Seminar 5

Transport

1. The actual Gibb's energy difference ΔG of a reaction or process $A \rightarrow B$ depends on the concentrations of A and B as $\Delta G = \Delta G^\circ + R\,T\,\ln([B]/[A])$. What is the Gibb's energy difference of transport of a nutrient against a 1000-fold concentration gradient? Can such transport be driven by ATP hydrolysis? What is the maximum gradient that could be maintained by a 100% efficient ATP-driven pump? What do you think is the maximum gradient in practise? Can you think of cases where gradients of this magnitude are needed?

2. The Na/K ATPase maintains inside-outside ratios of approx $[Na]_{in} / [Na]_{out} = 0.1$ and $[K]_{in} / [K]_{out} = 40$. For each ATP hydrolyzed, the ATPase pumps 3 Na^+ out and simultaneously 2 K^+ in, against their gradients. What is the ΔG for this process? What is the ΔG for transport of Na+ ions back into the cell? What gradient can be achieved by a Na^+-coupled nutrient transporter? Why would cells choose Na-coupled transport of nutrients rather than ATP-driven?

3. The insulin-dependent glucose transporter GLUT4 is passive and has K_M around 5 mM for extracellular glucose. What does a Michaelis-Menten constant mean for a passive transporter? Does the value make sense physiologically?

4. The transport of glucose by GLUT4 followed by phosphorylation by hexokinase (ΔG° = -17 kJ / mol) is considered to "trap" glucose in cells. If this system was allowed to go to equilibrium, what would the ratio $[\text{glucose-6P}]_{in} / [\text{glucose}]_{in}$ be? What about $[\text{glucose}]_{in} / [\text{glucose}]_{out}$? What situation do we expect in practice?

Compartmentalization and isoforms

9: There are three known forms of aldolase (A,B,C) in the human genome. In which tissues are they expressed? Why might it be useful for an organism to have multiple genes or splice variants encoding isoforms of enzymes (isozymes) with the same enzymatic activity?

8. There are two proteins in the human genome that produce carbamoyl phosphate. What are they? In what pathways to they occur? In what tissues and subcellular compartments are they expressed? Do these proteins have other functions?

9. The enzymes TYMS, SHMT1 and DHFR have been shown to form a complex in the nucleus during DNA replication (Andersson et al, *JBC* 287:7051-7062, 2012). What would be the benefit of colocalization of these three proteins?

Genomics: enzyme expression in activated T-cells

In this exercise we will look at some microarray gene expression data from Lund et al (*J Immunology* 171:5328–5336, 2003). The data derives from an experiment where human CD4+ T cells were stimulated to proliferate by antibodies against the T cell receptor. We will try to get some insights into the metabolic behavior of these cells based on the expression data.

1. Can you think of some metabolic pathways that should be induced / repressed in this experiment?

Gene level data

Relative mRNA abundance were measured by microarray at time 0 (unstimulated), and at 2h, 6h and 48h after stimulation. A total of 12,813 genes were measured, of which 1,365 were metabolic enzymes. Of these, 164 were reproducibly induced or repressed at least 4-fold over time 0, with a z-score > 3.

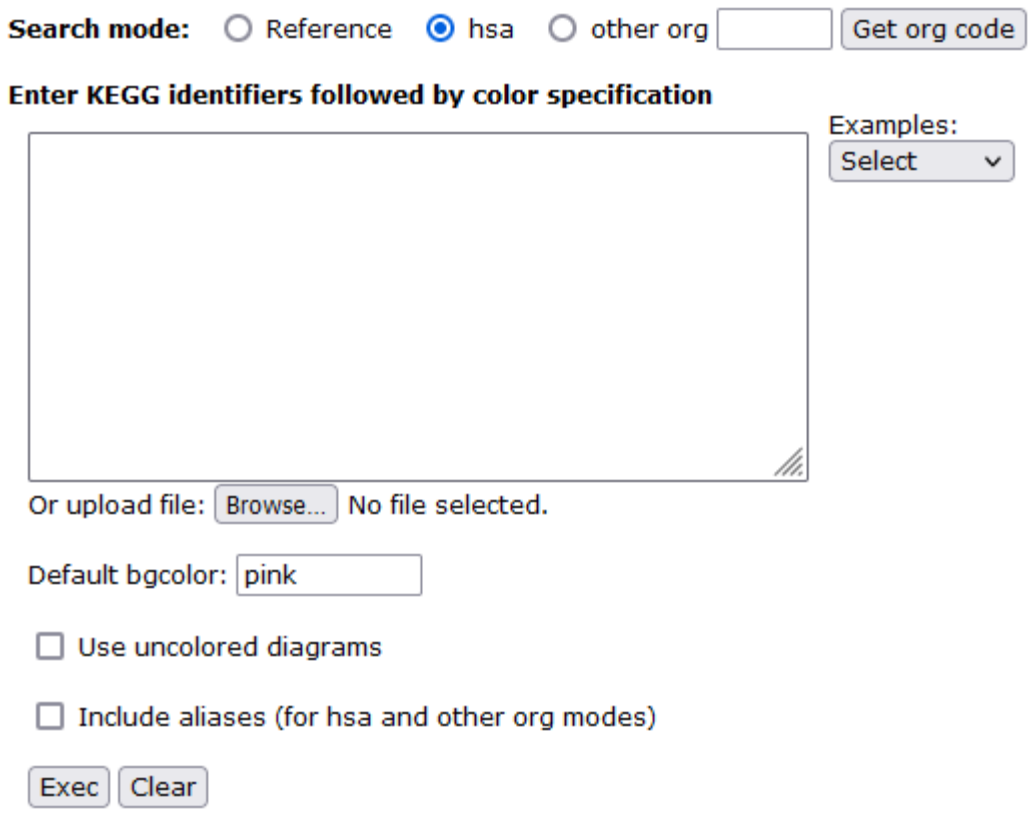
Download the gene expression data excel file from the course homepage. The “Expression data” sheer shows Entrez (NCBI) Gene ID, symbol and description for each gene in columns A—C, and fold changes over the 0h time point in columns D—M. The fold change data is color coded red for induced, blue for repressed, with a color gradient where 10-fold (or more) is most intense. The rows have been arranged by a clustering algorithm so that mRNAs with similar profiles are adjacent.

2. Take some time to look through the excel data. Do you recognize any metabolic enzymes? What pathways do they belong to? What is their expression pattern?

Pathway mapping using KEGG

To help interpret the data, we will use a tool available in the KEGG database to organize the results by metabolic pathways. The “KEGG input” worksheet provides a color code for each gene in column G, based on the log fold change at 48h (column B). Navigate to https://www.genome.jp/kegg/mapper/color.html (there is a link to on the course homepage). You should see the interface shown below. Next,

- Set “Search mode” to hsa (using homo sapiens gene IDs)
- Copy & paste the color codes from column G gene list into the text box (don’t include the header row).
- Click “Exec” to run the analysis



This will match the genes entered against the KEGG pathways, resulting in a listing of the KEGG pathways with the number of matched genes indicated in parentheses. There are a few gene IDs that don't map to the KEGG database, but we'll ignore this for now. Click on the number of genes to see the list of genes matching that pathway, and click the pathway name to see the pathway chart with enzymes highlighted (red for induced, blue for suppressed).

3. What pathways can you find that appear to be induced or repressed? Are they anabolic, catabolic? Note that one KEGG "pathway" is often a complex chart with many possible routes, and only some will match our gene expression data.

4. In some cases, you will see more highlighted reactions than there are genes matching the pathway. What's going on?

5. In many cases, pathways may look ambiguous. Discuss the limitations of this analysis. To what extent can we predict pathway activity from gene expression data alone? In this data, which cases appears clear / convincing, and which do not?

6. Exploratory data analysis *generates* rather than tests hypotheses. Choose a metabolic pathway that you think looks induced / repressed in this data. Can you suggest a follow-up experiment to test this hypothesis?