PS#3: We will begin at 10:30 am with Problem 1 in BH212

Assignments
Problem 1 - group 2
Problem 2 - group 1
Problem 3 - group 4
Problem 4 - group 3

Time limit: 25 min per group.

The beginning: Designate one person in each group to give a 5 min introduction: try to be succinct and remember to describe the biological significance of the question. You don’t need to describe the whole solution in these 5 min, but you can set the stage.

The discussion: Describe the solutions and the logic behind them.

The end: Designate one person in each group to give a short summary at the end of the problem discussion.
Q1. Using an S. cerevisiae in vitro splicing system, you find that a pre-mRNA substrate harboring a single 2’ deoxy substitution between the UACUAAC sequence and the CAG at the 3’ splice site of the substrate blocks splicing in vitro. The block is after the first chemical step (i.e. lariat-intermediate and cleaved 5’ exon accumulate).

Suggest at least two models to explain this observation, describe the predictions of each model and indicate the experimental test of each prediction.

![Diagram of RNA splicing](image)
Q2. A circular RNA can in principle be produced by RNA splicing. This can occur if the 3' OH of a cleaved 5’ exon attacks a 3’ splice site *upstream* of the 5’ splice site. Describe how one could detect such events in vivo. One suspects that cells have mechanisms to avoid such events.

Suggest at least two models for how such events might be prevented by cells, describe the predictions of each model, and indicate the experiment test of each prediction.
Q3. Formation of the “commitment complex” (in which U1 snRNP is bound to the 5’ splice site and BBP/SF1 is bound to the branchpoint) does not require ATP. However, binding of U2 snRNP to form the “A” complex does require ATP.

Suggest at least two models to explain this observation, describe the predictions of each model and indicate the experimental test of each prediction.
Q4. Mutations in U4 snRNA that artificially extend stem I of the U4-U6 duplex result in a cold sensitive phenotype. Selection of suppressors of this mutation identified numerous single amino substitutions in Prp8. One cluster occurs in a region of Prp8 that stimulates the helicase activity of Brr2 in vitro. Two other clusters lie elsewhere in Prp8.

Suggest at least two models to explain these observations, describe the predictions of each model and indicate an experimental test of each prediction.