Mutational Dissection

Introduction

Used for over 100 years to study phenotypic effect of genes through genetic mutation.

Using a mutagenesis to genetically disrupt normal gene activity.

Study consequent change in phenotype to understand normal biological function of genes.

Used to study specific genes or the additive effects of many genes on a particular process (e.g., brain development)

Disrupt one gene at a time a observe effect (if any)

There are **two** methods of dissection in terms of genetics used:

Forward Genetics

Identification of mutants precedes molecular analysis

"**genetics**" comes before molecular analysis

Reverse Genetics

Gene identified (by sequencing?) then phenotype of mutant studied

Molecular analysis comes before "**genetics**"

Three aspects of mutational dissection must be considered for each mutagenesis:

1) Selection of mutagen according to kinds of lesions desired.
2) Assay system that identifies relevant mutations according kinds of processes that one wished to study.
3) First phase of genetics and phenotypic characterization of recovered mutations.

The Mutational Assay System

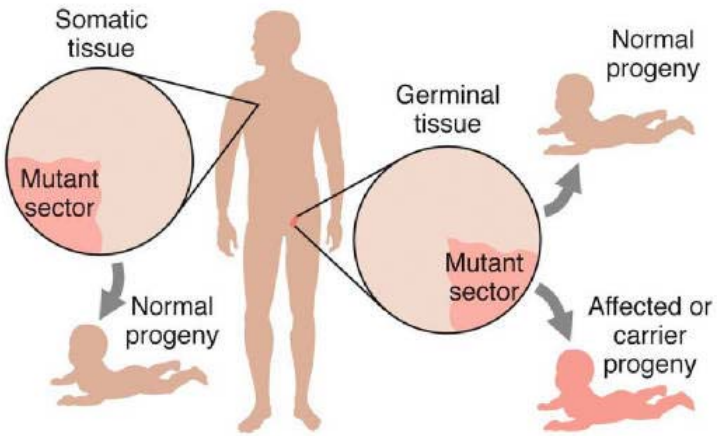
Key to mutational analysis is to generate candidate individuals with a phenotype of interest.

Combine appropriate system for detecting mutations with the appropriate mutagen.

Elements of an Appropriate Mutation-Detection System:

Type of Tissue/Cells

Genes can mutate in either **somatic** or **germinal** tissue.



Somatic Mutations occur in body cells (separte from germ cells).

If it occurrs in single cell in developing tissue, mutated cell is proginator of a population of identical mutant cells - called a **clone**.

Members of a clone stay close together during development; observable outcome is often a patch of phenotypically mutant cells called **mutant sector**.

In diploids, **dominant mutations** expected to show up in phenotype of cell; **recessive mutations** will not be expressed (masked by wild-type allele) *unless* 2nd mutation creates homozygous mutation.

If mutation occurs when cells are still dividing, mutant clone may arise; if mutation occurs in postmitotic cell, effect on phenotype *usually* negligible.

Somatic mutations **ARE NOT** passed on to progeny.

Germinal Mutations in germ-line set aside during development (cells that divide into gametes).

Idividual of **normal** phenotype may have undetected **mutant** gametes.

If mutated gamete participates in fertilization, mutation will be passed on to offspring.

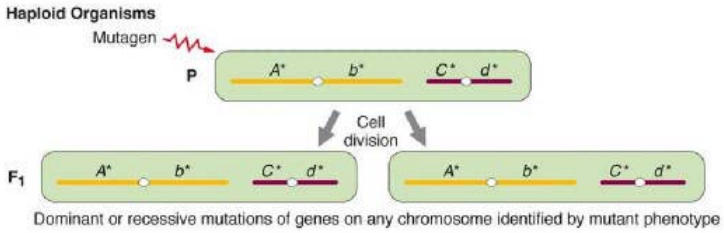
Mutation **only** detected if included in zygote.

Example: Mutation in allele determining curled ears appeared in germ-line of normal straight-eared cat and was expressed in progeny.

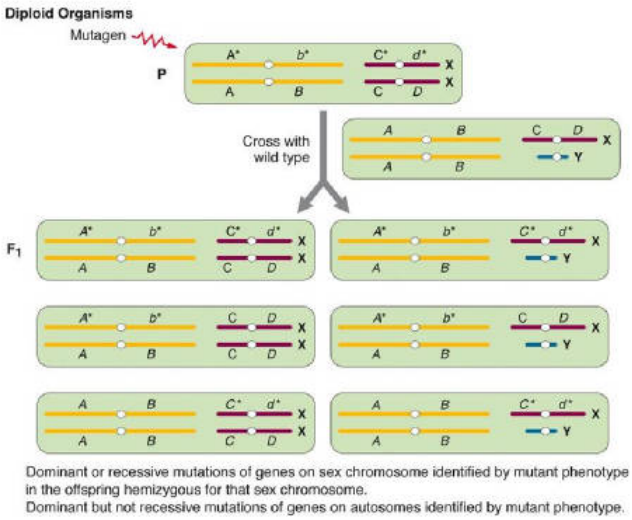
Dominant and Recessive Germ-line Mutations

Mutations can be either **dominant** or **recessive**.

In **haploid organisms** or **gametes** of diploid organisms dominant and recessive mutations can be ide ntified in F1 generation.



For autosomal genes in **diploid organisms**, F1 screens reveal only dominant mutations.



Autosomal recessive mutations in diploid organisms seen only in F2 or F3 generations (depending on if monoecious or dioecious).

Detecting Autosomal Mutations

In **self-fertilizing** species, new autosomal recessive mutation in F1 expected to be homozygous in 25% of F2 individuals.
In **cross-fertilizing species,** in order to observe homozygous autosomal recessive mutations, F3 individuals must be analyzed.

Recessive mutations of specifice gene may be recovered in F2 if F1 mutaginized progeny test crossed with individual carrying recessive mutant allele of gene.

However in this case only recessive mutations in genes (loci) that are mutant in the t ester strain can be recovered in second generation after mutagenesis

=> Specific locus test.

DIRECTED MUTATIONS AND PHENOCOPIES

- Hypothesis, first proposed by John cairns in 1988
- Progress in molecular biology has made it possible to target specific genes
- An understanding of cellular processes has enabled the development of direct mutation techniques
- Targeting techniques enabled evaluation of phenotypes displayed by individuals lacking the targeted gene
- Direct mutations can be achieved by two broad methods

1. inactivate the gene by targeted changes to its DNA

Targeted Gene Knockouts

Replacement of endogenous genes with ectopic genetically engineered DNA that inactivates the gene

Site-directed Mutagenesis and related techniques

Create mutations at any specific sites in a gene that has been cloned

2. Leave the gene intact, block the activity of the gene's mRNA or protein product

Phenocopying (Antisense RNA, ds-RNA interference and Chemical Library Screening)

Term derived by Richard Goldschmidt in 1935

Mimicking a mutant phenotype by manupilating environment

Gene is not mutated

Forward and Reverse Mutations:

Forward Mutation- change away from the wild type allele

Reverse Mutation- change towards the wild type allele

These terms are unrelated to “forward genetics” and “reverse genetics” discussed earlier

Genetic Selection versus Genetic Screens

Genetic Selections

- Preventing growth of organisms that do not contain the mutant allele

Examples of Genetic Selections:

1. Reversion to prototrophy
   - auxotrophic mutation which can be grow on minimal media supplemented with a compound downstream in the biosynthetic pathway
   - causes rare prototrophic revertants to grow on the media (Ex. leu-3)
2. Suppression of a mutant defect
   - identifies extragenic suppressor mutations (Ex. Nonsense mutation in auxotroph can be suppressed by mutated tRNA which reads anticodon)
3. Drug resistance
   - mutations that grow in presence of a drug that normally kills individuals (Ex. *E. coli* resistance to streptomycin)

Genetic Screens:

- both mutated and non- mutated individuals are recovered but the individuals carrying mutation are identified since they display the phenotype of the mutation

Examples of General Phenotypic Screens:

1. Biochemical (auxotrophic) mutations:
   - Biochemical mutants are normally auxotrophic – require specific additional nutrients for growth

   "Inborn errors of metabolism" is sometimes used to describe biochemical disorders such as PKU

   Ex: *Neurospora crassa* for auxotrophy and prototrophy
2. Morphological Mutations
   - Affect the appearance of an organism (shape, size, color)

   Ex. Drosophila mutants
3. Lethal Mutations
   - cause premature death of mutant individuals
   - recessive lethal mutations are more viable than dominant
4. Conditional Mutations
   - conditional mutant only causes mutant phenotype in a certain environment called restrictive condition

Ex. Temperature sensitive mutations

- wild type is produced in permissive condition

5. Behavioral Mutations

-affect behavior of an organism

-Ex. Drosophila wild type flies are phototrophic

Examples of Secondary Screens:

1. Modifier Mutations:

A mutation which one gene ameliorates (suppresses) or worsens (enhances) the phenotype in a second gene

· Mutagenized genotype is sensitized by introducing a known mutation into a gene that affects the process of interest

2. Mutations detected in somatic mosaics

Example: Studying eye development in Drosophila using mitotic crossing over

3. Screens based on gene expression

DNA regulatory sequences control transcription and are able to activate transcription of genes nearby

Example: Enhancer/Trap transgene

Analysis of the Recovered Mutations

Once mutations have been detected and isolated, it is important to be able to evaluate and draw conclusions about their properties.

Mutations; Mini-review:

Single genetic diferences (mutant vs wild-type) can be identified and mapped by comparing inheritance patterns to genetic markers.

Some mutant phenotypes are **recessive** to wild type, otheres **dominant**.

Multiple mutant alleles of same gene can arise - might fall into different phenotypic classes.

Some mutations are in protein-coding regions of genes; other mutations are not in coding regions.

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Classification Systems for Mutations

Important to know how a mutation in a given step of a process alters that process.

Using individual mutations to learn about the process requires knowing whether mutation is **loss-of-function** or **gain-of-function mutation**.

In mutational analysis one can infer loss- vs gain-of-function by phenotypic analysis of different dosages of mutant and wild-type genes.

Counting the Genes in a Biological Process

Typical mutation screen or selection recovers large number of mutations that represent multiple "hits" in smaller number of genes.

How many genes represented by this mutant collection?

Using **genetic transmissional and complementation** analysis.

Diagnostics for Loss-of-Function verses Gain-of-Function

Both gain-of-function and loss-of-function mutations can be **dominant** or **recessive**.

Knowing where any of the mutations map in the genome, we can determine whether chromosomal deletions or duplications of the gene exist.

Dominant Loss-of-Function Mutations

Single copy of wild-type allele is insufficient to make enough gene product to generate wild type phenotype.

Therefore, loss-of-function mutation also called haplo-insufficient

Gene dosage also used to distinguish between levels of loss-of-function.

Some loss-of-function mutations completely remove activity of gene product => Null Mutation

Others only decrease the gene product => Hypomorphic Mutation

Dominant Gain-of-Function Mutations

Two Examples:

1. Hypermorph: mutation that produces more gene activity per gene dose than wild-type, but in all other respects gene product is normal.

2. Neomorph**:** mutation that produces novel gene activity that is not characteristic of wild-type.

Example: if coding sequences of two genes fused in correct open reading frame in mutant, novel protein can be produced that may have cellular activity different from either parental protein.

Further Steps and Analysis of Mutational Dissection

Utilization of recombinational mapping for position cloning and Insertional mutagenesis for molecular "tagging"

Allows for molecular tagging of genes and their products

Understanding the "**genetics**" of genes and what they do

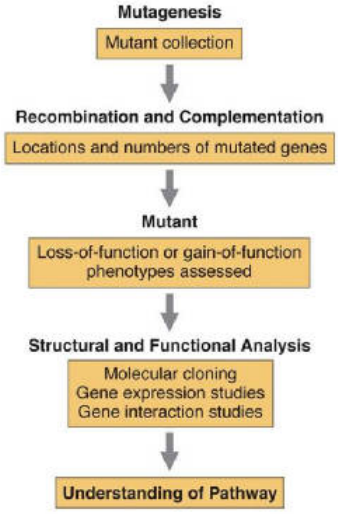
Provides the **basis** and **foundation** to understand the pathway of the gene or system

Allows us to ask the questions:

Where and **when** are these genes expressed?

What other products do these genes interact with?

How is the expression of these genes regulated?



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