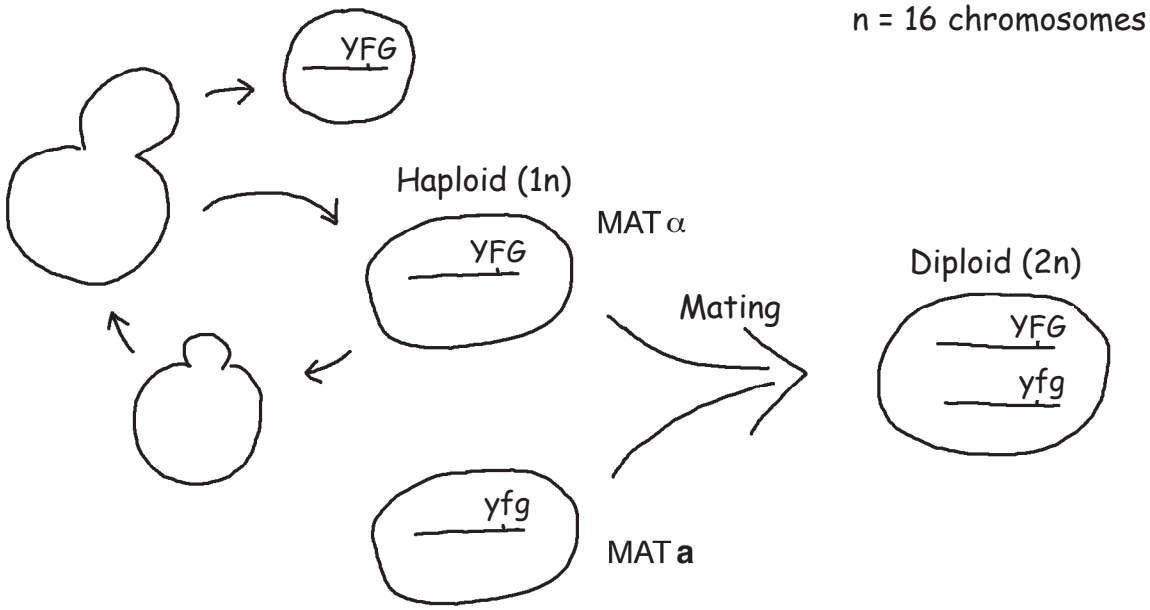
Lecture 2

In this lecture we are going to consider experiments on yeast, a very useful organism for genetic study. Yeast is more properly known as Saccharomyces cerevisiae, which is the single-celled microbe used to make bread and beer. Yeast can exist as haploids of either mating type **a** (MAT**a)** or mating type α (MATα). Haploid cells of different mating type when mixed together will mate to make a diploid cell.



Haploids and diploids are isomorphic – meaning that a given mutation will cause essentially the same change in haploid and diploid cells. This allows us to look at the effect of having two different alleles in the same (diploid) cell.

All yeast needs to grow are salts, minerals, and glucose (minimal medium). From these compounds, yeast cells can synthesize all of the molecules such as amino acids and nucleotides that are needed to construct a cell. The synthesis of complicated molecules requires many enzymatic steps. When combined, these enzymatic reactions constitute a **biochemical pathway**

Consider a simplified pathway for the synthesis of the amino acid histidine.

A → **B** → **C** → **D** → histidine → → Protein

Enzyme: (1) (2) (3) (4)

Each intermediate compound in the pathway (represented by A – D) is converted to the next by a different enzyme (numbered 1 – 4). For example, if there is a mutation in the gene for enzyme 3 then intermediate C can not be converted to D and the cell can not make histidine. Such a mutant will only grow if histidine is provided in the growth medium.

This type of mutation is known as an **auxotrophic mutation** and is very useful for genetic analysis.

	growth on minimal	growth on minimal + histidine
His^+ (wild-type)	+	+
His^-	-	+

Phenotype: All traits of an organism (with an emphasis on trait under investigation)

Homozygote: diploid with two like alleles of same gene

Heterozygote: diploid with two different alleles of same gene

Recessive Allele: trait is expressed in homozygote, but not in heterozygote

genotype	phenotype	Mate to :	diploid genotype	diploid phenotype
MAT**a** $His3^-$	His^-	MATα $His3^-$	$His3^-/His3^-$	His^-
MAT**a** $His3^-$	His^-	MATα $His3^+$	$His3^-/His3^+$	His^+

Based on the His^- phenotype of the $His3^-/His3^+$ heterozygote, we deduce that $His3^-$ is recessive to wild type.

Let's consider a different kind of mutation giving resistance to copper that occurs in a gene known as CUP1.

genotype	phenotype	Mate to :	diploid genotype	diploid phenotype
MAT**a** $Cup1^r$	copper resistant	MATα $Cup1^+$	$Cup1^r/Cup1^+$	copper resistant

Dominant Allele: trait is expressed in heterozygote

$Cup1^r$ is dominant to wild-type ($Cup1^+$).

The terms dominant and recessive are simply shorthand expressions for the results of particular experiments. If someone says a particular allele is dominant that means that at some point they constructed a heterozygous diploid and found that the trait was expressed in that diploid.

Note: Sometimes an allele will have more than one phenotype and may be recessive for one and dominant for another. In such cases, the phenotype must be specified when one is making statements about whether the allele is dominant or recessive. Consider for example, the allele for sickle cell hemoglobin in humans designated Hb^S. Heterozygous individuals (Hb^S/Hb^a) are more resistant to malaria, thus Hbs is dominant for the trait of malaria resistance. On the other hand, Hb^S/Hb^a heterozygotes do not have the debilitating sickle cell disease, but Hb^S/Hb^S homozygous individuals do. Therefore, Hb^S is recessive for the trait of sickle cell disease.

Once we find out whether an allele is dominant or recessive, we can already infer important information about the nature of the allele. The following conclusions will usually be true.

Recessive alleles usually cause a loss of gene function

Dominant alleles usually cause increased function or a new function

It turns out that the Cup^r allele actually carries more copies of the gene for a copper binding protein and therefore increases the activity of the gene.

Last lecture we defined the gene structurally as the DNA needed to encode a protein. We can now define a gene in a new way based on its function. Using the phenotypic difference between wild type and a recessive allele we can use a **Complementation test** to determine whether two different recessive alleles are in the same gene.

Say you isolate a new recessive histidine requiring mutation that we will call $HisX^-$. In principle, this mutation could be in the His3 gene or it could be in any of the other genes in the histidine biosynthetic pathway. In order to distinguish these possibilities we need a test to determine whether $HisX^-$ is a recessive allele of the same gene as $His3^-$.

To carry out a complementation test, one simply constructs a diploid carrying both the $His3^-$ and $HisX^-$ alleles.

An easy way to do this would be to mate a MAT**a** $HisX^-$ strain to a MATα $His3^-$ strain.

possibility	genotype of diploid	phenotype of diploid	complementation
HisX = His3	$His3^-/His3^-$	His^-	**No**
HisX ≠ His3	$His3^-/His3^+$, $HisX^-/HisX^+$	His^+	**Yes**

Having performed this test, if the two mutations don't complement we conclude that they are in the same gene. Conversely, if they do complement we conclude that they are in different genes.

This test only works for recessive mutations. Think about what the outcome would be if $HisX^-$ were dominant.

The complementation test can be thought of in the following way. If I have an allele with an observable phenotype whose function can be provided by a wild type genotype (i.e., the allele is recessive) — by mating to another recessive mutant, I can test whether the function that was lost because of the recessive allele can be provided by the other mutant genotype. If not, the two alleles must be defective in the same gene. The beauty of this test is that the trait can serve as a read-out of gene function even without knowledge of what the gene is doing at a molecular level.