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?

It is a genetic marker that can be used to identify a location in the chromosome.

what is CpG, and why does it matter for clock or bendless?

CpG islands are places in the genome with high Gc content and hypomethylated, and they are usuallt a sign that there is a promoter nearby. Thus, it is helpful to find them in this case, because it suggests that there are going to be genes activated by them (in this case clock and 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?
These genes are transcribed in opposite orientations.

- How can they determine the orientation of transcription of a gene with respect to the telomere to centromere direction of the chromosome?
Once they identify the cDNA, they can simply look where in the chromosome is the 5' of the cDNA and where is the 3' of the cDNA. The orientation of transcription will go 5' to 3'.

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.
They need a promoter, one/several ebhnacers, a polyA addition signal and the intron/exons necessary to complete the open reading frame of clock. It is possible that the bac 100 kb is missing a promoter or an enhancer (or both), because we know that the gene is transcribed in the 5' to 3' orientation, and the 100 kb BAc had the 5' of the chromosome cut out.

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)
Most likely the clock is a dominant negative that is able to bind to the wt clock (or to another protein in the pathway), but it disrupts it. So, that's the reason why it is better to have null/wt than clock/wt. For instance, it is possible that the wt gene has a dimerization domain and another domain to activeate some downstream effect. If the mutation keeps the dimerization domain but is missing the activation domain, it will bind to a normal protein and it will render it useless.

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.
1)The genome of Hamsters has not been sequenced.

2) there are inbred strains of mice that allow to distinguish which chromosomes come from the father or the mother when crossing different strains of mice. This allows to pinpoint where a mutaion is.

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.
You would make a construct with a promoter specific for the retina driving expression of the clock cDNA. Then you would inject this construct into a single cell embryo to make a transgenic mouse.

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 chunk missing, how much of the gene is missing, etc…
First you could do a few southerns to see if there is something grossly abnormal.

Then you could get the cDNA from the wt and the mutant mouse, and sequence them both in parallel (you would not do that with genomic DNA because it is too long).

If the cDNA looks ok, that would suggest that the problem is in the promoter/enhnacer/polyA etc… This would be much more difficult to figure out because you would have to sequence every nucleotide up and down 100 Kb to find out what's wrong.

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?

This suggests that the coding region is ok, but that the promoter/enhancer/polyA regions are messed up.