

DISCUSSION SESSION ON LECTURE #7: STUDY QUESTIONS

ASSIGNED PAPER: Villa F, Goebel J, Rafiqi FH, Deak M, Thastrup J, Alessi DR, van Aalten DM (2007) Structural insights into the recognition of substrates and activators by the OSR1 kinase. *EMBO Rep.* 8: 839-845.

1. Mutations in the genes that encode two related protein kinases, Wnk1 or Wnk4, are the apparent cause of a heritable human malady, Type II pseudohypoaldosteronism (PHA II, also known as Gordon syndrome), wherein ion imbalances lead to a chronic elevation of blood pressure. "Wnk" stands for "With No Lys"; which Lys are they referring to?
2. What biochemical and genetic evidence supports the conclusion that OSR1 (also known, more correctly, as OXSR1) and SPAK are downstream targets (i.e., phosphorylated and activated by Wnk1 and Wnk4)?
3. How are the actions of OSR1 and SPAK thought to affect the ion transporters and ion channels that are critically important in human electrolyte balance?
4. What is the CCT domain in OSR1 and SPAK and to what sequence motif does it bind with high affinity? In what proteins is that motif known to be found? How frequently do you think such a sequence is found on solvent-exposed regions of human proteins in epithelial cells?
5. What does this study reveal about where the peptide-binding site is located in the CCT domain of OSR1 and SPAK and about the nature of the peptide-CCT domain interaction? How this interaction might be modulated by phosphorylation is somewhat speculative; what further experiments would you do to confirm this mode of regulation?

Quantative Question: Given the decay rate / half-life of the radionuclide, ^{32}P , how many mCi will be present in a vial of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ that is shipped to you and is supposed to contain 10 mCi total as of today (Tues., 4 Dec. 2007), if you wait and don't open it, and instead use it only after final exams are over and you get back to the lab after the holidays and the BMB Asilomar Retreat, specifically on 15 Jan. 2008?