Phenotype and Function

Genetics can be used as a tool to study problems in biology and has made important contributions to the fields of development, cell biology and neurobiology. One of the goals in this type of research is to use mutations to reveal the normal function of a gene. However, to infer the function of a gene from mutant phenotypes, we need to know how the mutation perturbs the activity of the gene. Two questions are particularly important to address. (1) Does a recessive mutation reduce or eliminate gene function? (2) Does a dominant mutation reduce or alter gene function?

Before discussing the nature of mutations, two terms that are often used to describe phenotypes are penetrance and expressivity. These are related, but describe different features of phenotype. Penetrance refers to how many individuals of a given genotype express a given phenotype. We say that a phenotype is completely penetrant if all individuals show it and incompletely penetrant if a subset of individuals of a given genotype express the trait. Expressivity refers to the severity or degree of expression of a given phenotype. If the phenotype shows variable expressivity, the expression of the trait is variable among individuals of the same genotype. Factors that can contribute to incomplete penetrance and variable expressivity are differences in the genetic background of the individuals and environmental factors.

Recessive or dominant?

The first goal in understanding the nature of a mutation is to determine if it is dominant or recessive. In most cases mutant alleles are recessive to the wild-type alleles. There are also more complicated cases where two alleles are neither dominant nor recessive. If the heterozygote expresses the phenotype of both alleles, the alleles are called codominant. This is most frequently seen with molecular assays for gene function, for example, in ABO blood types. The phenotype of the heterozygote can also be intermediate between the phenotypes of the homozygotes. In these cases the alleles are referred to a semidominant or incompletely dominant.

Loss-of-function mutations

Recessive mutations almost always reduce or eliminate gene activity. Mutations that reduce gene function are called weak or hypomorphic. Mutations that eliminate gene function are called null or amorphic. Whether a mutation is hypomorphic or amorphic can be determined by genetic criteria.

Hypomorphic mutations reduce but do not eliminate gene activity. An example is the apricot allele (w^a) of the white gene. This mutation is recessive and results in apricot colored eyes. If we compare the

phenotypes of wild-type and w^a alleles, there are two interpretations of how the *white* gene functions. One possibility is that *white* produces eye color and the w^a allele reduces that production. Alternatively the white locus could inhibit eye color and the w^a allele increases the effectiveness of *white* to inhibit eye color. In 1932, Hermann Muller proposed that one could distinguish between these possibilities by changing the dose of the w^a allele. If *white* produces eye color then increasing the dose of w^a should increase eye color. However, if *white* inhibits eye color, then increasing the dose of w^a should lead to less eye color. When Muller conducted the dosage experiments with w^a, he found that as the dosage of w^a increased the mutant phenotype is less severe.

$$w^a/\text{Df}(w) > w^a/w^a > w^a/w^a/Dp(w^a) > w^a/+ = +/+$$
more mutant (less pigment)-------------------less mutant (more pigment)

    Amorphic mutations eliminate gene activity and behave similar to a deficiency of the locus. Recessive alleles of *white* (w) that lead to a white eye phenotype are considered to be amorphic. As w dosage increases, the mutant phenotype remains unchanged.

$$w/\text{Df} = w/w = w/w/Dp(w) > w/+ = +/+$$
more mutant (less pigment)-------------------less mutant (more pigment)

    Hypomorphic alleles can cause a range of mutant phenotypes. The *albino* gene of mice encodes tyrosinase, which is one of the enzymes that produces the fur pigment melanin from tyrosine. Mutant alleles of various strengths have been isolated. Patches of skin from these mice can be incubated with radioactive tyrosine and the amount of tyrosine incorporated into melanin can be measured. The table below is a comparison of the fur color phenotypes and levels of tyrosinase from mice carrying various *albino* alleles. C is the wild-type gene, c is the amorphic allele and the remaining alleles are hypomorphic.

Genotype	fur color	cpm of C^{14}-tyrosine incorporated
C/C	Black	1200 +/- 36
C/c	Black	617 +/- 33
c^{ch}/c^{ch}	grey	442 +/- 15
c^e/c^e	off white	98 +/- 11
c/c	white	47 +/- 5

From both the phenotypes and biochemical assays we can see that the different alleles have various activities: $C > C^{ch} > c^e > c$. This kind of graded set of alleles is called an allelic series.

When inferring the function of a gene, it is important to know the null phenotype, the phenotype in the absence of gene function. However, null alleles can often result in phenotypes that are difficult to interpret, and in these cases hypomorphic alleles can be more informative. For example, mutations that disrupt signaling through the Ras pathway in *C. elegans* cause a lethal phenotype that is not understood. However, the Ras pathway is also important for cell signaling during generation of a vulva, which occurs late in development well after the Ras pathway null mutants die. Because hypomorphic mutations in these genes result in no or partial lethality, animals homozygous for hypomorphic mutations can develop into adults with abnormal vulvae, revealing the role of these genes in cell signaling.

Dominant mutations: haploinsufficient or gain-of-function?
Haploinsufficient mutations
Dominant mutations can reduce (haloinsufficient alleles) or alter (gain-of-function alleles) gene function. Dominant mutations that cause a reduction or elimination of gene function define genes that are required in two doses. These mutations are known as haploinsufficient because the haploid dose of the gene is not sufficient for normal gene function. Thus, unlike the *albino* gene described above in which reducing gene function 50% does not have a phenotypic consequence, haploinsufficient mutations define genes in which a 50% reduction in gene function does affect phenotype. Animals heterozygous for a haploinsufficient mutation (m/+) have a phenotype similar to animals heterozygous for a deficiency for the locus (Df/+).

In *Drosophila*, there are about 50 *Minute* genes that are defined by haploinsufficient mutations. These mutations cause the same phenotype: homozygous flies die, and heterozygous flies have thin bristles and develop slowly. One of the Minute genes has been shown to encode a ribosomal protein.

Gain-of function mutations
There are three types of gain-of-function mutations. These mutations are usually, but not always, dominant to their wild-type alleles. We will only consider examples where gain-of-function alleles are dominant.

Hypermorphic mutations increase gene activity relative to their wild-type alleles. Adding wild-type gene activity to the hypermorphic gene activity leads to a more severe mutant phenotype. One example of

hypermorphic alleles are dominant alleles of the *C. elegans* gene *lin-12* [*lin-12(d)*]. The *lin-12* gene is required for certain cells to determine their fates normally.

genotype	Cell A	Cell B
lin-12(lf) / lin-12(lf)	B fate	B fate
lin-12(+) / lin-12(+)	A fate	B fate
lin-12(gf) / lin-12(gf)	A fate	A fate

One copy of *lin-12(gf)* transforms some B cells to the A fate. As the dosage of wild-type alleles is increased more B cells are transformed to the A fate.

$$lin\text{-}12(gf)/+/Dp\ lin\text{-}12(+) > lin\text{-}12(gf)/+ > lin\text{-}12(gf)/Df$$

more mutant   less mutant
more B cells transformed to A cells   fewer B cells transformed to A cells

Thus *lin-12(gf)* alleles increase *lin-12* gene activity, resulting in the transformation of B cells to the A fate. *lin-12* amorphic and hypomorphic alleles [*lin-12(lf)*] lead to cell fate transformations in the opposite direction, transforming cell A to a B fate. The two types of *lin-12* alleles that cause opposite transformations in cell fate have led to a model in which the *lin-12* gene functions as a binary switch in cell fate determination: if *lin-12* is active cells adopt fate A, if *lin-12* is inactive cells adopt fate B.

2. **Antimorphic** mutations, which are also called dominant negative mutations, alter gene activity to antagonize the activity of the wild-type allele. Wild-type gene function depends on the ratio of mutant gene dosage to wild-type dosage: the higher the ratio, the more severe the mutant phenotype.

mutant (*m*)/wild type (+) ratio	2:1	1:1	1:2
genotype	*m/m/Dp(+)*	*m/+*	*m/+/Dp(+)*
phenotype	more mutant		less mutant

Examples of antimorphic mutations are certain missense mutations in the DNA binding domain of the lac repressor. Because the lac repressor is a tetramer, mutant repressors can complex with wild-type repressors and poison the complex. In this case as the ratio of mutant : wild-type alleles increases, more defective tetramers are formed, and the phenotype becomes more mutant.

3. **Neomorphic** mutations lead to novel gene activity. These alleles differ from hypermorphic and antimorphic alleles in that they are insensitive to dosage. Dominant mutations in the *Drosophila Antennapedia (Antp)* gene can have a dramatic neomorphic phenotype. Flies with one copy of the dominant $Antp^{Ns}$ mutation have legs growing out of there heads in place of antennae. $Antp^{Ns}$ mutations are neomorphic because they are insensitive to changes in the dosage of the wild-type gene

$$Antp^{Ns} / Df = Antp^{Ns} / + = Antp^{Ns} / + / Dp(+)$$

The *Antp* gene is normally transcribed in the thorax where it promotes leg development. The $Antp^{Ns}$ mutation is caused by the insertion of a transposable element upstream of the *Antp* gene which leads to *Antp* expression in the head. This ectopic expression of the *Antp* gene promotes leg formation in the wrong place.

In the next series of lectures, we will discuss how genetics has been used as a tool to understand a developmental signaling pathway. What you have learned in this lecture will be used again later on to determine how mutations disrupt gene function in specific cases.