PS#4

We will begin at 10:30 am on Thursday 3/3 with Problem 1 in BH212

Assignments

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

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. Your labmate, Leonard, is trying to identify the interaction partners of his favorite viral protein, Spock. He has generated a virus strain in which a TAP (tandem affinity purification) tag is encoded at the annotated N terminus of Spock. Leonard is surprised to find that when he performs a Western blot on cells infected with this virus strain, he observes a single strong band corresponding to TAP-tagged Spock but it migrates as ~20 kDa larger than he had expected. Propose at least one explanation involving translation initiation, one involving elongation, and one involving termination for this aberrant migration of TAP-tagged Spock. What experiments could differentiate between these possible mechanisms?
Q2. A newly discovered virus, Jpee1 (pronounced ha-pee-wan) has a positive effect on the infected individuals. Those infected appear to be happier and therefore they are often participating in social events and parties, which facilitate virus transmission. However, under these conditions (at parties) the hosts are stressed (alcohol consumption, lack of sleep, etc.). Under these condition general cap-dependent translation is inhibited in the hosts. Although, Jpee1 mRNAs are capped, the virus has evolved mechanisms to overcome this block.

Sequencing information indicated an unusual 5'UTR structure on Jpee1 mRNA (Fig. 1, red structure is RNA hairpin). Additional information indicates that viral protein synthesis is stimulated by eIF2a phosphorylation.

Propose two or three different hypotheses for how Jpee1 mRNA 5'UTR structure ensures translation under global translation repression. Describe how you would experimentally test these hypotheses.
Q3. Dr. E.F. Teeyou received from his friends Drs. R. and A. Transpher a strain of E. coli that express red fluorescent protein (RFP) from a plasmid. Dr. Teeyou observed that the bacterial colonies were purple rather than red. He sequences the RNA from the bacteria and finds that the RFP mRNA contains no mutations. If he purifies the plasmid and transforms fresh E. coli from his lab the colonies are now red. He then suspects that the E. coli strain he received from Transphers’ lab has something wrong within the translation machinery. Indeed if he adds paramomycin to his E.coli strain he observes that the colonies go from being red to purple. Describe more than one model for what is wrong with the translation machinery in the Transpher lab bacteria.
Q4. You’ve constructed a yeast strain that expresses an intron-less GFP reporter. You recently identified an isolate that shows substantially reduced GFP protein expression. Because the strain otherwise behaves normally, you suspect that there was a mutation acquired in the GFP gene itself. After sequencing the entire gene (from promoter through transcription terminator), you identify a single point mutation located within the 5’ untranslated region.

The sequences of the 3’ ends of the wild-type and mutant 5’ UTRs (through the start codon) are shown below:

<table>
<thead>
<tr>
<th></th>
<th>5’-...AATTTTCAGCatg</th>
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<tbody>
<tr>
<td>WT</td>
<td>5’-...AATTTTCAGCatg</td>
</tr>
<tr>
<td>Mutant</td>
<td>5’-...AATTTTCATGCatg</td>
</tr>
</tbody>
</table>

The mutant mRNA is expressed at the same level as the wild-type mRNA, which leads you to wonder how the mutation affects translation. Propose more than one possible mechanism by which the mutation might inhibit translation.