Solution key - 7.012 Recitation 12 - 2010

Questions:

1. *Salmonella* is a bacterial genus that is highly related to *Escherichia*, the genus of *E. coli*. You study a *Salmonella* protein that is 100% identical to an *E. coli* protein.

i. At the nucleotide level, the *Salmonella* gene and the *E. coli* gene are only 87% identical. Explain how this is possible.

There are multiple codons for the same amino acids. This difference may result in difference in gene sequence although the protein produced may still be the same.

ii. Less than 5% of our genome is made up of genes. What is the other 95% made of?

Most of it is regulatory elements i.e. enhancers, promoters or introns that are ultimately spliced out.

iii. If you had the entire sequence of a genome of a new bacterium, how would you predict where the genes were in the genome?

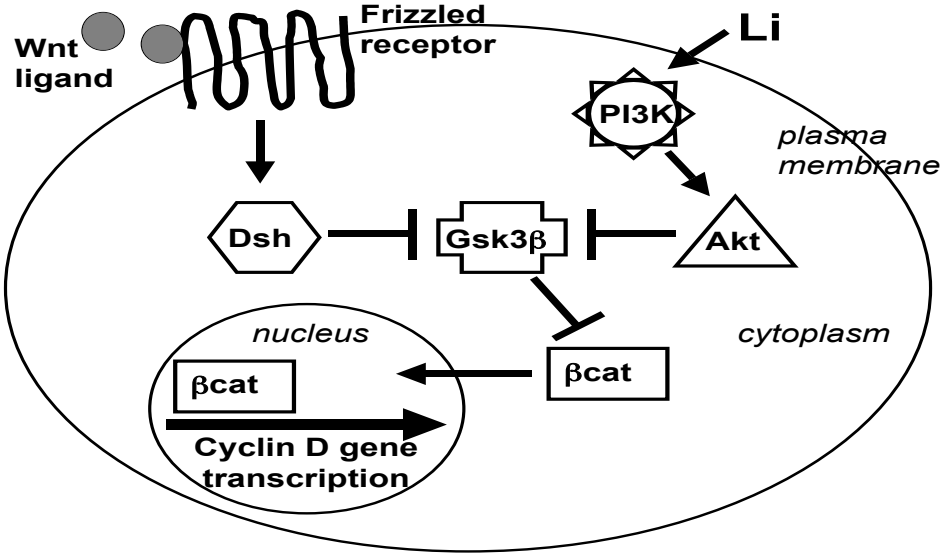
You could compare it by hunting for the open reading frames.

iv. Why could you not use the same strategy if you had the sequence of a genome of a eukaryote?

The eukaryotic genes, unlike the prokaryotic genes, contain exons and introns (which are ultimately spliced out or alternatively spliced out) making such a prediction difficult.

2. The *wnt* signaling pathway is one of the most important in biology. It is required for cell proliferation and its inhibition leads to programmed cell death. As diagrammed below.

- *Wnt ligands bind to frizzled receptors.*
- *These receptors bind to disheveled (Dsh) and activate its function.*
- *The Dsh inhibits GSK3β, a kinase.*
- *GSK3β phosphorylates the transcription factor β-catenin (βcat). The phosphorylated β- catenin is unstable and gets degraded.*
- *Thus wnt signaling inhibits GSK3β, promotes β-catenin stability and translocation to the nucleus.*
- *GSK3β can also be inhibited by addition of Lithium, acting through PI3 kinase/ Akt pathway.*
- *Cyclin D gene transcription is activated by β-catenin.*



Compare the expression of cyclin D protein levels in the pairs of cells (wild type and cells having a mutation / perturbation) described below and explain your reasoning. In each case **both cells of the pair are treated with wnt ligand.** *For your answers you should consider only those components that are*

shown in the schematic above or listed as bullet points in the explanation and explain only in the space provided.
State the changes that will be elicited by the following mutations.

a) Mutant cells having a Frizzled receptor that lacks its ligand binding domain.
This mutation will prevent the Frizzled receptor from binding to the wnt ligand. The Frizzled will not be able to activate Dsh which is required for the inactivation of GSK3β. Therefore GSK3β will remain constitutively active and will phosphorylate and promote degradation of β-catenin and prevent its nuclear translocation that is required for the transcription of cyclin D gene. So no cyclin D will be produced.

b) Mutant cells that express a constitutively active Dsh protein.
Dsh will always be active irrespective of the presence or absence of wnt ligand. The active Dsh will constitutively inactivate GSK3β. Hence the GSK3β will not be able to phosphorylate and degrade beta catenin, which will now translocate to nucleus to enhance the transcription of Cyclin D gene. So cyclin D will be constitutively produced.

c) Mutant cells in which β-catenin lacks its GSK3β phosphorylation site.
β-catenin will not be degraded by GSK3β irrespective of the presence or absence of wnt ligand. Hence it will always translocate to the nucleus to enhance the transcription of cyclin D gene. So cyclin D will be constitutively produced.

d) Wild type cells treated with Chiron 92060, a GSK3β inhibitor.
While wnt will still bind frizzled, it will be irrelevant. Chiron will inhibit GSK3β. Hence, the GSK3β will not be able to degrade β-catenin, which will now translocate to nucleus to enhance the transcription of Cyclin D gene. So cyclin D will be produced.