



UBL201-L Introductory Biology III - Molecular Biology

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Is UvrB essential to repair UV-induced DNA damage in Escherichia coli?

Aim of the experiment:

- To check if UvrB is essential for the repair of UV-induced DNA damage in E.coli cells.
- To study the various kinds of mutations(damage) caused by different wavelengths of UV light.
- To study the various mechanisms by which DNA repair occurs post UV damage.

Principle:

The principle of this experiment is to study whether UvrB is indispensable for the dark repair (NER) of DNA damage induced by UV light. The UV light causes the formation of thymidine dimers in the DNA which do not fit into the double helix and hinder replication and gene expression. One of the ways by which these thymidine dimers are repaired is by the UvrABC system. The UvrABC system is a nucleotide excision repair system. The UvrA protein scans the DNA for the presence of thymine dimers. The UvrB protein binds to the UvrA protein and the UvrC protein binds to the UvrB protein. The UvrC protein then cleaves the DNA at the 8th phosphodiester bond from the 5' end and the 4th phosphodiester bond from the 3' end of the thymidine dimer. The UvrD protein (a helicase) then removes the damaged DNA strand and the DNA polymerase I fills the gap. The DNA ligase then seals the nick. The UvrB protein is essential for the repair of the thymine dimers. If the UvrB protein is not present, the thymidine dimers will not be repaired and the cell will die (if other repair mechanisms are also impaired). The UvrB protein is essential for the repair of UV-induced DNA damage in E.coli cells.

The mechanism of photoreactivation is hindered in our experiment by performing it in the dark.

Results and discussions:

We performed the experiment by making 10-fold serial dilutions up to 10^{-5} of both E.coli TG1 cells and E.coli TG1 Δ UvrB cells and plating $2\mu l$ of each dilution on LB agar plates. The plates were then exposed to UV light for 0s, 5s, 10s and 15s and 20s. The plates were then incubated at 37°C for 24 hours whilst ensuring darkness throughout the process such that photoreactivation is prevented. The results are shown in the table below:

Concentration of E.coli cells	Exposure: wild type					Exposure: knockout				
	0s	5s	10s	15s	20s	0s	5s	10s	15s	20s
10^{-1}	+	+	+	+	+	+	+	-	-	+
10^{-2}	+	+	+	+	+	+	-	-	-	-
10^{-3}	+	+	+	+	+	+	-	-	-	-
10^{-4}	+	+	+	+	-	+	-	-	-	-
10^{-5}	+	+	+	-	-	+	-	-	-	-

The '+' sign indicates that the E.coli cells survived and the '-' sign indicates that the E.coli cells died(or did not grow). We can consistently see that the E.coli TG1 cells survived for most exposure times and the E.coli TG1 Δ UvrB cells died for most exposure times. This shows that the UvrB protein is essential for the repair of UV-induced DNA damage in E.coli cells.

Images:

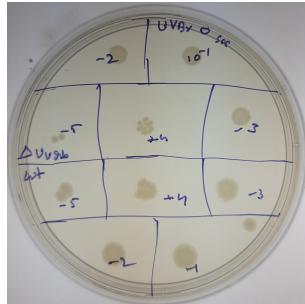


Figure 1: E.coli TG1 and TG1 Δ UvrB cells exposed to UV light for 0s

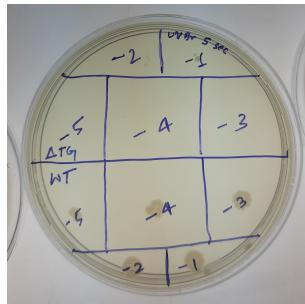


Figure 2: E.coli TG1 and TG1 Δ UvrB cells exposed to UV light for 5s

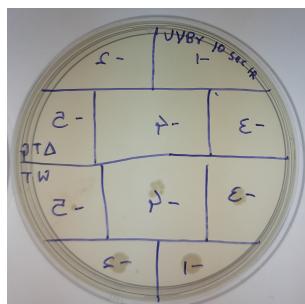


Figure 3: E.coli TG1 and TG1 Δ UvrB cells exposed to UV light for 10s

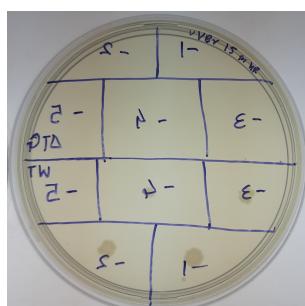


Figure 4: E.coli TG1 and TG1 Δ UvrB cells exposed to UV light for 15s

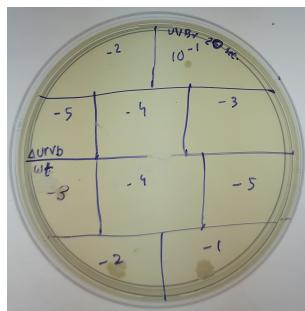


Figure 5: E.coli TG1 and TG1 Δ UvrB cells exposed to UV light for 20s

Sources of error:

There are certain dilutions in wild type cells which due not show growth. This is found only in very low dilutions, hence it can be attributed to lack of cells in the dilution due to which no growth was observed.

However, in the case of the knockout cells, there are certain dilutions which show growth. This has occurred in cells with high concentrations. This can be attributed to the fact that the cells were not exposed to UV light properly as they were shielded by other cells due to high cell number.

In case of 20s exposure, the cells were exposed to UV light for 20s but they were also exposed to the light for a few seconds while the plate was being removed from the hood. This could have caused the cells to repair the damage caused by the UV light and hence show growth. Again this has occurred in cells with high concentrations.

Answer the following questions:

Question 1:

How does nucleotide excision repair differ from photoreactivation?

Answer:

Nucleotide excision repair is a mechanism by which DNA damage is repaired by removing the damaged DNA strand and replacing it with a new strand. The NER mechanism is controlled by the UvrABC system. First, a UvrA-UvrB complex scans the DNA, with the UvrA subunit recognizing distortions in the helix, caused for example by pyrimidine dimers. When the complex recognizes such a distortion, the UvrA subunit leaves and an UvrC protein comes in and binds to the UvrB monomer and, hence, forms a new UvrBC dimer. DNA helicase II (or UvrD) then comes in and removes the excised segment by actively breaking the hydrogen bonds between the complementary bases. The resultant gap is then filled in using DNA polymerase I and DNA ligase.

Whereas, photoreactivation is a mechanism by which DNA damage is repaired by breaking the thymidine dimers using light of wavelength 380-500nm. It is controlled by DNA photolyases which recognize the “kink” in the DNA, and bind to the site. When excited by blue light (380-500 nm wavelength), the photolyase along with its co-factor FADH- traps the energy from photons and transfers it to pyrimidine dimer and eventually break apart the dimer.

Hence, the main difference between NER and photoreactivation is that NER is a dark repair mechanism whereas photoreactivation is a light repair mechanism where different enzymes are used to repair the damage caused by UV light.

Question 2:

Which DNA polymerase is used in nucleotide excision repair (Both in prokaryotes and mammalian cells)?

Answer:

In prokaryotes, DNA polymerase I (although κ or III can also substitute for it) is used in nucleotide excision repair whereas in eukaryotes, DNA polymerase δ , ϵ and/or κ are used in nucleotide excision repair.