MCB 142 Discussion

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1 Reverse Genetics

1.1 Overexpression

Introduce the gene downstream of a highly active promoter (e.g. P_{mec} :: *ced*3 mentioned in lecture). In yeast, cells can be transformed with a plasmid containing this gene construct.

1.2 Knockdown

Use the RNAi machinery of the cell to generate partial loss-of-function in a gene. In *C. elegans*, this procedure can be as simple as feeding the worms with bacteria containing your RNAi gene.

2 Cell Cycle and Cancer

2.1 Conditional alleles

Temperature-sensitive mutations produce proteins that function normally but become unstable at suboptimal temperatures. These mutations are useful for studying essential genes, such as cell cycle genes, whose loss-of-function mutations normally result in lethality. The restrictive temperature is the temperature at which the mutation causes loss-of-function. The permissive temperature is the temperature at which the mutation does not affect phenotype.

2.2 Tumor suppressors

The wild-type function of tumor suppressors is to inhibit progression of the cell cycle. A loss-of-function in a tumor suppressor can lead to cancer.

2.3 Oncogenes

The wild-type function of oncogenes is to promote progression of the cell cycle. A gain-of-function in an oncogene can lead to cancer.

3 Pathway Analysis

3.1 Loss-of-function versus gain-of-function

Loss-of-function alleles are usually recessive, and gain-of-function alleles are usually dominant. However, there are certain exceptions to the rule, such as haploinsufficient genes and dominant negative mutations. To be absolutely sure, cross your mutant strain with a line that contains a deficiency at your gene locus. (You will need to map the gene first in order to do this test.)

- amorph: m/+ = Df/+
- hypomorph: m/+ > Df/+
- hypermorph: m/Df > m/+
- neomorph: m/Df = m/+

4 Practice Problems

4.1 Vulval development

The six vulval precursor cells (VPCs) adopt different cell fates (primary, secondary and tertiary) depending on their proximity to a signaling anchor cell. The VPC closest to the anchor cell develops into a primary VPC, which then becomes the vulva. Cells adjacent to the primary VPC develop as secondary VPCs.

lin-12(null) mutants lack secondary VPCs. *lin-12(gf)* mutants cause all six VPCs to adopt the secondary fate. A *lin-15(lf)* mutant has multiple primary VPCs, which leads to multiple vulval openings (a phenotype known as Muv). The *lin-12(lf) lin-15(lf)* double mutant also results in the Muv phenotype, with all six VPCs adopting the primary fate. Propose a model to explain these results.

4.2 Sex determination

her-1 mutants also show abnormal sex determination: *her-1(gf)* hermaphrodites develop into males while *her-1(lf)* males develop as hermaphrodites. *tra-2(lf)* hermaphrodites develop as males, while *tra-2(gf)* males develop as hermaphrodites. The *her-1(lf) tra-2(lf)* double mutant shows the following phenotype: hermaphrodites develop as males. Propose a model to explain this result.

A *xol-1(gf)* is able to promote male development in hermaphrodites. A *xol-1(gf) her-1(lf)* double mutant male develops as a hermaphrodite. Propose a model to explain this result.

We know that TRA-1 functions downstream of TRA-2. What phenotypes would you expect from the following double mutants:

- *tra-1(gf) xol-1(gf)*

- *tra-1(lf) her-1(lf)*