

DISCUSSION SESSION ON LECTURE #8: STUDY QUESTIONS

ASSIGNED PAPER: Yang J, Roe SM, Prickett TD, Brautigan DL, Barford D (2007) The structure of Tap42/alpha4 reveals a tetratricopeptide repeat-like fold and provides insights into PP2A regulation. *Biochemistry* 46: 8807-8815.

- (1) This paper addresses assembly and regulation of the so-called type 2A phosphothreonine-, phosphoserine-specific phosphoprotein phosphatase (PP2A). This enzyme is typically considered to comprise a heterotrimer. What functions are the three classes of subunits (A, B, and C) in this enzyme thought to perform?
- (2) In humans, the A subunits of PP2A are encoded by two genes (α and β), the B subunits are expressed from at least four gene families (four B isoforms, five B' isotypes, three B'' isoforms, and two B''' isotypes), and the C subunits are encoded by two genes (α and β). Excluding splice variants of each gene product, hypothetically, how many potential forms of heterotrimeric PP2A are there?
- (3) The C subunit of PP2A is not restricted to association with its classical A and B subunits. What connection does the PP2Ac subunit have to the *S. cerevisiae* Tap42 protein and its mammalian homolog (called "alpha-4")?
- (4) What is a tetratricopeptide repeat (TPR) and in what classes of other protein molecules that we have already encountered in this course are they found and what function(s) do they serve?

Quantitative Problem: You measure the activity of a certain phosphoprotein phosphatase in a crude extract of hepatocytes. You take an amount of the extract that contains 100 units of the phosphatase activity and subject it to ion exchange chromatography, collecting twenty 1-ml fractions of the eluate. You assay all the fractions for activity and find that Fr. #3 has 1.5 units of activity, and no other fraction has detectable activity (and extensive washing does not remove any more activity from the column). You start mixing different fractions together and re-assay, and you find that when Fr. 8 and Fr. 12 are mixed together, it yields a solution with 250 units of activity. Provide the most parsimonious explanation for the above observations by answering the following questions: (a) What is in Fr. #3? (b) What is in Fr. 8 and Fr. 12? (c) How can the total activity in the 8-12 mixture be greater than the number of units you started with? (d) What would happen if the 8-12 mixture were passed over the same kind of column again? Would there be activity in any of the fractions? (e) What would happen if the 8-12 mixture were passed over a size exclusion (gel filtration) column instead? Would there be activity in any of the fractions? (f) Suppose you mixed all 20 of the fractions from the original ion exchange column together, would you expect to get 100 units of phosphatase activity, or 250 units, or something different? Why?