Eukaryotic Transcriptional Regulation:

You are studying the regulation of a eukaryotic gene involved in memory called Absent Minded. Animals with mutations in this gene have severe short- term memory loss. You want to understand what controls the levels of Absent Minded gene expression to see if you can increase its expression in normal animals and therefore increase short-term memory. Using mutational mapping of the promoter, you identify two 10 bp regions of the promoter that when mutated strongly reduce expression of the Absent Minded promoter *in vivo*. You purify the proteins that bind these promoter elements and call the proteins ABR1 and ABR2.

To understand how these two proteins regulate Absent Minded gene expression, you do an *in vivo* experiment and an *in vitro* experiment

In vivo

To be sure that these proteins are involved in activation of the Absent Minded promoter, you fuse the Absent Minded promoter (Upstream Activating Sequences and Promoter) to the LacZ gene and test the levels of expression in cells that have mutations that fail to produce the ABR1 and/or ABR2 proteins. You get the following results:

ABR1 Gene	ABR2 gene	Transcription Units
Wildtype	Wildtype	2000 Units
Mutant	Wildtype	50 Units
Wildtype	Mutant	200 Units
Mutant	Mutant	50 Units

- Based on these data, what can you conclude about the function of ABR1 and ABR2 at the Absent Minded promoter.

Both proteins are required to achieve high levels of activation *in vivo.* When ABR2 is present but not ABR1 then there is only basal levels of transcription, this suggests that ABR2 requires ABR1 function in vivo to activate transcription. When ABR1 is present but not ABR2, there is a 4 fold increase in transcription activity over the basal level indicating that ABR1 on its own can alter transcriptional activity *in vivo*.

In vitro

Your next step is to purify the ABR1 and ABR2 proteins. You test their ability to activate transcription *in vitro* by ***first*** adding the purified activators to a plasmid containing the Absent Minded Gene (Upstream Activating Sequences, Promoter, and Full Open Reading Frame). You then add purified RNA Polymerase II and auxiliary factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH). You get the following results:

ABR1 Protein	ABR2 Protein	Absent Minded Transcription (pmoles/minute)
-	-	500
+	-	500
-	+	3000
+	+	3000

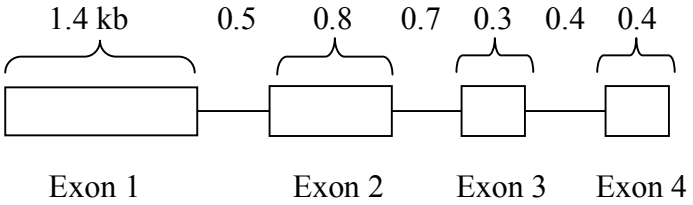
- Based on the differences between the reconstituted *in vitro* transcription experiments and the *in vivo* promoter fusion experiments, propose a function for the ABR1 regulator. **Hint:** Think about differences between prokaryotic and eukaryotic transcription and the kinds of functions eukaryotic transcriptional activators have. Explain your reasoning.

The *in vitro* experiment shows the opposite of the *in vivo* experiment. ABR2 can provide full activation on its own without ABR1 being present and ABR1 has no effect on the transcription rate *in vitro*. How do we reconcile these two seemingly contradictory experiments? The key is from the hint—what is

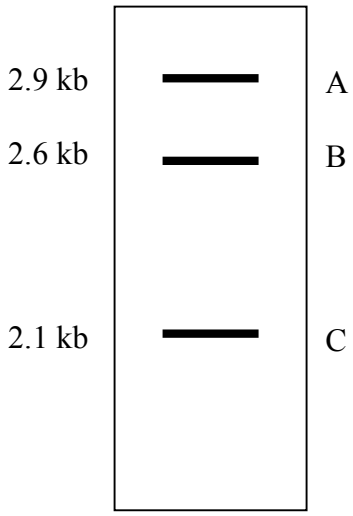
different about eukaryotic transcription…….nucleosomes. The eukaryotic transcriptional machinery has to deal with the presence of nulceosomes. They do this by using histone acetyl-transferases and histone remodeling machines to loosen DNA and move nucleosomes. So one model to explain our data is that ABR1 is a histone acetyl-transferase that is required *in vivo* (with nucleosomes) but not *in vitro* (no nucleosomes) for ABR2 function. ABR2 must bind to the DNA to activate transcription. In the *in vivo* experiment, ABR1 must be present to loosen the DNA around the nucleosomes and provide access to the DNA for ABR2. *In vitro,* there are no nucleosomes and so ABR1 is not needed and has no affect on transcription on its own. ABR2 can bind and activate high levels of transcription on its own when no nucleosomes are around. The increase in *in vivo* transcription by ABR1 alone is likely do to the change in nucleosome binding that allows a higher level of basal transcription.

You are interested in studying the expression of the *mudblood* gene. The structure of the genomic coding region for the *mudblood* gene is shown below (Exons are boxes; Introns are lines). You isolate all the mRNAs from cells expressing this gene, run the mRNA on a gel, and then transfer the mRNAs to a membrane in order to do a northern blot. In order to study the mRNAs made by this gene, you make a radiolabled DNA probe that corresponds to the DNA sequence found in exon 1. After hybridizing this probe to the membrane you made, you observe three different bands on the autoradiograph of your northern blot. Your results are shown on the right.

Mudblood Genomic Region



Northern Results
with Exon 1 Probe:



- What process is occurring to produce this pattern?

Alternative Splicing

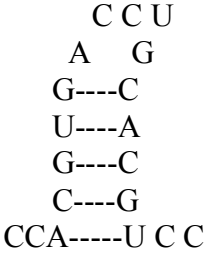
- What can you conclude about the composition of bands A, B, and C that you observe on your northern blot?

Band A: Exon 1-4 spliced together
Band B: Exon 1,2, and 4 spliced together
Band C: Exon 1,3 and 4 spliced together

We have seen in class that although RNA is single stranded, it has the ability to fold in to extremely complex structures. The following RNA sequencecan fold up into a hairpin structure

5' CCACGUGACCUGCACGUCC 3'
1 2 3

- Draw the structure of this hairpin.



- When the G residue marked 1 is mutated to a C, the RNA no longer folds into a stable hairpin. However, if a second mutation is introduced changing the C residue labeled 3 to a G, then a stable hairpin again forms. Briefly explain.

When the G changes to a C, it can no longer pair with the C residue at position number 3. This lack of pairing will prevent a stable hairpin from forming. If the C at position 3 is then changed to a G, it will restore the pairing and allow the stem-loop to form.

- If a deletion of the ACC sequence that begins at position 2 is made, then no folded structure is seen. Why do you think this occurs?

The ACC acts as a linker to form the loop part of the stem-loop. If these nucleotides are deleted, the sequence cannot fold back on itself.