

Then gene-b(lf) is epistatic to gene-a(lf) or gene-b(lf) > gene-a(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:**

\[ \begin{align*}
A & \rightarrow \beta & \rightarrow \gamma & \rightarrow \delta \\
\text{alpha} & - \text{starting molecule(s)}, & \beta & \text{gamma} & - \text{intermediates}, & \delta & - \text{end product}
\end{align*} \]

**Assembly pathway:**

\[ \begin{align*}
A, B, C & \text{and D} & \equiv & \text{gene products} \\
[AB], [ABC] & - \text{intermediates} & [ABCD] & - \text{assembled product}
\end{align*} \]

**Regulatory/Binary switch pathway**

A, B, C - gene product 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
- P differentiation genes are NOT expressed
- Q differentiation genes ARE expressed

**Developmental pathway vs. Regulatory switch pathway**

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(+).

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

\[ A \rightarrow B \rightarrow C \rightarrow D \]

- In \( A(\text{lf}) \), alpha accumulates (an alpha phenotype)
- In \( B(\text{lf}) \), beta accumulates
- In \( C(\text{lf}) \), gamma accumulates

Is “temporal” order equivalent to causal order?

- \( A(\text{lf});B(\text{lf}) \) double mutant = \( A(\text{lf}) \) alone: alpha accumulates.
- \( A(\text{lf});C(\text{lf}) \) double mutant = \( A(\text{lf}) \) alone: alpha accumulates.
- \( B(\text{lf});C(\text{lf}) \) double mutant = \( B(\text{lf}) \) 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 \( \text{lf} \) 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)
       \[ \frac{\text{GFP\text{-}balancer}}{\text{nonGreen}} \equiv \text{Green} \]
     - e) Tightly linked morphological marker mutant (e.g. dpy-20 for \( \text{fem-3} \) mutant) that segregate with the gene mutation of interest.
Regulatory/binary switch pathways (C. elegans somatic sex example)

- Remember the wild-type function of each gene because some gene orders may not make sense
  \[ \begin{array}{c}
  \text{tra} & \longrightarrow & \text{fem}
  \end{array} \]

- \( \text{lf} \) (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 \( b(\text{lf}) \) is epistatic to \( a(\text{lf}) \), \( b(+) \) functions after \( a(+) \).

<table>
<thead>
<tr>
<th>Somatic phenotype</th>
<th>fem-1(\text{lf})</th>
<th>♂</th>
<th>WT function - ♂ Development</th>
<th>tra-1(\text{lf})</th>
<th>♀</th>
<th>WT function - ♀ Development</th>
</tr>
</thead>
</table>

What sex is specified in the absence of both fem-1 and tra-1 products?

\[ \begin{array}{c}
\text{tra-1(\text{lf}); fem-1(\text{lf}) is ♀(soma)}
\end{array} \]

Thus male somatic development can occur even in the absence of the masculinizing product \( \text{fem-1}(+) \).

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?
Can the opposite model -- tra-1 acting as a negative regulator of fem-1 explain the phenotypic data?

Since tra-1(lf) is epistatic to fem-1(lf), tra-1(+) must act after fem-1(+) and tra-1(lf) is epistatic to fem-2(lf) and fem-3(lf).

tra-1(lf) is epistatic to fem-2(lf) and fem-3(lf).

Thus the three fem genes act at the same level in the pathway.

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

tra-1(lf) is epistatic to fem-2(lf) and fem-3(lf).

tra-1(lf) is epistatic to fem-2(lf) and fem-3(lf).

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 ♀

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

What sex is specified in the absence of both tra-2 and fem-1 products?

tra-2(lf), fem-1(lf) is ♀

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(+).
How can tra-2(+) promote female development but not be essential for it?

<table>
<thead>
<tr>
<th>Gene</th>
<th>WT function</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>her-1(lf)</td>
<td>♀</td>
<td>WT function</td>
</tr>
<tr>
<td>tra-2(lf)</td>
<td>♂</td>
<td>WT function</td>
</tr>
</tbody>
</table>

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  
\textit{her-1}(lf); \textit{tra-1}(lf) \textit{♂} phenotype

Triple mutants  
\textit{tra-2}(lf); \textit{fem}(lf); \textit{tra-1}(lf) \textit{♂} phenotype
\textit{her-1}(lf); \textit{tra-2}(lf); \textit{fem}(lf) \textit{♀} phenotype, etc.

Quadruple mutant  
\textit{her-1}(lf); \textit{tra-2}(lf); \textit{fem}(lf); \textit{tra-1}(lf) \textit{♂} phenotype, etc.

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

\textit{tra-1}(lf) has a \textit{♂} phenotype \textbf{M1} masculinizing starting substrate  
\textit{fem-1,2,3}(lf) a \textit{♀} phenotype \textbf{F1} feminizing intermediate accumulates  
\textit{tra-2,3}(lf) a \textit{♂} phenotype \textbf{M2} masculinizing intermediate accumulates  
\textit{her-1}(lf) a \textit{♀} phenotype \textbf{F2} feminizing intermediate accumulates

Will a synthetic/assembly pathway explain somatic sex determination?  
1) Epistatic gene order is reversed.  
2) A dependent sequence of event.  
\therefore Arrest phenotypes - intermediates accumulate.

But does a 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? \textbf{M1} and \textbf{M3} specify \textit{♂} fate.  
3) How are \textbf{gf} mutations explained?  
4) Predicts that dosage compensation should be dependent on sex determination pathway. \textit{It is not.}  
5) Not consistent with molecular data.
Genetic pathway for somatic sex determination and dosage compensation in *C. elegans*

Molecular Characterization of the pathway

[Diagrams and tables related to the genetic pathway are shown here.]
tra-1 is a sexual fate regulator in many cell types/tissues

Hermaphrodite Specific Neuron (HSN) Developmental Pathway

Neuronal vs. non-neuronal Fates

HSN vs. PhB Fates

Regulatory switch

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) if phenotype.

- her-1, fem-1, fem-2, and fem-3 are needed for ♂ development.
  Are there 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 readouts 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

Case 1

\[
\begin{array}{c|c|c}
& fem-3(gf) & fem-1(lf) \\
fem-1 & ON & OFF \\
fem-3 & ON & OFF \\
\end{array}
\]

\[ \therefore \text{fem-3}(gf) \text{ is epistatic to fem-1}(lf) \]

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

Case 2

\[
\begin{array}{c|c|c}
& fem-1(lf); fem-3(gf) & fem-1(lf) \\
fem-1 & OFF & ON \\
fem-3 & ON & OFF \\
\end{array}
\]

\[ \therefore \text{fem-3}(gf) \text{ can not bypass the need for fem-1}(+) \text{ in directing } \varphi \text{ germline development.} \]

Two possible orders.

a) fem-1 \( \leftrightarrow \) fem-3

fem-3 is an obliged (+) regulator of fem-3. gf mutation does not compensate for the need of fem-1 action on fem-3.

b) fem-3 \( \leftrightarrow \) fem-1

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:
   i) gene-a null --- phenotype A
      gene-b null --- phenotype B
      gene-a; gene-b double null - New phenotype C
   ii) gene-a null - Incompletely penetrant phenotype A
      gene-b null - Incompletely penetrant phenotype A
      gene-a; gene-b double null - Highly penetrant phenotype A
Synthetic phenotypes with non-null alleles does not necessarily imply redundancy

1) femin-4 null --- 5% female
femin-5 null --- 10% female
femin-4, femin-5 double null --- 100% female (synergistic interaction)

Conclude that femin-4 femin-5 fate Redundant function

2) femin-4 hypomorph - 5% female
femin-5 hypomorph - 10% female
femin-4, femin-5 double hypomorph - 100% female

Can not distinguish femin-4 femin-5 Sequential function

FROM femin-4 femin-5 Redundant function

Additional information is needed to understand the mechanismic basis of redundancy

1) If femin-4 and femin-5 are paralogous genes then they perform the same the function by the same mechanism.
2) femin-4 and femin-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 biochemical functions
      i) that eventually cover an a common process
      Parallel femin-4 femin-5 Process
      ii) 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 under test are involved in the same biological process.
  let-60G(Let) double with femin-1 (Fem) = Lethal
  But what does this mean?
- Hypomorphic alleles may give you different results than null alleles.
- Can not deduce gene order when of mutations of both genes are used
- For binary switch pathways (2 phenotypic outcomes) branches and feedback is difficult to discern.
  - 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 phenotypic effect, usually with two loci. (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.
  • See Phillips, Nature Reviews Genetics, 2008 9:855-867