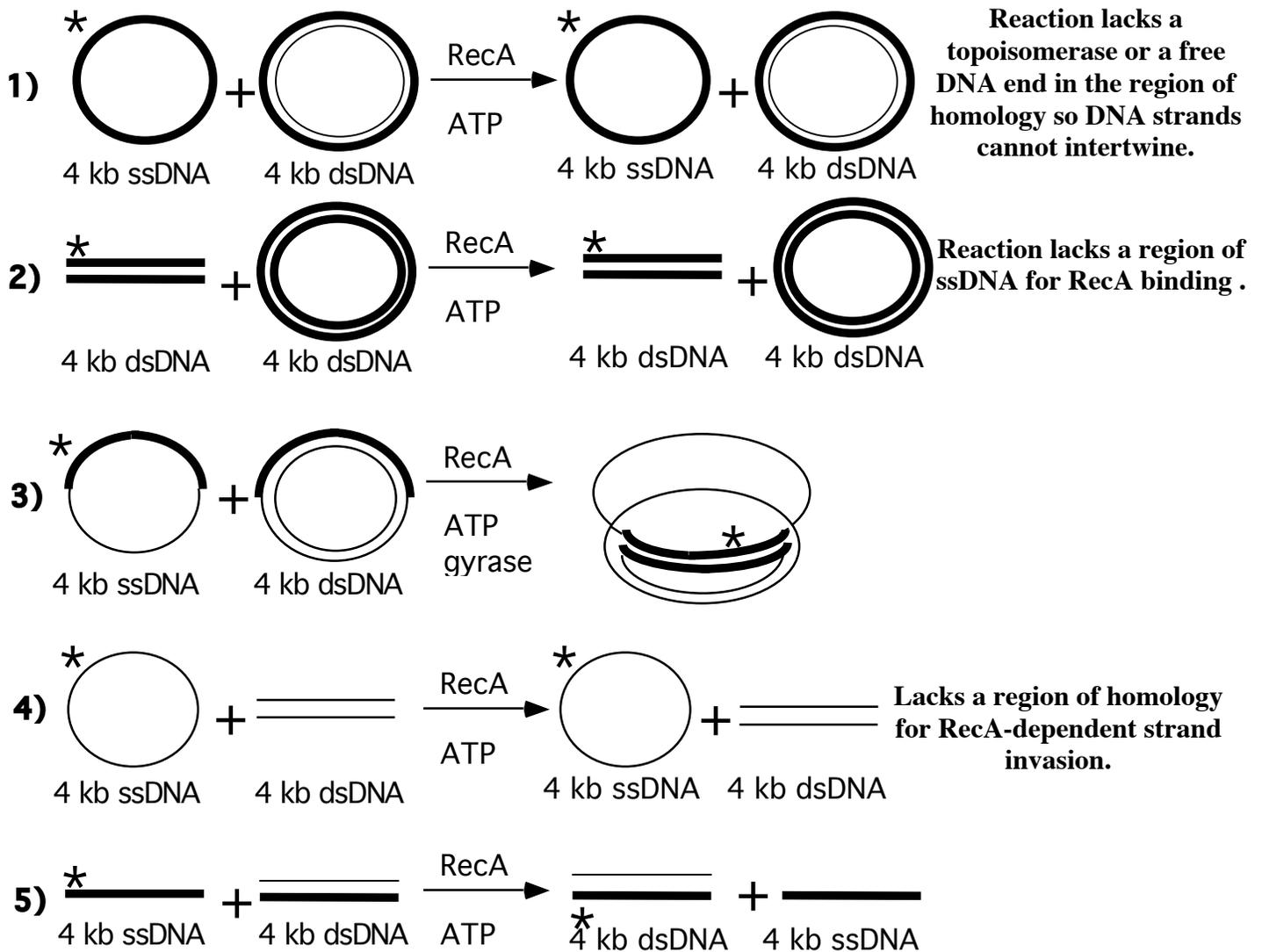
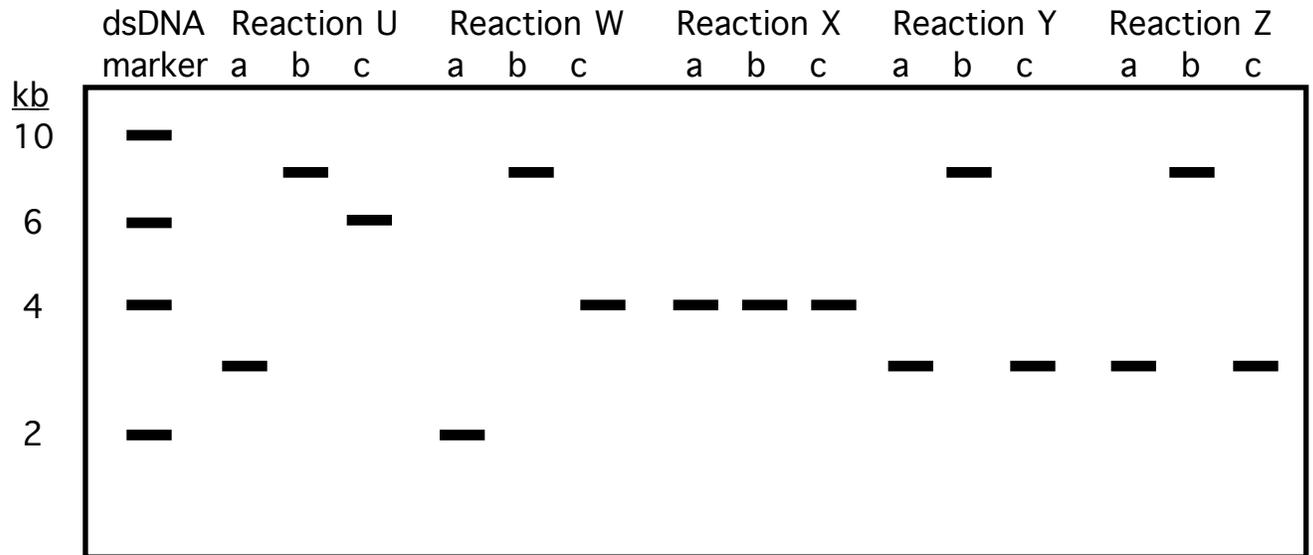


**Question 1.**

**1A.** You are studying the function of RecA *in vitro* along with a lab partner. You run several reactions to examine the requirements for RecA activity. For each reaction below give the expected structure of the DNA products after RecA has been removed (by phenol extraction.) Regions of homology are shown as **thick lines**. Radiolabelled DNA strands are indicated by the \*.



**1B.** After you carry out these reactions, your partner runs a non-denaturing acrylamide gel to analyze the products. For each reaction, she runs 3 lanes. Lane (a) is a control reaction run without RecA. Lane (b) is the complete reaction loaded directly into the gel. Lane (c) is the complete reaction that has been phenol-extracted to remove protein prior to loading on the gel. She exposes the resulting 15-lane gel to X-ray film. She has to leave at this point, and after developing the film, you sit down to write up the lab report. Unfortunately, you realize that you do not know what order she loaded the reactions in. The film is as follows:



Which reaction numbers (1-5) correspond to the reactions on the film (U-Z)? Explain the differences between lanes a, b, and c for each reaction.

**U (Reaction 3)**

**a: Circular ssDNA, apparently runs at ~3 kb. Same size template as reactions Y and Z.**

**b: RecA binds and forms a new DNA product combining the 2 DNA's; RecA remains bound and you see a band of ~8 kb.**

**c: Final product, stable without RecA, combines two substrate DNA's in a D-loop structure that runs at ~6 kb.**

**W (Reaction 5)**

**a: Initial labeled DNA is linear ssDNA, runs faster than circular at ~2kb.**

**b: RecA binds and forms a new DNA product combining the 2 DNA's; RecA remains bound and you see a band of ~8 kb.**

**c: Final labeled product is 4 kb dsDNA. □**

**X (Reaction 2)**

**a: 4 kb linear, labeled dsDNA**

**b and c: No ssDNA, RecA can't bind, same size labeled DNA molecule (4 kb) observed before and after reaction.**

**Y and Z (Reactions 1 or 4)**

**a: These have the same size circular ssDNA, apparently runs at ~3 kb**

**b: RecA binds both DNA molecules, you see a band for 2 DNA's + protein shift (~8kb)**

**c: Same radiolabelled DNA molecule is present at the end of the reaction because of the reasons indicated in (1a).**

**Question 4.** For your graduate thesis, you are working on a putative eukaryotic analog of the RecBCD complex in *S. cerevisiae* called the Mre11/Rad50/Xrs2 complex or MRX. Previous work in the field has demonstrated that all of the genes encoding this complex are required *in vivo* for the timely formation of 3' overhangs during meiotic recombination.

4A) The purified MRX complex has been demonstrated to have exonuclease activity *in vitro*. Given its functional similarity to the RecBCD complex, what 2 other enzymatic activities do you suspect it might have?

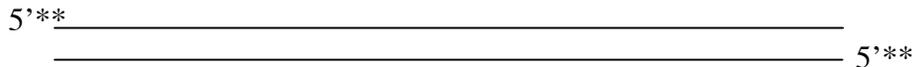
Helicase activity and endonuclease activity.

4B) To study the MRX complex further, you wish to establish an *in vitro* assay for exonuclease activity. Describe how you establish your assay. Specifically, what type of DNA substrate might you use and how might you discriminate between 3'→5' and 5'→3' activity?

Many types of answers are acceptable. You need a way to measure nuclease activity in a direction specific manner.

ONE way to do this is the following:

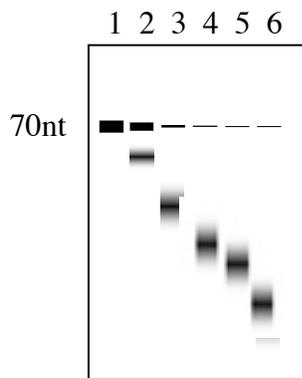
1. Use two annealed 70 nt ssDNA primers. 5' end label using T4 polynucleotide kinase and radioactive dATP. (Like in the paper we discussed.)



2. Incubate this substrate with your putative exonuclease. Take timepoints to determine exonuclease activity over the time of your reaction.

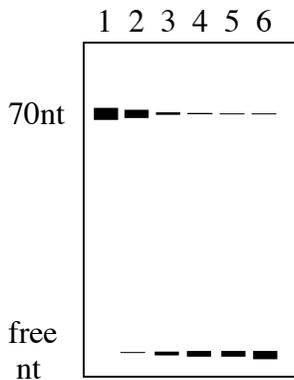
3. Run the resulting products on a denaturing SDS page gel (a sequencing gel). If you have a 3'→5' exonuclease, you will see the following results: □  
Degradation from the opposite end of the label will result in shorter and shorter □  
fragments. □

See next page for gel results:



1. t=0 minutes
2. t=10s
3. t=30s
4. t=60s
5. t=2minutes
6. t=5minutes

If you have a 5'→3' exonuclease, you will have the following results:



1. t=0 minutes
2. t=10s
3. t=30s
4. t=60s
5. t=2minutes
6. t=5minutes

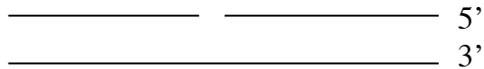
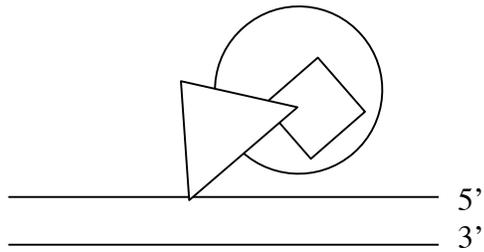
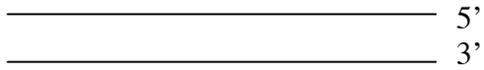
The 5'→3' exo activity will rapidly result in the generation of free nucleotides which will run at the bottom of your gel.

4C) Oddly, you find that the MRX complex has 3'→5' exonuclease activity. You were expecting 5'→3' exonuclease activity since a 3' overhang is generated. Assuming that your *in vitro* assay really does reflect the *in vivo* activity of the complex, how might you explain the need for the MRX complex function in generating 3' overhangs? Propose a model, include a drawing of how this would function on DNA.

HINT I: What are the two other activities you proposed in part 1?

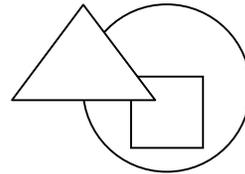
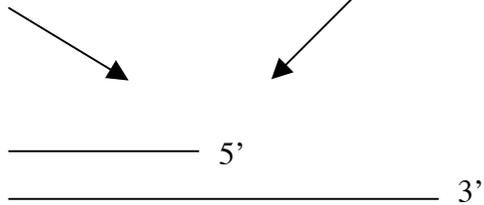
HINT II: How does DNA polymerase accomplish lagging strand synthesis, moving in the overall direction of 3'→5' when its catalytic activity moves in the 5'→3' direction??

Double  
Strand  
Break



Helicase  
activity

Exonuclease activity



MRX

1. Endonuclease activity  
generates a nick.

2. Either the helicase activity peels  
off the top strand to reveal a 3'  
overhang, OR the exonuclease  
activity can now chew 3'->5'  
to generate the 3' overhang.

4D) During the course of your studies, you find that a particular point mutation in the Xrs2 protein causes DNA damage sensitivity *in vivo*, but normal meiotic recombination. When you test the Xrs2 mutant *in vitro* in your assay, you find that it is wild-type for all activities. How can you explain its *in vivo* phenotype? What might this mutation be affecting?

This mutation could affect a residue that is critical *in vivo* for activation of the MRX complex. In response to DNA damage, the cell activates a signaling cascade that leads to the activation of proteins involved in repairing DNA damage. This particular mutation does not affect the meiotic recombination function or *in vitro* activity, but is essential for activation of MRX in response to DNA damage.