

Papers Discussion April 9, 2008

Figures will be assigned to groups that will lead the discussion for that particular figure at the beginning a class. This includes understanding the goals, reagents, procedures and conclusions reached in the figure. In addition, below are some questions that must also be covered.

For each figure, the following protocol of discussion should be followed.

- 1) Goal(s) of the experiment. What are the questions that are being addressed?
- 2) Set-up of the experiment. Why were the reagents used? Describe the technique(s) employed.
- 3) Go through the actual data.
- 4) Author's conclusions.
- 5) Personal interpretation and opinions.

PAPER #1

"Snapshots of DsbA in action: detection of proteins in the process of oxidative folding." *Science* (2004) 303:534-537.

Figure 1

Describe the screen that discovered the Val¹⁵⁰ and Pro¹⁵¹ mutations. What is AMS? And why is it helpful here?

Figure 2

What do the solid arrowheads correspond to in lane 4?
What is the evidence that supports this claim?
Describe a pulse-chase procedure.

Figure 3

Describe the 2D electrophoresis procedure (note the gel was run differently than the method discussed in class).
Why does each spot between the open arrowheads have a corresponding DsbA spot?

Figure 4

Describe the constructs and disulfide content of the substrates.

“Co-translational folding of an alphavirus capsid protein in the cytosol of living cells.”

Nature Cell Biology by Nicola, Chen & Helenius

Overall:

Why use a viral protein as a model substrate for studying protein maturation?

Figure 1:

Why are the lysates in b so clean?

Why do you see C protein after 0.25 min of translation?

Why use recombinant vaccinia virus for Δ WT expression?

Figure 2:

How did they calculate that cleavage occurs 10 sec after cleavage site exits the ribosome?

Contrast cycloheximide and puromycin's mechanism of protein synthesis inhibition.

Figure 3:

What is the significance of ATP depletion?

Why is only p97 interacting with hsp70 and not p62 and E1?

Figure 4:

What is a bacterial spheroplast?

What is the translation rate in all panels? How do these values compared to other estimates for bacterial translation rates?