LECTURE 7: TETRAD ANALYSIS; GENE CONVERSION, continued

Reading: Ch. 5, p 142-151; Ch. 6, p 192-194

Problems: Ch. 5, solved problem III, also 28a, 29ab, 30, 32, 34, 35; Ch. 6, #30, 31

Announcements:

*You must be enrolled in the section that you are attending. If you are not enrolled, you must contact both GSIs involved and we will work with the MCB Undergraduate Affairs office to get you into a section. You may not get your first choice. Friday sections tend to be over-enrolled.

*You must take the quiz in the section in which you are enrolled. If you have a conflict, you must arrange to take the quiz in a different section before you regular section meets.

First, let's review how to calculate map distance in yeast:

Consider and example of two linked genes, *ARG3* and *URA2*.

P: *arg3 ura2* x *ARG3 URA2*

Diploid: *arg3 ura2* / *ARG3 URA2*

Meiotic products:

PD (*arg3 ura2*; *arg3 ura2*; *ARG3 URA2*; *ARG3 URA2*) = 127

NPD (*arg3 URA2*; *arg3 URA2*; *ARG3 ura2*; *ARG3 ura2*) = 3

T: (*arg3 ura2*; *arg3 URA2*; *ARG3 ura2*; *ARG3 URA2*) = 70

Recombination frequency, RF = [(NPD + 1/2T) / total number of tetrads] x 100. In our example, this is ([3 + 1/2(70)]/200) x 100 = 19 map units. We will modify the equation to obtain a better estimate of map distance. If you draw out the possible crossover events between two linked genes, you can see the different tetrads that result; see Fig. 5.17 (p. 147).

No crossovers --------> PD

Single crossover --------> T

Double crossover (2-strand) --------> PD

Double crossover (3-strand) --------> T

Double crossover (3-strand) --------> T

Double crossover (4-strand) --------> NPD

You can see how we can modify the equation to make it more accurate. Remember that half (2/4) the strands recombine if there is a single crossover event and that 4 strands recombine if there is a double crossover event (even if all of the strands don't participate, some participate more than once.)

<u>Map distance</u> = (total rec. events / total tetrads) x 100 = [(1/2[SCO] + DCO) / total tetrads] x 100

Map distance = ([1/2 (T – 2 NPD) + 4 NPD]/ total tetrads) x 100

Map distance = (1/2 T + 3 NPD) / total tetrads x 100

For our example above, map distance = ([1/2 (70) + 3 (3)] / 200) x 100 = 22 map units

This modified equation makes 2 assumptions: (1) there are no more than two crossovers in the interval and (2) there is no chromosomal interference (all types of DCOs occur with equal frequency.

ORDERED TETRADS AND GENE-CENTROMERE DISTANCE

In *Neurospora crassa*, meiosis occurs within the tight confines of a narrow ascus, resulting in the formation of **ordered tetrads**. Because of the precise positioning of each meiotic product within the ascus, one can infer the arrangement (and segregation) of each chromatid of homologous chromosomes during Meiosis I and II. This gives information about the distance between the gene and its centromere. (Meiosis II is followed by mitosis; each pair of genetically identical daugthers sits adjacent to one another. Each ascus is thus made of up 8 haploid ascospores.)

Consider a gene required for ascospore color (ws+ gives black spores and ws gives white spores):

P: ws+ x ws
Diploid: ws+ / ws (immediately undergoes meiosis)

If no recombination between ws gene and the centromere occurs, then the resulting ascospores are arranged in a neat array with black and white spores clearly segregated from one another (they separated during meiosis I), each type cleanly segregated to either side of the imaginary line separating the 4th and 5th ascospores. This is called **a first division segregation pattern**. Since the daughters of the mitotic division lie right next to one another, we can simplify the two possible configurations to:

(ws+ ws+ ws ws)
(ws ws ws+ ws+)

If recombination occurs between ws and the centromere, then a **second division segregation pattern** is observed. Now, both types of spores are found on either side of the imaginary line between the 4th and 5th ascospores. Now there are four possible configurations:

(ws+ ws ws+ ws)
(ws ws+ ws+ ws)
(ws+ ws ws ws+)
(ws ws+ ws ws+)

When an ascus shows a second division segregation pattern, we know that half of the chromatids are recombinant and the other half have not participated in crossovers. Thus, we can calculate the distance of a gene from its centromere simply by dividing the percentage of second division octads by 2.

Gene-centromere distance = ([# of second division octads / total octads] x 100) / 2

To examine linkage of two genes in Neurospora, we can use the same formulas as we did for Baker's yeast.

Gene conversion is the unidirectional transfer of genetic information.

Gene conversion is any deviation from the expected 2:2 segregation of parental alleles. Let's consider an example in the yeast *S. cerevisiae*. A diploid yeast cell is heterozygous at the gene B (B/b). When induced to sporulate, the resulting tetrads usually segregate 2:2 for the parental alleles. Occasionally, a tetrad results that doesn't share this segregation pattern, but instead segregate 3:1. These tetrads cannot be classified as PD, NPD, or T. The deviations from the expected are the result of gene conversion at the B locus. In Neurospora, 6:2, 2:6, 5:3, 3:5, and 3:1:1:3 (aberrant 4:4) gene conversion octads can be observed. The latter "half-chromatid conversion" octads can only be explained if the two strands of the DNA double helix carry information for two different alleles (**heteroduplex DNA**) at the end of meiosis.

Consider a 5:3 octad: + + + + + m m m

The box indicates a pair of spores that arose by a mitotic division. Remember that an octad contains four pairs of cells and that each pair of cells should be genetically identical. Thus, two strands of double helical DNA on the chromatid of the haploid cell that gives rise to these non-identical daughters must have contained information for both alleles of the gene (one for the + allele and one for the m allele) after meiosis. (In 6:2 and 2:6 octads, the mismatch in heteroduplex DNA is repaired prior to mitosis).

Gene conversion is not explained by mutation, since the allele that is converted always changes to the other allele segregating in the cross, not to a new allele.

Gene conversion is associated with recombination of flanking markers.

What else happens when gene conversion happens on a chromosome? Consider that gene B above is tightly linked to genes A and C. If a diploid cell (ABC / abc) is induced to sporulate, then the majority of the progeny are PDs (ABC, ABC, abc, abc). However gene conversion does occur at low levels, giving rise to the following combinations of alleles when the tetrads are examined.

1	2	3	4
ABC	ABC	ABC	ABC
ABC	**ABc**	AbC	**abC**
aBc	**aBC**	abc	**Abc**
abc	abc	abc	abc

Half of the tetrads that exhibit gene conversion at B also show recombination of flanking markers A and C, and the other half do not show recombination. It is only among the subset of tetrads that experienced gene conversion at B that the recombination frequency of the tightly linked A and C genes is that high.

This type of data contributed to our current understanding of the molecular mechanisms of recombination and what happens at the level of the DNA when to chromosomes recombine. You will review this in future molecular biology courses you take, but if you want a head start, there is a nice diagram in your book, p. 196 – 199 (that also illustrates nicely the concept of "heteroduplex" DNA).