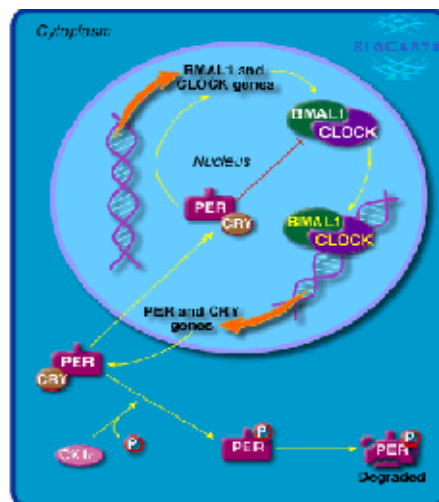


Homework 2

Friday 9/30-/05

Biological background:

Organisms from flies to humans have daily circadian rhythms entrained with the 24-hour cycle of day and night that regulate many physiological systems. In mammals, there appears to be a master regulator of circadian rhythms in the hypothalamus, as well as additional peripheral mechanisms. The basic framework of the molecular pathways in the cell that produce and maintain these daily rhythms has been elucidated. The central feature of the circadian pathways are two transcription factors, Bmal1 and Clock, that are expressed in a 24 hour cycle and that act together as a heterodimer to regulate the expression of other genes involved in maintaining the circadian rhythm. Bmal1 and Clock activate transcription of multiple Period (per) and Cryptochrome (Cry) genes. When the activity of Bmal1 and Clock is maximal, Per and Cry genes are transcribed, and then translated in the cytoplasm. The translated Per and Cry genes form a complex in the cytoplasm that is transported back into the nucleus. In the nucleus, Per and Cry form a feedback loop that inhibits transcriptional activation by Bmal1 and Clock, lowering their own expression in the process. At the same time, Per and Cry increase transcription of Bmal1 and Clock genes, to initiate the next phase of the cycle once again. This cycle runs autonomously in the cell, causing jet lag during travel, but can be regulated to retrain to changes in the light cycle. Factors that regulate the cycle include casein kinase 1 epsilon (CK1e) that phosphorylates Per2 and induces its degradation. Mutation of PER2 in humans has been genetically linked to changes in human sleep patterns. Further exploration of the factors that regulate circadian rhythms may enable manipulation of the system in sleep disorders.



Tools:

1. Gene/Protein sequence search:
NCBI Entrez: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein>
2. Pairwise alignment:
<http://www.ebi.ac.uk/emboss/align/index.html> (Nucleotide and protein; local and global)
3. Multiple alignment:
ClustalW: <http://www.ebi.ac.uk/clustalw/index.html>

Questions:

1. Retrieve the protein and gene genebank record of Clock (circadian locomoter output cycles kaput) in mouse (*Mus musculus*). Go to NCBI Entrez. Search “Protein” for “NP_031741” (protein id: 7106459). Search “Gene” for “Clock” (gene id: 12753).

Which chromosome is this gene located on? Which chain does the coding region of Clock locate? How many amino acids does Clock have?
Cut and copy the protein and cDNA sequence of Clock, using the FASTA format. (How do you find the cDNA sequence for Clock?)

2. Retrieve the protein and gene genebank record for CLOCK in human (*Homo sapiens*) (protein id: 4758010), a homolog of Clock in mouse.
3. Use the EBI pairwise alignment tool to do a global and a local alignment of the nucleotide sequences of Clock and CLOCK with default setting for all variables. What is different between the two outputs? Please explain th difference
4. Use the EBI tool to do a global and a local alignment of the protein sequences of Clock and CLOCK. What does the result tells you?
5. Retrieve the protein sequence of clk in fly (*Drosophila melanogaster*) (protein id: 62472090). Use the EBI alignment tool to do a global and a local alignment of the protein sequences of clk and Clock (mouse). What can you learn from the result?
6. Now let’s play with the different parameters used in Dynamic Programming for pairwise alignment. Do a local alignment of protein sequences of clk (fly) and CLOCK (human), using the Blosum62 scoring matrix with the gap opening penalty as 1 and extension penalty as 1. Save your result. Then do the local alignment again with gap opening penalty set to be 10. Which results make more biological sense? Why?
7. To get a taste of multiple sequence alignment. Retrieve protein sequences of the following proteins with ID: 62472090 (clk); 7106459(Clock) and 4758010 (CLOCK). Use ClustalW to align clk, clock and CLOCK with default settings. Do you see anything that is not captured by the pairwise alignments?