Cell autonomous vs. cell non-autonomous gene function

In multicellular organisms, it is important to know in what cell(s) the activity of a gene is required.

- while RNA expression can be highly informative, gene products are often regulated post-transcriptionally and post-translationally.
- gene may encode a signaling molecule or function in the synthesis/activity of a signaling molecule.

Genetic approaches to assess in which cell(s) gene activity is required.

- mosaic analysis (refers to stochastically generated genotypic changes; worms & flies)
- cell type specific knockout
- cell type specific RNAi
- cell type specific rescue

Employing cell type specific promoter

Issues
- Promoter/enhancer driver lines give unexpected cell type expression (e.g. Luo et al., 2020 Neuron, 106:37-65)
- Expression typically non-physiological
Cell autonomy/non-autonomy (mosaic analysis) analysis is used to define the anatomical focus of gene action

- The cell(s) in which removal of wild-type gene activity results in a mutant phenotype (indicates necessity) and/or
- The cell(s) in which the presence of wild-type gene activity is sufficient for a wild-type phenotype.

Cell autonomy/non-autonomy analysis is required in metazoans to provide an organismal context for gene and pathway function.

Cell autonomy/non-autonomy analysis is most informative for biological processes involving cell-cell communication.
Two possible outcomes of mosaic analysis define the gene as being involved in a) cell-autonomous or b) cell-nonautonomous processes

a) Cell autonomy: where phenotype and genotype of cells are concordant
   - a genotypically mutant cell displays a mutant phenotype.

b) Cell non-autonomy: when phenotype and genotype of cells are not concordant
   - a genotypically mutant cell may cause a genotypically wild-type cell to exhibit a mutant phenotype.
   and/or
   - a genotypically wild-type cell may rescue a genotypically mutant cell.
Cell autonomous gene action suggests that the product is involved in signal reception, signal transduction, or does not participate in a process involving cell-cell interactions.

Cell non-autonomous gene action suggests that the product is a signaling molecule or participates in the synthesis of a signaling molecule or metabolite.
Mosaic Analysis requires

1) A method to produce a lineage, patch or group of mutant cells (usually \(lf/lf\)) in an otherwise wild type (usually \(lf/+\)) animal.
   a) *C. elegans* - use mitotically unstable free duplications/transgenic arrays
   b) Drosophila - use mitotic recombination (X-ray) or mediated by FLP-FRT recombination
   a) Mouse - chimeras, using homozygous mutant ES or iPS cells, or Cre-lox recombination

2) A cell autonomous marker to allow genotypically mutant and genotypically wild-type cells to be identified, independent of gene that is under investigation.

For *C. elegans*, \(ncl-1(lf)\) mutation affects the size of the nucleolus - scored by Nomarski microscopy (in live animals) or Nuclear GFP driven by the \(sur-5\) promoter - scored by fluorescence microscopy (in live animals).
Mosaic analysis in C. elegans

C. elegans chromosomes are holocentric – multiple “centromeres” along the length of the chromosome.

Chromosomal fragments (free duplication or transgene arrays) can be propagated mitotically and meiotically so long as there is at least one sequence that behaves like a “centromere”.

Mitotic stability depends on the length of the chromosome fragment.
Embryonic Lineage tree showing origins of sexually dimorphic larval cells

<table>
<thead>
<tr>
<th>cell(s)</th>
<th>male fate</th>
<th>hermaphrodite fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>V5 (LR)</td>
<td>ray 1 (Lr)</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>V6 (LR)</td>
<td>rays 2,3,4,5,6 (Lr)</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>T (LR)</td>
<td>rays 7,8,9 (Lr)</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>P10.p</td>
<td>hook</td>
<td>hypodermis</td>
</tr>
<tr>
<td>P5.p, P6.p, P7.p</td>
<td>hypodermis</td>
<td>vulva</td>
</tr>
<tr>
<td>B</td>
<td>spicules</td>
<td>no divisions</td>
</tr>
<tr>
<td>M</td>
<td>posterior diagonal muscles</td>
<td>vulval muscles</td>
</tr>
<tr>
<td>Z1, Z4</td>
<td>single-armed gonad</td>
<td>two-armed gonad</td>
</tr>
<tr>
<td>P4</td>
<td>sperm only</td>
<td>sperm and oocytes</td>
</tr>
</tbody>
</table>
tra-1 mosaic analysis (in XX animals): autonomy

tra-1(lf) is masculized, \( \therefore \) tra-1(+) promotes \( \Phi \) fate.  \{Zinc finger transcription factor\}

1) Assess cell autonomous marker (genotype)
*tra-1* mosaic analysis (in XX animals): autonomy

*tra-1(If)* is masculized, ∴*tra-1(+) promotes ♀ fate.

1) Assess cell autonomous marker (genotype)
2) Assess sexual phenotype
3) Are genotype and phenotype concordant?

♂ tail is intersexual
tra-1 Mosaic analysis (cont)

Dp lost in MS and Descendants

Ncl + + + + + + + + + + + - + + + + + + +
tra-1 Mosaic analysis (cont)

Cell Autonomy

Ncl  +  +  +  +  +  +  +  +  +  +  -  +  +  +  +

♂  ♂

Dp lost in MS and Descendents

Post-embryonic fates of sexually dimorphic cells

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<tr>
<th>cell(n)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>V9 (LR)</td>
<td>ray 1.5,0</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>V6 (LR)</td>
<td>rays 2,3,4,5,6 (f)</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>P1 (LR)</td>
<td>rays 7,8,9 (f)</td>
<td>hypodermis, alae</td>
</tr>
<tr>
<td>P10 (P)</td>
<td>hook</td>
<td>hypodermis</td>
</tr>
<tr>
<td>P5, P6, P7, P</td>
<td>hypodermis</td>
<td>vulva</td>
</tr>
<tr>
<td>P</td>
<td>vulva</td>
<td>ovaries</td>
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Cell non-autonomy - caused by loss of a developmental signaling event; Z1/Z4 in hermaphrodite generate the AC, which signals via EGF to P5.p, P6.p & P7.p to induce the vulval cell fate.

No vulva, P5.p, P6.p & P7.p instead have Male Fate
Mosaic analysis of *her-1* (in *XO* animals): Non-autonomous action

*her-1*(If) is feminized, *∴her-1*(+) promotes the ♂ fate.  

{peptide hormone}

I) *her-1*(-) cells follows ♂ fate
Mosaic analysis of *her-1* (in *XO* animals): Nonautonomous action

*her-1(If)* is feminized, ∴*her-1(+) +♂* promotes the ♀ fate.

I) *her-1(-) -cells follows ♀ fate*

Should be female fate, genotypically loss of function, but instead have male fate

Masculinizing influence of *her-1(+) -cells (HER-1 peptide hormone)*
**her-1 Non-autonomous action (cont)**

II) *her-1* (+) cells follow ♀ fate

- Ncl $\text{+ + + + + + + + + + +}$

Should be male fate, genotypically WT, but instead have female fate

- $\text{♀}$ $\text{♀}$ $\text{♀}$ $\text{♀}$ $\text{♀}$

Feminizing influence of *her-1* (-) cells

Dp lost in P1 and descendents
Model for non-autonomous *her-1* action

- **a** *her-1+* AB lineage dimorphic cell
- **b** *her-1-* P lineage dimorphic cell
- **c** *her-1+* Non-autonomous masculinization
- **d** *her-1-* Non-autonomous feminization
sdc-1 encodes a zinc finger transcription factor.

Does sdc-1 act cell autonomously or non-autonomously in the control of somatic sex determination?
Mosaic analysis of Zinc-finger gene product sdc-1(If) (in XX): Non-autonomy

sdc-1(If) is masculinized, ∴ sdc-1(+) promotes ♀ fate.

Should be female fate, genotypically WT, but instead have male fate

her-1 gene transcribed

Masculinizing HER-1 peptide hormone made by sdc-1(-) cells
It is activity in the context of the pathway that determines autonomy/non-autonomy.

If gene-$A$ acts non-autonomously – does not mean that its gene product is the signaling molecule. In $sdc-1$ case, it is acting as a transcriptional repressor of a signaling molecule.

A and B will act non-autonomously
C and D will act autonomously