Site-specific recombination

1. Query: What is the chemistry of strand breakage during conservative site specific recombination? (**I know we went over this a bunch of times, but I am confused on serine vs. tyrosine. I thought it was just the site specificity but the notes make it seems like a bunch more.)**

The site specific recombinases (both the serine family and tyrosine family) break strands using transesterification chemistry. That is, they break the 5'-3' phosphodiester bond in DNA. But at the same time they also form a phosphodiester bond, between the phosphate in DNA and the active site serine or tyrosine of the recombinase (3'-phosphotyrosine or 5'-phosphoserine). Hence the energy of the phosphodiester bond is conserved during strand breakage. The strand joining step is also transesterification, again conserving the energy of the phosphodiester bond (see below; query 2) This is the reason why these recombinases are called conservative site-specific recombinases.

2. Query: What is the chemistry of strand joining during conservative site specific recombination?

Transesterification. The 5'-OH group (tyrosine family) or the 3'-OH group (serine family) formed during strand cutting will attack the 3'-phopshotyrosine or the 5'-phopshoserine bond across DNA partners. The result is the breakage of the phosphodiester bond between DNA and the recombinase and the reformation of a 5'-3' phosphodiester bond.

3. Query: What is the difference between breaking a phosphodiester bond by transesterification and by hydrolysis? What is the mode of strand cleavage mediated by the conservative site specific recombinases?

As I explained above, transesterification preserves the energy of the phosphodiester bond, you break one but also make one at the same time. Hydrolysis (attack by water) breaks the phosphodiester but does not make one. The energy of the bond is lost. The products can be a 3'phosphate and a 5'-OH or a 5'-phopshate and a 3'OH. You will see this mode of strand cleavage when we discuss DNA transposition.

4. Query: What is the cleavage nucleophile during Integrase/Tyrosine family site specific recombination? What are the two DNA ends formed as a result of strand cutting?

Tyrosine is the cleavage nucleophile in tyrosine family.

The products are 3’-phosphotyrosie and 5’-OH (tyrosine family).

[Serine is the cleavage nucleophile in serine family. 5’-phosphoserine and 3’-OH (serine family) are formed by strand cleavage].

5. **Query**: Do the enzymes of the tyrosine family make single or double stranded cuts?

Tyrosine family recombinases make single stranded cuts.

6. **Query**: What is the nucleophile for the Resolvase/Invertase family site specific recombination? What DNA ends are formed as a result of strand cutting?

Nucleophile: serine; products; 5’-phopshoserine plus 3’-OH.

7. **Query**: What is the nucleophile for strand joining during the Invertase/Resolvase reaction?

3’-OH.

8. **Query**: Do the Invertase/Resolvase enzymes make single strand or double strand cuts?

Double stranded cuts.

10. **Query**: Is a Holliday intermediate formed during the Resolvase/Invertase reaction?

Holliday intermediate is not formed during serine family recombination.

11. **Query**: Do the invertases and resolvases follow the same chemical mechanism or different chemical mechanisms during recombination?

They follow the same mechanism, serine mediated double strand breaks; rotation of the cut strands through 180 degrees; strand joining.

12, **Query**: Can you explain how site specific recombination is used to bring about different biological consequences?

You should know the examples we discussed in class and explained in the notes. For example, how does site-specific recombination control plasmid amplification in yeast, how does it help bacterial chromosome segregation etc. etc.

13. **Query**: How does site specific recombination help a bacterial pathogen to escape the host

immune system, at least temporarily?

We discussed the example of Salmonella bacterium controlling the expression of one of two types of flagellin genes. This is achieved by inverting a DNA segment containing a promoter. In one orientation, the promoter is in the right orientation to drive the expression of one type of flagellin gene (A) and the repressor for the second type of flagellin gene (B). In the opposite orientation, the promoter is in the 'off' configuration for expression of the A gene and the repressor for B. Lack of repressor will turn on the expression of flagellin gene B.

14. **Query**: Can you explain DNA inversion and DNA deletion (excision or resolution) by site-specific recombination?

A. **DNA inversion between head-to-head sites**:

Imagine some sequence within the site at the left and the site at the right: GACC/CTGG. Y our can see that these sequences are in inverted (head-to-head) orientation; so the two sites are also in the inverted orientation. By site, I mean the full site composed of two half-sites; each half site has a recombinase subunit bound to it. Each full site has two recombinase subunits bound, so the two strands of DNA within each site can be broken and then exchanged. To distinguish the two strands I have colored them red and green.

5'GACC3'----------5'**GGTC**3'
3'CTGG5'----------3'**CCAG**5'

I am marking all 5' and 3' ends so you can keep track of strand polarity.

As an example, imagine the strand breaks to occur after 5'GA3'
3'CT5'

5'GA3' 5'CC3'----------5'**GG**3' 5'**TC**3'
3'CT5' 3'GG5'----------3'**CC**5' 3'**AG**5'

To complete recombination, I have to join the left 5'GA3' to the right 5'**CC**3'.
3'CT5' 3'**GG**5'

And I have to join the left 5'CC3' end to the right 5'**TC**3' end.
3'GG5' 3'**AG**5'

The result is:

5’GA**CC**3’----------5’GG**TC**3’
3’CT**GG**5’----------3’CC**AG**5’

Note that the sites are regenerated after recombination. Note also that in the inverted DNA segment the top and bottom strands are switched. The green dashed line is now at the top. This is because DNA is duplex, with strands of opposite polarity, and recombination is the exchange of duplex DNA. You cannot do inversion in a single strand of DNA or RNA (you will be joining 3’ to 3’ or 5’ to 5’).

When someone talks of ‘anti-sense’ RNA, for example, he/she is talking of the ‘complement’ of the sense strand.

B. DNA excision between head-to-tail recombination sites

Try to write out a similar sequence for the head-to tail sites.

5’GACC3’----------5’**GACC**3’
3’CTGG5’----------3’**CTGG**5’

Since the sites are in the same orientation, you will be excising the DNA between the sites as a circle. You will join left 5’GA3’ end to the right 5’**CC**3’ end.

3’CT5’ 3’**GG**5’

5’GA**CC**3’
3’CT**GG**5’.

Note all the dotted DNA is deleted (as a circle) with one recombination site 5’**GA**CC3’ on it.
3’**CT**GG5’

Some clarification of semantics:

Head-to-head sites mean sites in inverted orientation. The arrowhead will point to each other, and the tails will also point to each other on a circular DNA molecule. So if one wants to call the site tail-to tail that is fine too.

Similarly, head-to-tail sites are in direct orientation. On a circular molecule, this will be also tail-to-head.