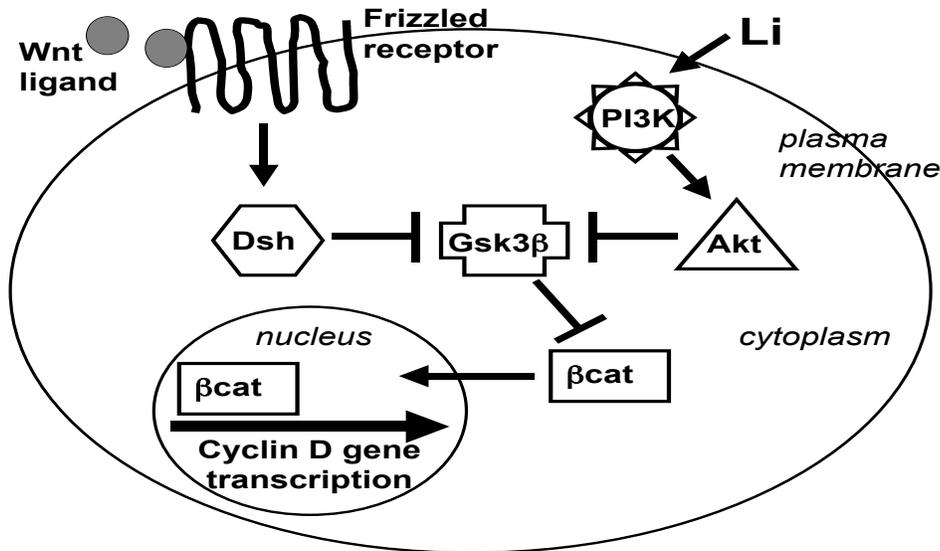


SOLUTION KEY- SECTION 12

1. 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 *GSK3 β* , a kinase.
- *GSK3 β* phosphorylates the transcription factor β -catenin (β cat). The phosphorylated β -catenin is unstable and gets degraded.
- Thus *wnt* signaling inhibits *GSK3 β* , promotes β -catenin stability and translocation to the nucleus.
- *GSK3 β* 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 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. You may ignore the effect of Lithium while answering the questions. **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 GSK3 β . Therefore GSK3 β 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 GSK3 β . Hence the GSK3 β 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.

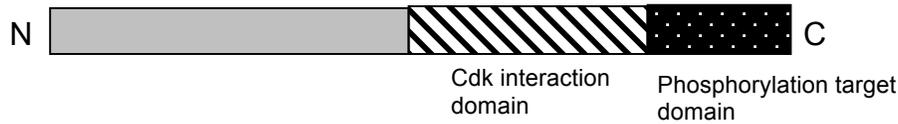
c) Mutant cells in which β -catenin lacks its GSK3 β phosphorylation site.

β -catenin will not be degraded by GSK3 β 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 GSK3 β inhibitor.

While *wnt* will still bind *frizzled*, it will be irrelevant. *Chiron* will inhibit $GSK3\beta$. Hence, the $GSK3\beta$ 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.

3. The cyclin D protein has the following structure.



a) What effect would the deletion of the Cdk-binding domain of Cyclin D have on cell proliferation? Explain.

The cyclin will not be able to bind to and activate the CDK. Hence the CDK will not be available to control the regulatory effect of proteins such as RB, p53 on cell cycle. Hence no cell division will be observed.

b) Using a temperature sensitive variant of Cyclin D, you find that the cells fail to divide at the non-permissive temperature. The arrested cells have a diploid DNA content ($2n$) in a single nucleus. At what cell cycle checkpoint (or stage of cell cycle) is cyclin D likely to act? Explain.

At G1/S phase since the DNA content is still $2n$, meaning that it has not undergone DNA duplication/replication ($4n$).

c) How would the Cdk protein levels vary during the normal cell cycle?

The level of CDK protein remains the same through all the phases of cell cycle. However it becomes functional only when it binds to and is activated by its specific Cyclin.

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