Discussion Questions for **Pestova et al, Nature 332-334 (2000).**

**Background**

We have seen in Bioreg that GTPases –for example EF-Tu that delivers the elongator aa-tRNAs to the A-site of the ribosome –provides fidelity control of amino-acyl-tRNA selection. This study by Pestova and coworkers uncovers a second GTPase that is required during eukaryotic translation initiation. Think broadly about how this GTPase, eIF5B, could provide for an additional point of control in eukaryotic translation initiation and its relationship to its more ancient bacterial counterpart.

**Questions**

1) What is the controversy being addressed?

2) Enumerate the evidence that GTP hydrolysis by TC (eIF2-MET-tRNAi-MET, GTP) is not sufficient for subunit joining. What is the role of eIF5?

3) Do the findings suggest eIF5 and eIF5B work together or in different steps?

Are their clear precursor product relationships that argue for the latter?

4) What are the different methods for detecting subunit joining in this study?

(Hint: one is direct but not a functional assay, the other is a functional assay but indirect)

5) What is the evidence that eIF5B is a GTPase? Does this study provide clues for the molecular entity that assists in GTPase activation of eIF5B? Could this mechanism be related to how EF-Tu GTPases are activated?

6) Is GTP hydrolysis by eIF5B necessary for subunit joining? Is it sufficient? How about peptide bond formation? What is the evidence for or against? Why must eIF5B hydrolyze GTP?

7) Compare and contrast the activities of prokaryotic IF2 and eukaryotic eIF5B. How are they similar? Why are they different?

8) Does the paper provide evidence for an additional step of eukaryotic translation initiation that could be regulated? How might you envision regulation could be achieved? Said differently, what is missing from this assay that could provide some level of regulation? (Hint: have the mRNAs used in this study had the “nuclear experience?” How are they transcribed?)