Grundlagen Praktikum II: Yeast Genetics

Introduction

Genetics is the science of inheritance. The basis of inheritance are the genes encoded in the DNA of an individual. In this section of the "Grundlagen Praktikum" we will use the **budding yeast** (Bäckerhefe) ***Saccharomyces cerevisiae*** to explore some concepts of genetic inheritance and how to use genetics to understand biological processes.

Key learning goals are:

- Understanding meiosis and how it leads to the segregation of genes and phenotypes in offspring.
- Understanding the interaction of genes in their influence on a phenotype (genetic epistasis).
- Understanding the methodology to analyze genetic experiments (in yeast).

The "awesome power of yeast genetics"

Yeast is a workhorse of cell biology because it can be genetically modified very easily. While also fission yeast (*Schizosaccharomyces pombe*) is an important genetically tractable model organism, budding yeast is the historically more popular experimental system. It was the first eukaryote transformed by plasmids, the first eukaryote for which a precise gene knock-out was constructed and the first eukaryote for which the genome was sequenced completely (in 1996). Nevertheless, the role of many of its more than 6000 genes still remains unclear and it remains an important study system in cell biology.

Since yeast can grow and replicate as a **haploid** organism, the phenotype of **recessive** mutations can be readily observed. On the other hand, a **sexual cycle** and **diploid** state also exist (Figure 1), which allows for genetic analyses using **complementation**, **recombination** and **epistasis** analysis. We will come back to this concepts below.

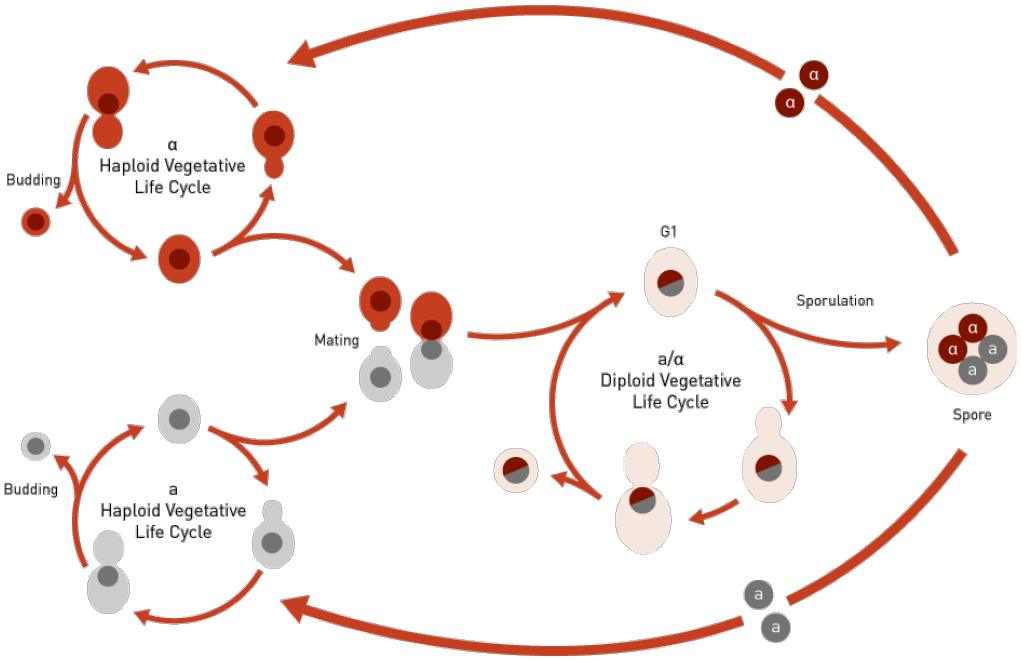

*Figure 1: **The yeast life cycle:** Yeast can grow as a haploid or diploid organism. Haploids of the opposite mating type (a and alpha) can **mate** to produce diploids. Diploids **sporulate** to produce haploid spores which can survive harsh conditions and reenter the cell cycle once conditions improve.*

Yeast has the special advantage over other genetic model organisms that the individual phenotype of all four gametes of meiosis can be examined. This is due to the fact, that meiosis in yeast produces a so called tetrad: the four gametes contained in a sac called the ascus. These tetrads can be dissected with a microneedle and the growth and phenotype of the spores can be analyzed. This allows the very precise analysis of the phenotypic effect of gene inheritance. In order to fully understand the experiments we are going to carry out in the practical and in order to achieve the learning goals, it is crucial to review the process of meiosis and understand exactly what happens during this special cell division.

Meiosis

Meiosis is the reduction cell division which creates four haploid cells from a single diploid cell (Figure 2). First, one round of DNA replication generates a 4n cell, which carries each chromatid in four copies. This is followed by two rounds of cell division yielding four haploids (1n) cells. For a single gene, two gametes will carry the maternal and two the paternal copy.

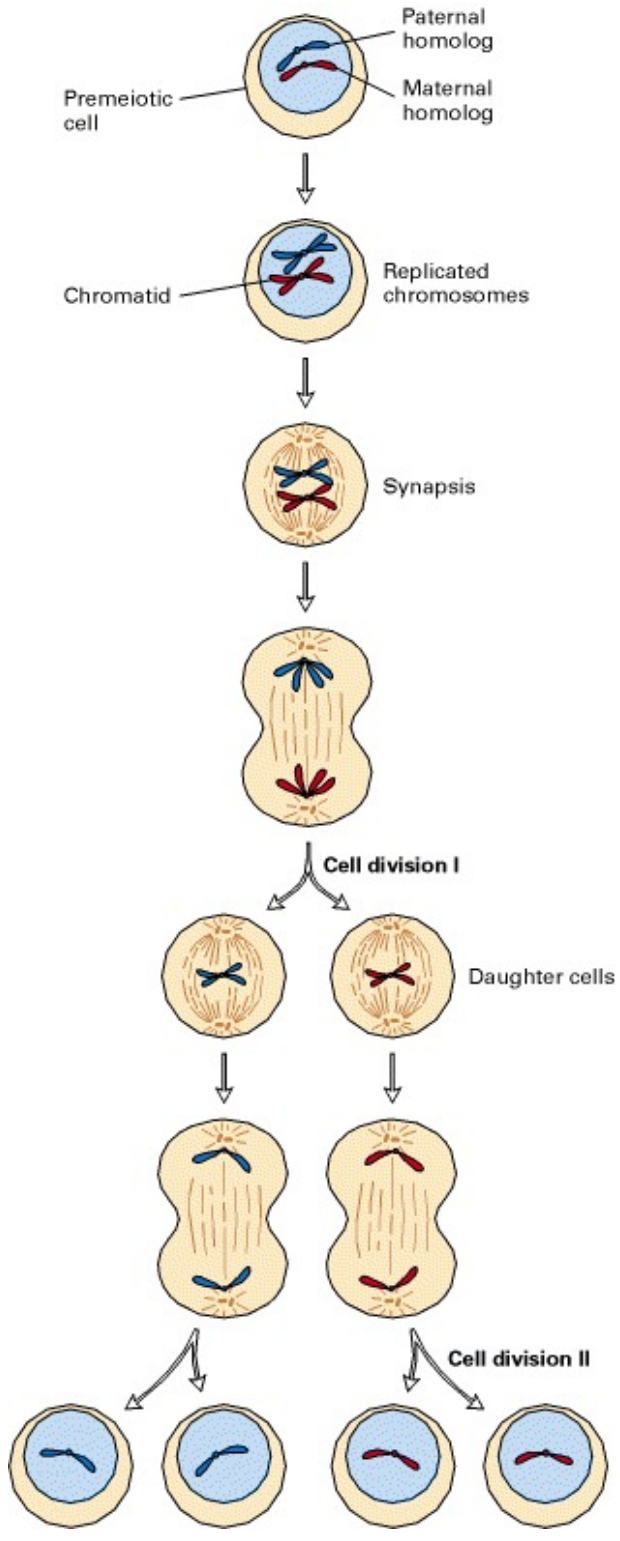

Figure 2: A premeiotic germ cell has two copies of each chromosome (2n), one maternal and one paternal. Chromosomes are replicated during the S phase, giving a 4n chromosomal complement. During the first meiotic division, each replicated chromosome aligns at the cell equator, paired with its homologous partner; this pairing off, permits genetic recombination. One homolog goes into one daughter cell, and the other homolog goes into the other cell. The resulting 2n cells undergo a second division without intervening DNA replication. During this second meiotic division, the sister chromatids separate and these now independent chromosomes are randomly apportioned to the daughter cells. from Molecular Cell Biology. 4th edition.)

Now let's examine what happens if the fate of two genes is followed through meiosis. Consider that the two genes are located on different chromosomes and the organism is heterozygous for each, because it was derived from a mating of cells with the genotypes AB and ab (Figure 3). In the first meiotic division, the homologous chromosomes are separated **randomly** to the two daughter cells (Mendel's principle of **Random Assortment**). This can result in two possible outcomes: (i) AB and ab (Figure 3A) or (ii) Ab and aB (Figure 3B). These are then further segregated into haploid gametes. In the first case, the games have the genotype AB, AB, ab and ab. These genotypes are identical to the parental genotypes. This outcome of a tetrad is called

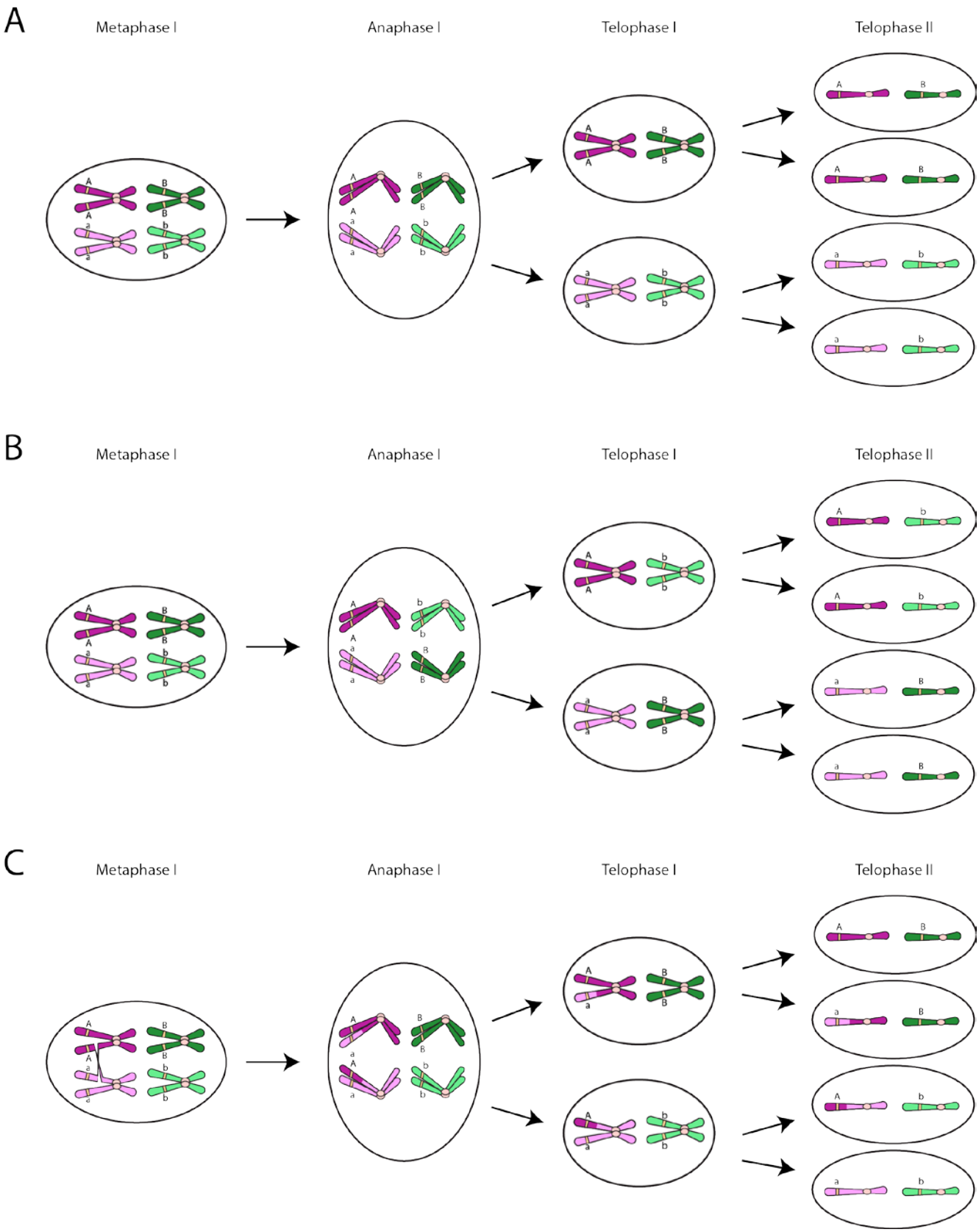

Figure 3: Principles of meiotic chromosome behaviour. A: parental ditype. B: nonparental ditype. C: tetratype.

parental ditype (two different genotypes which are the same as in the parents). In the second case instead, the spores have the genotypes Ab, Ab, aB, aB. These are recombinant genotypes which did not occur in the parental strains. This is called the **nonparental ditype** (two different genotypes which are not the same as in the parents). The probability of these two outcomes (i) and (ii) is the same if the two genes analyzed are located on different chromosomes. A third outcome is possible, if **crossing over** and **meiotic recombination**

occur between the centromere and the gene of interest during meiosis I leading to different alleles on the two chromatids of each chromosome (Figure 3C). Figure 3C shows only one example of such an outcome, which generates spores of the genotypes AB, aB, ab, Ab. This meiosis generated four different spores, two of parental and two of nonparental genotype. This is called a **tetratype**.

However, if two genes are located on the same chromosome (Figure 4), even a nonparental ditype can only be generated by crossing over and meiotic recombination occur between the two genes. How could a nonparental ditype be derived from the diploid cell shown in Figure 4? Draw the crossing over on a piece of paper. Since nonparental ditypes can be generated only by crossing overs, the proportion of tetrads with a nonparental ditype over parental ditype is a measure of the **linkage** between two genes, i.e. a measure for the frequency of crossing over between the two genes which translates (roughly) to their distance from each other on the chromosome. The father apart they are, the more likely it is that a crossing over occurs between them. If nonparental ditypes are less frequent than parental ditypes, the two genes are said to be linked and are located on the same chromosome. Since crossing overs are very frequent in yeast, even genes located on the same chromosomes can segregate independently and appear unlinked if their distance from each other is sufficiently large.

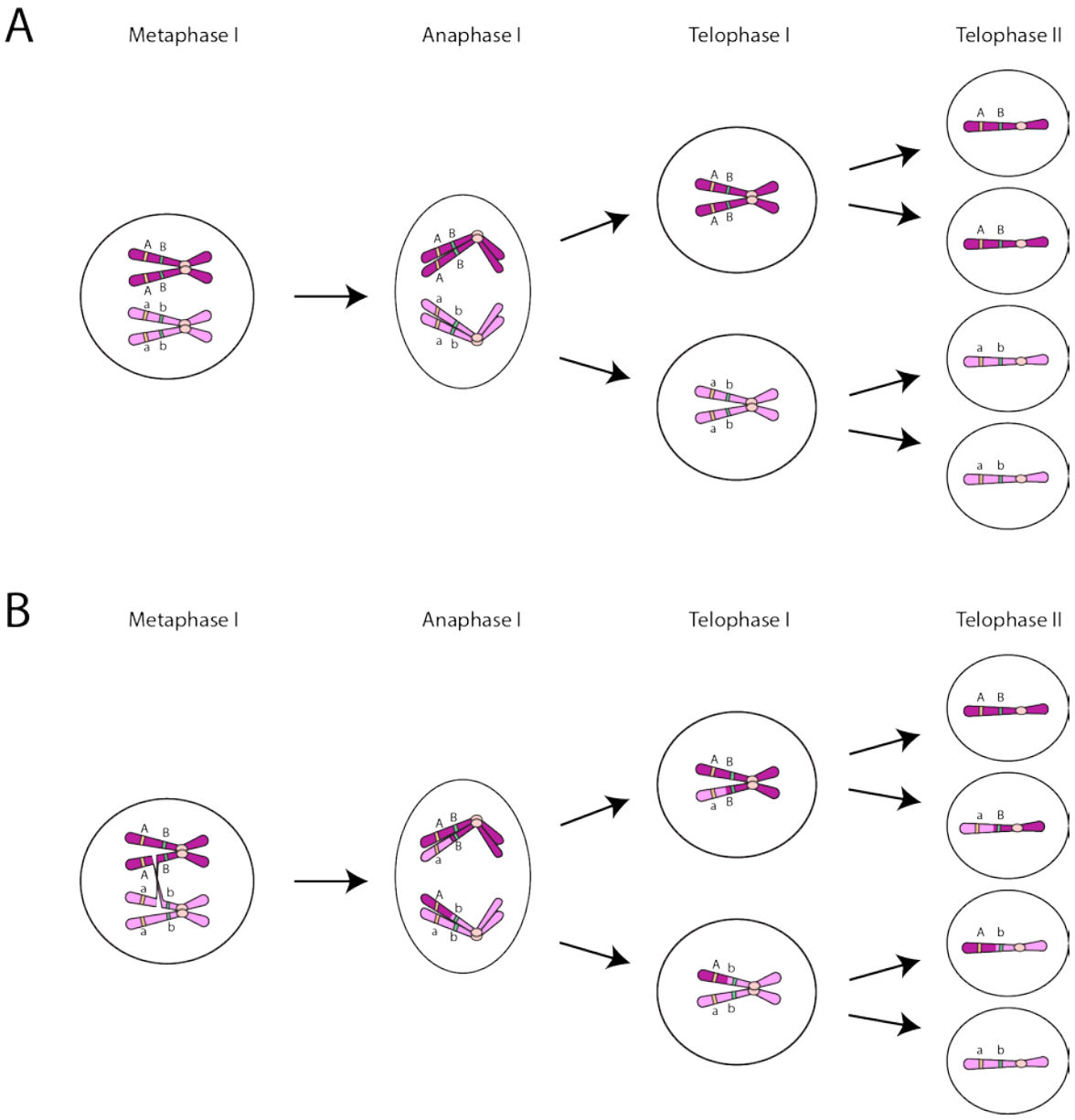

Figure 4: Principles of meitoic chormosome behaviour. A: parental ditype. C: tetratype.

Genetics screens using yeast

The identification of genes and there function has relied heavily on genetic screens.

Forward genetic screening using random mutagenesis

In **random mutagenesis** screening, a **mutation inducing agent** (e.g. UV light or ethylmethane sulfonate (EMS)) is applied and the resulting population containing different mutants is screen for a **phenotype of interest**. A phenotype that is easily scored is growth (vs. no growth) on e.g. different media. For example, the ability to grow on a certain food source could be tested. Let's look at an example. Yeast cells can use different sugars as carbon source. Their favorite sugar is glucose, but they can also metabolise e.g. galactose. Galactose is a C-4 epimer of glucose. To enter glycolysis is has to undergo three metabolic steps that are specific to galactose (Figure 2a). The genes responsible for these metabolic steps could be identified in a screen testing the growth of yeast cells on galactose as the only carbon source in the medium. When these genes where mutated, the yeast was unable to grow on the galactose medium. Mutants with defects in the galactose metabolism enzymes can be said to be **conditionally lethal**, because they cannot grow on galactose but are perfectly viable on other carbon sources. Conditional mutations are also a way around the problem of analyzing genes whose function is essential for basic cell function like e.g. genes required for cell division. To study such **essential genes**, geneticist rely on **conditional mutants** like for example **temperature sensitive mutants**. Such mutant cells are phenotypically normal if grown at room temperature but their mutant phenotype becomes visible upon shift to higher (thermosensitive) or lower (cold sensitive) temperatures where they can no longer grow. The phenotype of the growth arrested cells can then be analyzed to understand the function of the mutated gene.

Once a set of mutants that show the interesting phenotype is identified, the next step in classical genetics is the grouping of the identified mutants in **complementation groups**. These identify (for recessive genes) the number of different genes that where mutated. For example, two mutant strains identified in the screen for mutants that can no longer grow on

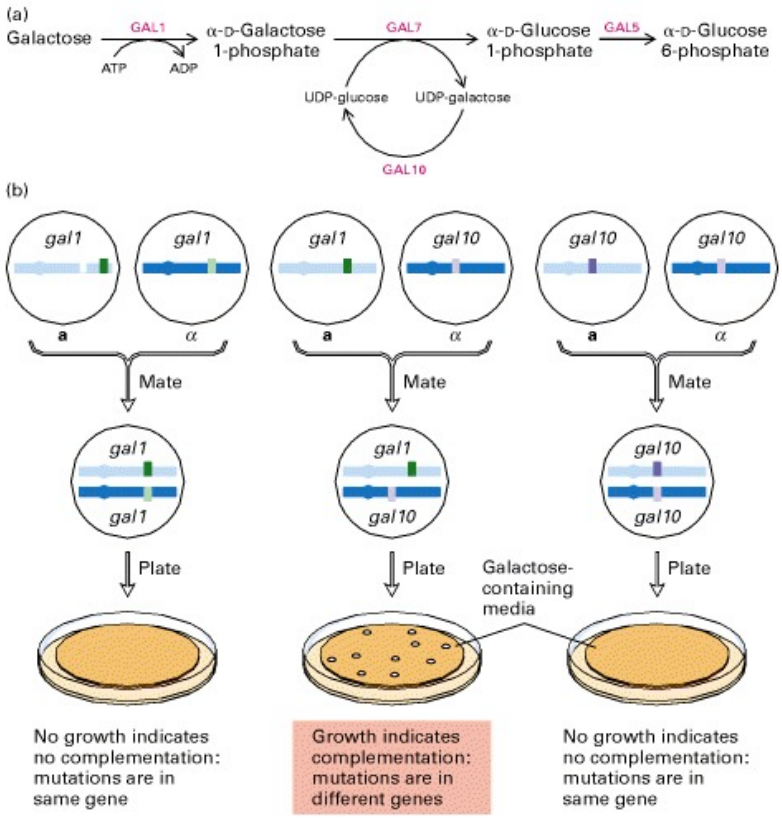

Figure 5: Complementation analysis in S. cerevisiae. (From: Molecular Cell Biology. 4th edition.Lodish H, Berk A, Zipursky SL, et al.New York: W. H. Freeman; 2000.) (a) Pathway used by yeast cells to metabolize galactose to glucose, which then enters the glycolytic pathway. Yeast cells must produce all four enzymes (red) in order to grow on galactose. GAL1=galactokinase; GAL7=galactose 1-phosphate uridyl transferase; GAL10=UDP-galactose 4-epimerase; GAL5=phosphoglucomutase. (b) Complementation tests can be performed with yeast by mating haploid a and α cells to produce diploid cells. This example shows the results that would be obtained in complementation tests of Gal− strains carrying different mutations (indicated by vertical colored lines) in the GAL1 and GAL10 genes, which encode two different enzymes required for galactose metabolism. Both of these genes are located on yeast chromosome II

galactose are mated. If the two mutations affected the same gene, the diploid cells will remain defective in this gene and still be unable to grow on galactose (Figure 2). If, however, the two mutations affected different genes, the diploid cells will be heterozygous for both mutations but carry a wildtype allele for each. Therefore, these cells will be able to grow on galactose. The two mutations thus are in different complementation groups and in this case in different genes. In this way, it was found that galactose metabolism in yeast requires 5 different genes (The four enzymes shown in Figure 2a plus a transporter, GAL2, which imports galactose into the cell).

In the next step, the function of the individual genes in the pathway of interest needs to be determined. **Double mutants** can be used to analyze the interaction of genes and mutant with each other and deduce e.g. the order in which different genes function within a molecular pathway. For example, in the secretory pathway, different mutations block secretion at different steps. The phenotype of the double mutant will indicate in which order two genes function. For example, if mutation A in the secretory pathway leads to accumulation of a marker protein in the ER, but mutation B leads to accumulation in the Golgi, the phenotype of the double mutant AB can indicate, whether the two mutations function in the same or in different pathways. If the double mutant has the same phenotype as mutation A, this indicates that gene B functions downstream of A in the same pathway. In this case, A is said to be **epistatic** to B since its phenotype is dominant over B.

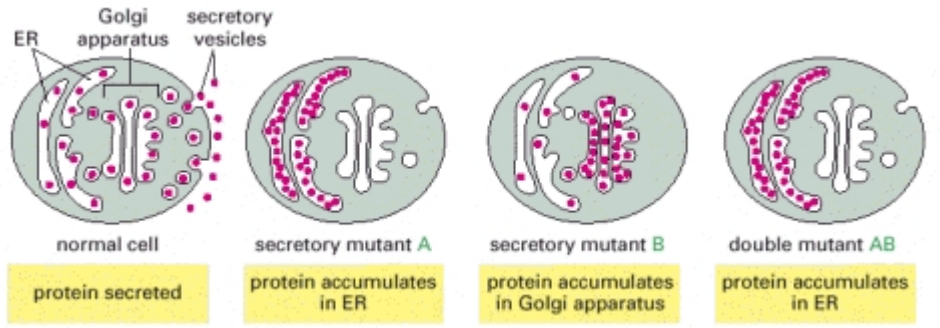

Figure 6 Using genetics to determine the order of function of genes. (From: Molecular Cell Biology. 4th edition. Lodish H, Berk A, Zipursky SL, et al.New York: W. H. Freeman; 2000.) In normal cells, proteins are loaded into vesicles, which fuse with the plasma membrane and secrete their contents into the extracellular medium. In secretory mutant A, proteins accumulate in the ER. In a different secretory mutant B, proteins accumulate in the Golgi. In the double mutant AB, proteins accumulate in the ER; this indicates that the gene defective in mutant A acts before the gene defective in mutant B in the secretory pathway.