BB101: 2022 Autumn semester

Quiz #3: Each correct answer is worth 1 or 2 marks (check each one for how many marks). You will not be getting any partial marks so think and answer carefully. You must answer only within the space provided. Time for taking this quiz is 15 minutes.

You are now expert in biology and have decided to work on cholera, a gastro-intestinal disease that affects many of us in India. Cholera outbreaks currently account for 1.3 to 4.0 million cases and cause between 21 000 and 143 000 deaths worldwide. So, it is certainly a problem that is worth studying. Cholera is caused by the cholera toxin (CT), sent out of the cell by *Vibrio cholerae*. It is synthesized by the *cct* operon. You read that the regulators of the *cct* operon are ToxR and ToxS proteins, embedded in the cell membrane of *Vibrio cholerae*.

Qs 1. Tox R and ToxS are activators of the *cct* operon, with ToxR directly turning the operon on. In its role as an activator, which protein does ToxR activate and how might it activate this protein? (1 mark)

ToxR activates RNA polymerase. It can do so by increasing its binding to the promoter or enhancing initiation.

Qs 2. Do you think that eukaryotes could use the strategy of having a transcriptional activator on their cell membrane? Explain your answer briefly. (1 mark)

Eukaryotes have cells with a nucleus that contains the DNA. So, their transcriptional activators are found inside the nucleus. Due to the separation between the cell membrane and the nucleus, eukaryotic cells use signal transduction pathways to relay the signal from the cell surface to the nucleus. Eukaryotic cells cannot use the strategy of having a transcriptional activator on their cell membrane.

To treat cholera using drugs that target GPCRs, you do your research and synthesize the drugs given in the table below. You test the action of each drug on cell signalling pathways.

Drug name	Effect of drug on cAMP levels after GPCR activation
CholeraGo 1	increase
CholeraGo 2	decrease
CholeraGo 3	no change

Qs 3. Which drug will you use to treat cholera? Explain your answer briefly. (1 mark)

I will use CholeraGo2. We discussed during the tutorial that Cholera Toxin (CT) results in activation of cAMP and this results in effects on downstream proteins leading to loss of salts and water from the cells. CholeraGo2 will reverse this effect of CT.

Qs 4. You want to design a switch in *Lactobacillus* (normally found in probiotics such as yoghurt) that will sense the cholera toxin in the intestine and kill *Vibrio cholerae*. The idea is to have genetically modified *Lactobacillus* in our intestines that are natural killers of *Vibrio cholerae* when they detect these bacteria. Describe your switch, with diagrams, showing clearly how the switch works to kill *Vibrio*

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cholerae. Don't forget to mention the signalling molecule, the components of the operon, how the operon is switched on or off and the protein products of the operon. (2 marks)

The switch has three components: the signal, the operon and the proteins made by the operon.

The signal will be Cholera Toxin (CT), as this is a highly specific molecule of *Vibrio cholerae*.

The operon will be one of two types:

1) A repressor protein will bind to the operator of the operon when CT is not present and when CT is present, it will bind to the repressor which will fall off the operator, allowing transcription of the operon genes.

   OR

2) An activator protein will bind to the promoter, enhancing the activity of RNA polymerase only when CT is present.

The proteins made by the operon will be proteins that affect the quorum sensing/virulence genes of *Vibrio cholerae*. They could also kill *Vibrio cholera*e directly, but this killing property has to be specific for *Vibrio cholera*e only and not other bacteria.

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