Unit 2

Molecular mechanisms of crossing over including models of recombination:

All DNA is recombinant DNA. Genetic exchange works constantly to blend and rearrange chromosomes, most obviously during meiosis, when homologous chromosomes pair prior to the first nuclear division.

During this pairing genetic exchange between the chromosomes occurs. This exchange is called Crossing over. It is one of the result of homologous ~~chromosome~~ recombination.

Homologous recombination is an essential cellular process catalyzed by enzymes. Recombination provide genetic variation, retrive sequences lost through DNA damage by replacing the damaged section with an undamaged DNA strand from homologous chromosome. Recombination also provides a mechanism to restart stalled or damaged replication fork.

The key steps of homologous recombination shared by these models include:

1. Alignment of two homologous DNA molecules. DNA molecules can have small regions of sequence difference and may, for example, carry different sequence variants known as alleles of the same gene.

2. Introduction of breaks in the DNA. The breaks may occur in one strand DNA strand or both DNA strand.

3. Strand Swap: Formation of initial short regions of base pairing between the two recombining DNA molecules. This pairing occurs when a single-stranded region of DNA originating from one parental molecule pairs with its complementary strand in the homologous duplex DNA molecule. The step is called Strand invasion. As a result of Strand invasion the two DNA molecules become connected by crossing over DNA strand. This cross structure is called a Holliday junction.

4. Branch migration: Movement of the holliday junction. A holliday junction can move along the DNA by the repeated melting and formation of base pairs. Each time the junction moves, base pairs are broken in the parental DNA molecules while identical base pairs are formed in the recombination intermediate. This process is called Branch migration.

5. Resolution: Cleavage of the holliday junction. cutting the DNA strands within the holliday junction regenerates two separate duplex DNA molecules and therefore finishes genetic exchange. This process is called Resolution.

The steps involved in Holliday model of homologous recombination

1) Recombination is initiated by the introduction of a nick in each DNA molecule at the identical location.

2) The holliday junction generated by strand invasion can then move along the DNA by branch migration

3) If the two DNA molecules are not identical ~~between~~ at points branch migration through these regions of sequence difference generates DNA duplexes carrying one or few sequence mismatches. Such regions are called heteroduplex DNA.

4) Finishing recombination requires resolution of the Holliday junction by cutting the DNA strands near the site of the cross. Resolution occurs in one of two ways and therefore gives rise to two distinct classes of DNA products.

cut site one:

The resulting DNA molecules ~~will have~~ their structure or products are refferred to as "Splice" recombinant product, because the two original duplexes are now "splice together". This type of recombinat~~ion~~

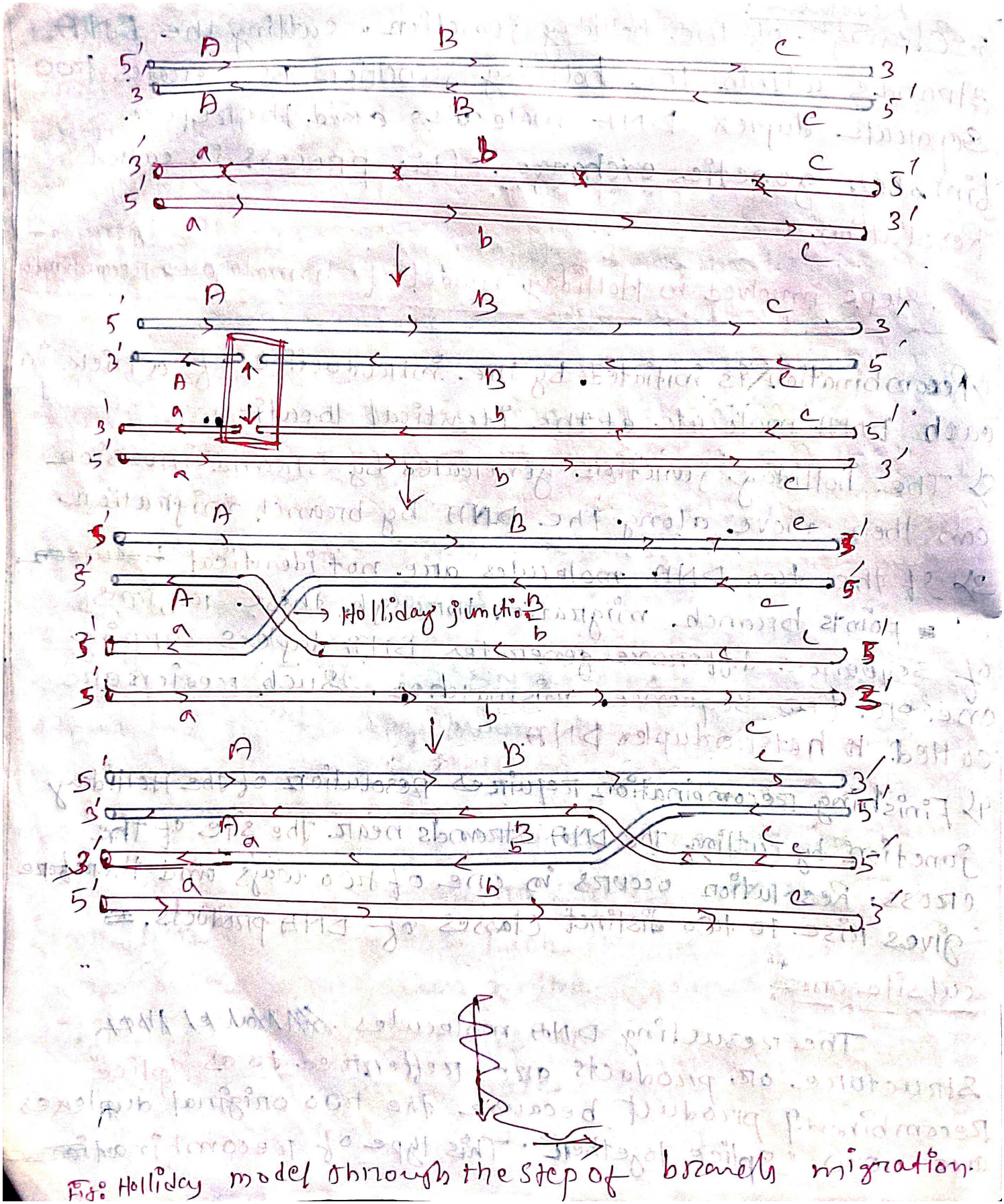

Fig: Holliday model through the step of branch migration.

is also called the cross over product.

cut site two:

The resulting DNA molecules contains a region or "patch" of hybrid DNA. These molecules are thus known as patch product. These molecules are, therefore also known as non-cross over products.

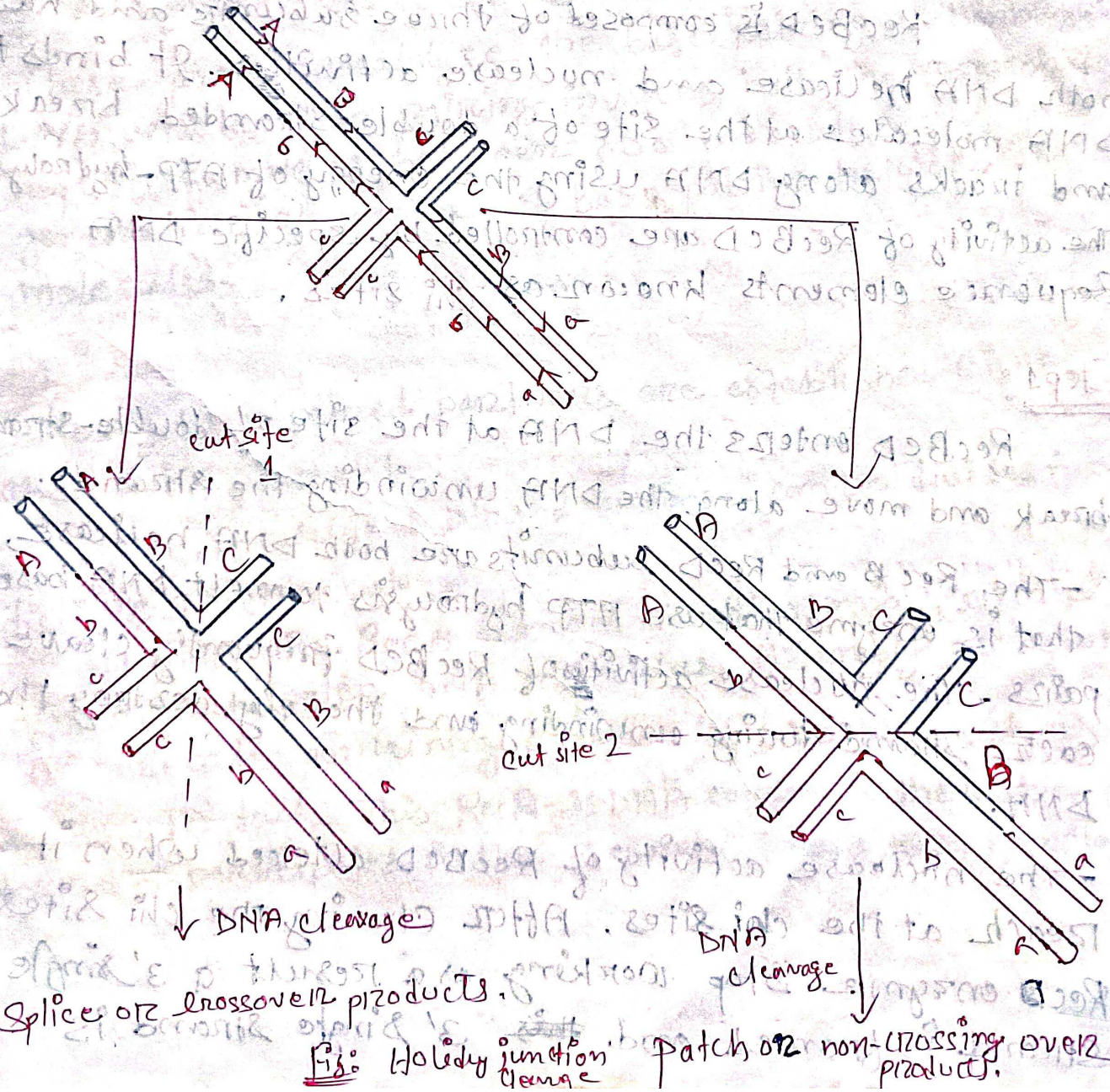

Fig: Holiday junction cleavage

RecBCD Helicase/Nuclease processes Brooken DNA molecules for Recombination:

~~DNA molecules with ssDNA~~

The RecBCD enzyme processes broken DNA molecules to generate these regions of ssDNA. RecBCD also, helps load the RecA strand-exchang protein onto these ssDNA.

RecBCD is composed of three subunits and has both DNA helicase and nuclease activities. It binds to DNA molecules at the site of a double-stranded break and tracks along DNA using the energy of ATP-hydrolysis. The activity of RecBCD are controlled by specific DNA sequence elements known as chi sites.

Step 1:

RecBCD enters the DNA at the site of double-strand break and move along the DNA, unwinding the strands.

-The RecB and RecD subunits are both DNA helicase, that is enzyme that use ATP hydrolysis to melt DNA base pairs. The nuclease activities of RecBCD frequently cleave each strand during unwinding and thereby destroy the DNA.

-The nuclease activity of RecBCD altered when it reach at the chi sites. After cleaving the chi sites RecB enzyme stop working as a result a 3' single strand is formed and ~~this~~ 3' single strand is

extended. This extended 3' is called

Step 2:

RecA protein assembles on Single-Stranded DNA and promotes strand invasion.

RecA protein is the founding member of a family of enzymes called strand-exchange protein. These proteins catalyze the base pairing of homologous DNA molecule. pairing involves both the search for sequence matches between two molecules and the generation of regions of base pairing between these molecules.

Step 3:

Newly base-paired partners are established within the RecA filament.

RecA-catalyzed strand exchange can be divided into distinct reaction stages.

First, the RecA filament must assemble on one of the participating DNA molecules. Assembly occurs on a molecule containing a region of ssDNA, such as an ssDNA tail. This RecA-ssDNA complex is the active form that participates in the search for a homology.

Step 4:

RuvAB complex specifically recognizes Holliday junctions and promotes branch migration.

After the strand invasion step of recombination is complete, the two recombining DNA molecules are connected by a DNA branch known as a Holliday junction.

RuvA recognizes and binds to Holliday junctions and recruits the RuvB protein to this site.

RuvB ATPase provide the energy to drive the exchanges of base pairs that move the DNA branch.

Step 5:

RuvC cleaves specific DNA strands at the Holliday junction to finish Recombination.

Resolution by RuvC occurs when RuvC recognizes the Holliday junction and specifically nicks two of the homologous DNA strands that have same polarity.