

DISCUSSION SESSION ON LECTURE #6: STUDY QUESTIONS

ASSIGNED PAPER: Ballon DR, Flanary PL, Gladue DP, Konopka JB, Dohlman HG, Thorner J (2006) DEP-domain-mediated regulation of GPCR signaling responses. Cell 126: 1079-1093.

(1) What are DEP domains and in what kinds of proteins are they found?

(2) RGS (Regulator of G-Protein Signaling) proteins are negative regulators of GPCR-initiated signaling. At what level (i.e., by what mechanism) do RGS proteins act to down-regulate signaling evoked by the activation of a heterotrimeric G protein?

(3) A GAP ("GTPase-activating protein") promotes the hydrolysis by water of the GTP bound to a GTP-binding protein. What is necessary, chemically, to orient a water molecule and enhance its ability to attack a phosphate group? What is the most likely transition state for this reaction?

(3) What biological process does the yeast GPCR Ste2 trigger? How was endocytosis of this receptor prevented to enhance the amount of the receptor that remains at the plasma membrane? Why was this strategy valuable?

(4) What hypothesis can you envision to explain why each of the two DEP domains in the yeast RGS protein Sst2 are necessary for its function?

Quantitative Question: Tyrosine is one of the twenty naturally-occurring amino acids. Based on your knowledge of the approximate pK_a values of the ionizable groups in (free) tyrosine, what is (a) the approximate pI value (isoelectric point) for this amino acid? Protein-tyrosine kinases are enzymes that are able to phosphorylate the side chain of tyrosine (when it is a surface-exposed residue in a polypeptide chain). Based on the fact that the approximate pK_a 's of the two ionizable groups present on an esterified phosphate (see box below) are $pK_{a1} = 1$ and $pK_{a2} = 7$, what is (b) the approximate pI value for (free) phospho-tyrosine?

