Questions to the Antoch et al paper:

1. Describe the elements that are presented in figure 1:
   -what is D5Mit307, and what is it useful for?
   -what is CpG, and why does it matter for clock or bendless?
   -How does it help you to know where a CpG island is?
   -What does it mean that the arrows for bendless and clock are facing each other?
   - How can they determine the orientation of transcription of a gene with respect to the telomere to centromere direction of the chromosome?

2. Describe all the (putative) elements that are present in Bac54, that allow the researchers to rescue the mutation. Let's assume that BAC 54 100 Kb has ALL the introns and exons of the wild type clock gene: why do you think that BAC54 work works but BAC 54 100 Kb doesn't.

3. In addition to the mutant that they found, there is another called clock Null that does not have ANY of the coding region of clock (basically the whole gene is out). The null/null homozygote is screwed, but the heterozygote Null/wt is quite normal. Moreover, when they put a single copy of a BAC 54 into the Null/Null it rescues the rhythm pretty well. In contrast to this, the clock/wt heterozygote is screwed, and when they put a single copy of the transgene of the BAC 54 it rescues only partially the rhythm. How is it possible that having no gene at all (Null) gives a milder phenotype than having a partially mutated gene (clock mutation described in the paper). Elaborate on what kind of mutations they may be dealing with, and why it behaves like this. Please, don't just say " it is because it is a XXX mutant". Elaborate on the potential molecular mechanisms. (Hint: we talked about this at length a couple of weeks ago)

4. They had these mutants in hamsters for more than 20 years, but they decided to start from scratch in mice. Exactly, what kind of tools do you have in mice that you don't have in hamsters? Why are these tools critical for these kinds of experiments? Elaborate on this.

5. Now that you know that the missing gene is clock, you would like to test if having a mouse that expresses clock ONLY in the retina would have a normal circadian rhythm. How would you manage to do this (a mouse with clock being expressed in the retina and absolutely nowhere else in the body)? Elaborate on your answer.

6. You have found that the clock mutation was on chromosome 5, on its long arm, and you have narrowed to 1000 kb. You do the BAC rescue and find that there is a BAC that rescues the rhythm. How do you identify exactly what was wrong in the mutant gene that you have managed to rescue? (is it a small mutation or a big chunck missing, how much of the gene is missing, etc…

7. You have done all the work mentioned in question 6, and the sequence of the gene seems to be absolutely normal. Moreover, you take the cDNA from the **mutant** mouse,

you engineer a BAC such that you replace the coding region of the wildtype genome with the mutant coding region and it rescues the mutant mouse! What is going on here?