GENOME553 Winter 2009

Paper for Tuesday 20 January 2009
Mody, A, Weiner, J and Ramanathan, S. 2009 *(in press Nature Cell Biology)* Modularity of MAP kinases allows deformation of their signaling pathways.

Questions for Thought

As you read this paper, **write down questions** you have about the logic or rationale for each experiment, the method employed, and the conclusions drawn. Come up with at least three questions. Turn in your questions as homework at the beginning of class. During class we will discuss your questions along with the QfT below. We will emphasize the questions in bold; the other questions are meant to help you think about each issue.

Thus far, we discussed how genetic analysis is used to identify and order the components of a particular pathway. Biological systems tend to use molecules and pathway elements over and over again. This paper addresses how pathways may diversify to accommodate different inputs and generate multiple outputs.

1) Before reading this paper: **What biological mechanisms may allow the evolution of two functionally diverse pathways from a common ancestral pathway?** How can this be achieved without losing fitness on the way? **How do biological systems achieve output specificity for two pathways with different inputs but shared signaling components?** How do biological systems insulate closely related pathways?

2) **What are the major scientific questions the authors aim to address?** How do they relate to question 1)? How did the goal of these experiments affect their choice of genes? Why are these pathways an ideal model for studying these questions (think input/output)? Why choose yeast to study this problem?

3) How do the authors support their conclusion that the MAPK are highly conserved yet allow quick pathway rewiring and acquisition of new components?

4) **Creating chimeras is common method to analyze the structure-function relationship for a given protein. Why?** If successful, what aspects of protein function can this method elucidate and differentiate? What is a prerequisite for this method? What is a common problem with creating chimeras?

5) **Why is this study able to circumvent this problem?** How does their phylogenetic analysis help? How does their analysis of conserved residues and their location in the protein structure help? Do you think that their strictly quantitative analysis of these features was important for their success?

6) What is their prediction for how specificity of MAPK is achieved and how does this differ from conventional wisdom?

7) Methods: **The authors test their strains containing the chimeras with several assays. Why? What distinguishes the assays from each other?** What is the point of using both high-and low copy plasmids? How could expression of a gene from a plasmid differ from expression of a copy that is integrated in the genome? What essential experimental controls that the authors using to assure that their assays will work correctly?

8) **Why do you think expression levels matter for their results?** What conclusions do you draw from the constitutive activity of some constructs? The lack of activity?

9) **Why is it important for their scientific goal to test if cross-wiring is due to direct or indirect mechanisms?** How do they distinguish between these two mechanisms and what do their results indicate?

10) How could high-level expression interfere with activation of pSTL1? Why can some hybrids rescue cell growth on high osmolar medium, but do not mediate reporter activity? How would you test your predictions?

11) Evolutionary dogma holds that change is incremental, one small change with little phenotypic effect at the time. **How do their findings/conclusions fit/not fit this assumption? How does the observed modularity alleviate the problem of fitness loss for diversifying pathways?** How do their findings with regard to specificity fit their predictions? Why is this surprising? Why also expected (for some evolutionary biologists)?

12) The authors note that further analysis of their hybrids will complement traditional biochemical approaches. How? **How will their continued analysis aid the further investigation of these networks?** What do their findings imply for synthetic protein design?