

DISCUSSION SESSION ON LECTURE #9: STUDY QUESTIONS

ASSIGNED PAPER: Dentin R, Liu Y, Koo SH, Hedrick S, Vargas T, Heredia J, Yates J 3rd, Montminy M (2007) Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature 449: 366-369.

- (1) How do the alternative co-activators— CBP and TORC2 —that associate with DNA-bound CREB differ in the mechanism by which they regulate CREB-dependent gene expression?
- (2) What is the chemical basis behind each of the different assays used to monitor the CREB-dependent expression of genes and/or their products?
- (3) What factors (signals) control the association between TORC and DNA-bound CREB? What is SIK2? What is LKB1?
- (4) This work seeks to elucidate the molecular basis for the control of CREB function by insulin. What are their conclusions?

Quantitative Problem: You enter into a research agreement with a pharmaceutical company to test the efficacy and specificity of a new protein kinase inhibitor, (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-ol (see box), that they have developed that is purportedly highly selective for blocking the catalytic activity of one of the three transmembrane receptors (VEGFR2) for the vascular endothelial growth factor (VEGF), a 412-residue angiogenesis inducer. This compound is a potential chemotherapeutic agent against certain cancers because solid tumors must become highly vascularized to grow. The company sends you a solution (which turns out to have an  $A_{260\text{nm}}$  of 0.3 when you dilute a small aliquot of it 100-fold), but forgets to tell you the concentration. Based on reasonable model compounds, (a) guess-timate the molar extinction coefficient of this inhibitor at 260 nm and pH 7; and, (b) using your estimate, the Beer-Lambert Law, and a cuvette with a 1-cm path length, calculate the approximate concentration of the inhibitor solution you were sent.

