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Bioc471a/571a Homework 7 - Due at the start of class October 23

All 13 of the questions listed below refer to material presented in Lecture 17 "Gene Expression Lab Practicum." Choose any five of the questions and answer them on a separate page referring to them by number (2 pts each).

1. What is the difference between the yeast Two-hybrid screen and the yeast One-hybrid screen with regard to the research objective (i.e., what do you need as materials for each strategy and what is the outcome)?

   The yeast two hybrid screen is designed to identify relevant protein-protein interactions using a bait protein to fish out target proteins. A postive signal is identified by in vivo reconstitution of an active transcription complex. The one hybrid screen is designed to fish out DNA binding proteins that recognize a DNA sequence cloned upstream of yeast reporter genes.

2. What could the scientist do if the pancreatic library she was screening did not contain any clones corresponding to the 5' end of the ORF (the library was made with oligo dT)? Describe two approaches and the advantages/disadvantages of each.

   One approach would be to make a new library using gene specific promers. A second approach would be to use 5' RACE to pull out the missing sequences (which could then be used as a new probe to rescreen the library).

3. Based on the observation that IBx4 reporter gene worked almost as well as the insulin reporter gene in this assay, what does this suggest about the mechanism of Igs in insulin gene regulation? Which experiment with PanC cells supports this conclusion?

   Dumb question (too confusing).

4. One interpretation of the terminal deletion experiments is that protein domains required for Igs function (SLH interactions) are encoded by the deleted amino acids. What is an alternative explanation that might explain the loss of Igs activity?

   An alternative explanation is that the protein is misfolded and therefore it is misleading because the "real" interaction domains are present in the deletion but are non-functional.

5. Why is it necessary to engineer in the ATG and TAG codons into the coding sequence of the N-terminal and C-terminal deletion mutants, respectively? How would you do this?

   It is necessary to engineer in both start and stop codons when creating terminal deletions, this is best done with PCR primers.

6. What explanation for the "super-shifted" band in the EMSA assay in the presence of Dib-TC protein extract best fits the researcher's hypothesis? What is an alternative explanation and how would you distinguish between the two?

   The simplest explanation is that SLH proteins present in the Dib-TC extacts interact with Igs protein-DNA complexes resulting in the super shifted band. An alternative explantation is that inclusion of a crude protein extract introducesnon-specific DNA binding proteins. This could be tested by using anti-SLH antibodies to determine if they are in the shifted band, or use the antibodies to deplete the Dib-TC extracts of SLH protein and see if the band disappears.

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7. What do the data from the Gain of Function experiments indicate with regard to the relative "strength" of the two putative activation domains? Assuming the researcher's model is correct, what is the most likely biochemical explanation for this difference?

   Dumb question (too confusing).

8. Do the gain of function experiments prove that Igs proteins interact with SLH proteins? Explain. How is it thought that "activation domains" stimulate transcriptional initiation rates?

   Dumb question (too advanced).

9. What is the purpose of adding the Flag epitope sequences to the N-terminus of the Igs coding sequence? Why not just use anti-Igs antibodies for these co-immunoprecipitation studies?

   The purpose of adding the Flag epitope is to permit specific identification and precipitation of the recombinant Igs protein deletants. These tag sequences are a better approach than Igs antibodies because all proteins are the same independent of deltions and the Igs antibody might not interact with, or interfere with, the Igs-SLH protein complex.

10. How would you construct the internal Igs deletants IgsD31-99 and IgsD31-204 used in the co-immunoprecipitation experiments? How would you confirm unambiguously that the deletions you created did not inadvertently cause a "frame shift mutation?"

    Dumb question (too confusing).

11. What are psi- packaging cells and why are they required for retroviral expression systems? Do the infected DiB-TC cells produce recombinant virus? Explain.

    Dumb question (too easy).

12. What is the source of the two bands in the Ponceau-stained nitrocellulose filter and why are these the only protein bands observed?

    Dumb question (too technical).

13. What do the results of the Igs 658 and Igs 31-204 co-immunoprecipitation results suggest about the role of activation region 2 in SLH binding?

    The intensity of the bands suggest that activation region 1 is the major functional domain. It is likely that the contribution of activation region 2 residues to the overall affinity of the Igs-SLH interaction is minimal.