Homologous recombination: a general picture

For a general description of homologous recombination showing the consequence of recombination between two homologous DNA regions: circular vs linear DNA and circular versus circular DNA, go the link below:

http://www.wwnorton.com/college/biology/mbio/animations/main.asp?chno=ch09a01

Holliday junction: Animations of the process of branch migration

The RuvAB proteins constitute the helicase that drives directional branch migration of the Holliday junction in E. coli. RuvB is the active helicase that hydrolyzes ATP to produce the energy that is required for the process. Without energy, the movement of the branch point would be a random walk, and will not be useful in producing large patches of heteroduplex DNA.

The RuvA protein is responsible for binding the branch point and recruiting the RuvB helicase, RuvA is a dimer, so two hexameric RuvB complexes are bound to the junction. You can imagine that the RuvA dimers act as stators and the RuVB hexamers act as the motors to form an active DNA pump at the junction. Homoduplex DNA will be sucked into the pump (through the inlet), and heteroduplex DNA extruded from it (through the outlet).

We shall illustrate these points in class. Please see the animations of branch migrations by using the following links:

http://www.shef.ac.uk/mbb/ruva

http://www.shef.ac.uk/mbb/ruva/ruva-020.html

http://www.shef.ac.uk/mbb/ruva/ruva-010.html

http://www.shef.ac.uk/mbb/ruva/ruva-040.html

Note that branch migration is facilitated in the anti-parallel (H-form) Holliday junction compared to the parallel (X-form) junction. This is because in the latter (parallel form, each step of branch migration is associated with unstacking and restacking the bases). The requirement of base unstacking step is eliminated in the antiparallel junction, thus branch migration is energetically less constrained.

Resolution of Holliday junctions

As we discussed in class, the Holliday junction can be resolved in two ways. One will retain flanking markers A, B and a, b in our drawings in the parental configuration. The other will cause them to be in the exchanged or crossed over configuration: A,b and a, B. The link below is useful in seeing how this occurs.

http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation35.html

Note: The above animation also shows how recombination is initiated in E. coli by the action of the RecBCD enzyme and the chi sequence that triggers the strand breakage event. The RecA protein mediates the invasion by the single strand with a 3' end of the homologous partner DNA. In the model here, the D loop strand is not digested away (as we described for the Meselson-Radding model) but is involved in pairing with the partner duplex. Hence this is a true Holliday model, generating symmetric, rather than asymmetric, heteroduplex.

The major Holliday junction resolving activity in e. coli is the RuvC protein, which acts as a dimer.

The RuvABC system represents a branch migration/junction resolution machine.