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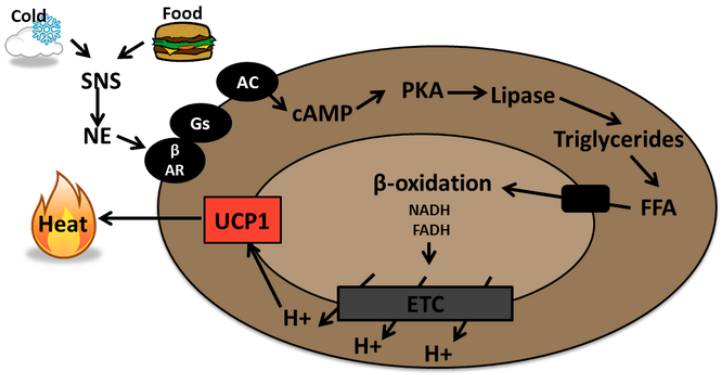
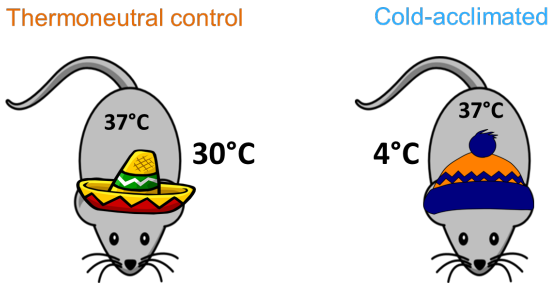
RNA sequencing of brown adipose tissue to assess lipolytic pathways and adaptive thermogenesis

Introduction:

Adipose tissue is an important organ that helps maintain the energy status of the whole animal. For example, when adipose tissues have adequate lipid stores, adipose tissues release a protein called leptin that travels to the brain. In the hypothalamus, this protein causes a decrease in eating behaviour. Alternatively, in times of cold stress, a specialized adipose tissue called brown adipose tissue (BAT) burns its triglyceride stores to produce heat. The pathway for this process can be seen below.



Brown adipose tissue takes advantage of the heat-producing protein UCP1 in order to perform this function. As mentioned above, lipids are the fuel for thermogenesis- specifically, we can see in the above image that Triglycerides are broken down into free fatty acids (FFA in the image above). This process is called lipolysis and essentially, it fuels adaptive thermogenesis in the brown adipose tissue. In order to understand genetic changes in the lipolysis pathway, we performed the following study.



Methods:

Mice were housed in cages at a temperature of 30°C (thermoneutral control mice) or 4°C (cold-acclimated mice). The thermoneutral treatment ensures that mice do not have any need to perform thermogenesis to maintain their body temperate of 37°C. Conversely, cold-acclimated mice will have maximally-activated heat production pathways in their brown adipose tissue. Since lipolysis provides the fuel for adaptive thermogenesis, the lipolytic pathway is required and genes associated with that pathway should be enhanced in the cold-acclimated group as compared to the thermoneutral control mice. To test this, we have

performed an RNA sequencing experiment to measure the expression level of the genes as mRNA abundance in the cells is measured as a proxy for gene expression. Recall that one method of increasing the number of proteins with a specific function in a cell is to increase the transcription rate, which is the rate at which mRNA are produced from the gene. Refer to the Central Dogma of Molecular Biology below. As the need for gene products (proteins) increases, the transcriptional rate increases, creating more templates for protein synthesis. By having more mRNA templates available, there will be a higher number of them being translated into protein at any one time. Thus we can develop the following hypothesis.

Hypothesis: there will be a greater number of lipolysis-related mRNA in our brown adipose tissue from the cold-acclimated mice compared to thermoneutral mice as the adipose tissue attempts to create fuel for adaptive thermogenesis.

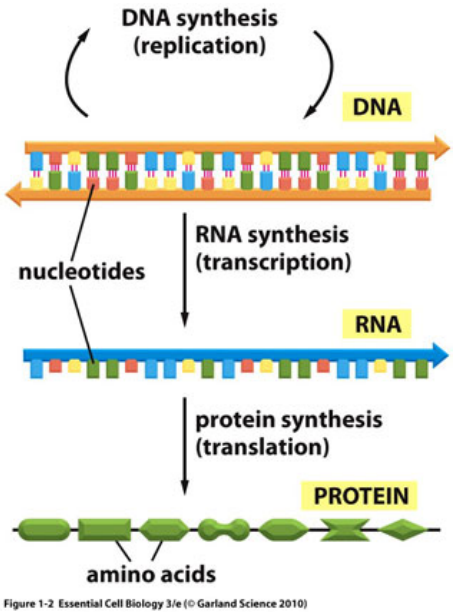


Figure 1-2 Essential Cell Biology 3/e (© Garland Science 2010)

Progress:

We currently have the RNA-Seq data and are interested in assessing known pathways. These pathways are composed of sets of genes known to play functional roles in those pathways. For example, beta-adrenergic receptors (“β-AR”) and hormone sensitive lipase (“Lipase”) are both genes expected to increase in expression with increased lipolysis and their gene products can be seen on the first diagram on page 1. Since we intend to query multiple such pathways, it would be useful to have a tool that allows us to output gene lists depending on the pathway we are interested in. We could then extract the relevant expression results from the RNA-Sequencing data and create a heatmap to show increases or decreases of expression in genes associated with those lists.

Thus the first part of this project will be automating the above process. My colleagues have suggested using a Python package called Bioservices, link:

https://bioservices.readthedocs.io/en/master/

This tool creates lists of genes and outputs them depending on the animal (*Mus musculus* for our situation) and the pathway of interest. One such database that this tool can connect to is called KEGG Pathway, link:

https://www.genome.jp/kegg/pathway.html

Specifically, here's the page that shows the lipolytic pathway of mouse adipose tissue:

https://www.genome.jp/dbget-bin/www_bget?pathway:mmu04923

I would like to output a list of the genes shown in the pathway on the link above, and though KEGG does not have a gene list output service, Bioservices is supposed to do just that. Since we have no expertise with python code, we are hoping that someone can assess the utility of this package and potentially perform some tweaking to adapt it to our needs. Any information on alternative or more adaptable methods would also be useful. I am happy to meet outside of lectures to offer more specific instructions and help answer logistical questions.