Deoxyribose Nucleic Acid (DNA) is the code that dictates how organisms function living on Earth. DNA is composed of nucleic acids and different sequences code for different proteins. This is important because proteins, primarily enzymes, are the sole reason why life is allowed to exist. According to Dr. Richard Wolfenden, uncatalyzed reactions that create DNA and RNA would take 78 million years to form (University of North Carolina School of Medicine, 2008). Every cell on Earth came from another cell, which is a process done by DNA polymerase that occurs during the synthesis phase of the cell cycle. Once a new cell is created, new proteins are required for proper maintenance of bodily functions, known as homeostasis. To get to proteins from DNA, transcription, and translation must occur. Transcription is the process of using RNA polymerase to turn DNA into mRNA, and translation is the process of turning that mRNA into the amino acid chain, which is done inside a ribosome. (Brown T., and Brown T. Jr., no date). The number of rings determines whether the nitrogenous base is a purine with two rings or a pyrimidine with one ring. The purines, A and G, form weak hydrogen bonds with the corresponding pyrimidines, T and C, on the opposite strand of DNA (Albert 2020). The weakness means they can be easily split apart during the replication and transcription phases. These fundamentals of the central dogma, DNA to RNA to proteins, of life revolve around two base pairs: adenosine (A) to thymine (T) and guanine (G) to cytosine (C). However, a recent discovery has opened a new realm of science with the creation of two nucleotides.

These newly synthesized nucleotides were discovered by researchers at The Scripps Research Institute in an article, *A semi-synthetic organism with an expanded genetic alphabet*. The newly discovered nucleotides are called deoxy-NaM and deoxy-5SICS, which are shortened to X-Y respectively. These unnatural base pairs (UBPs) have major differences from the natural A, T, G, and C base pairs. By having a repulsive characteristic to water, the UBPs are opposite of

the natural nucleotides in that they are nonpolar (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). This characteristic causes water to push itself away from the nucleotides, which means the UBPs are hydrophobic. For all cells, natural nucleotides enter the cell by nucleotide transporter proteins, however, the synthesized nucleotides are unable to go through those channels at similar rates. Another difference between the new nucleotides and the old ones is the rate of degradation; the UBPs degrade much more rapidly. To fix this, oligonucleotides containing unnatural bases were put into a plasmid and replicated using polymerase chain reactions (PCR) (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). The paper discussed techniques used to determine if *E. coli* could successfully replicate a piece of DNA with UBPs.

In determining the effectiveness of UBPs, the researchers first examined if and how UBPs entered the cell. Figure 2d graphs the intracellular levels of varying nucleotides in *E. coli* over a set time. The bumps on the graph indicate the presence of nucleotides and just before the 7.0 acquisition time, there is a bump for a trial where there is a presence of UBPs and IPTG. The IPTG is a chemical that regulates the expression of the transporter protein, which is encoded in the ACS plasmid (pACS). The plasmid that contains the X and Y UBPs is found in the INF plasmid (pINF) (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). This bump proves that UBPs are able to enter the cell, but require the pACS because of the need for a transporter protein. For, without the transporter protein, the intracellular levels of the Y nucleotide were basically undetectable. Another way UBPs were implemented into the cells was through the use of electroporation. Electroporation is accomplished by shocking the *E. coli* with a low electrical current to cause the bacteria to be more permeable and to replicate in much larger numbers. This increased replication can be seen

in figure 2c, where bacteria without the shock, replicated in much lower numbers (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). Those two methods were crucial to represent how UBPs were implemented into bacterial cells while addressing the problems.

The next thing represented by the data in the article was to show that replication was feasible with evidence of UBPs remaining inside the DNA of *E. coli*. There were two ways that represented plasmid replication. Those two methods were through the use of PCR and sequencing, which are depicted in figures 2e and 2f. PCR shows the amplification of the plasmids. In figure 2e, the only condition that had a band of DNA that contained the UBPs was when IPTG, dXTP/dYTP, and SA were present (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). More plasmids present in the gel resulted in a darker band forming. The other way that proves the plasmids were replicated was through the utilization of sequencing. During sequencing the presence of UBPs caused the process to stop. Under conditions where there was no IPTG or UBPs, sequencing of the plasmid continued. This continuation of sequencing is caused by no detectable UBPs. For *E. coli* to replicate pINF, both the transporter protein (coded by pACS) and UBPs needed to be present.

The last thing discussed in the article was whether the UBPs caused damage to *E. coli* and how long the bacteria were able to survive. As mentioned above, in figure 2c, bacterial replication with UBPs is possible, however, when UBPs were present, bacterial growth was slower when compared to no UBPs present. After about eighteen hours, the bacteria with UBPs replicated faster. (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). This is represented by the OD_{600}value. A higher OD_{600}value equates to more bacterial growth. This is because more light is absorbed in a cloudy solution caused by an

increased presence of bacteria. The longevity of the E. coli is represented in figure 3, which shows the bacteria with UBPs lasted about 24 doublings (Malyshev D., Dhami K., Lavergne T., Chen T., Dai N., Foster J., Correa I., and Romesberg F., 2014). Through the combination of examining the OD_{600}value and the growth rate of E. coli with UBPs, it can be seen that UBPs cause no harm to bacteria.

In conclusion, *E. coli* could successfully replicate a piece of DNA that contains UBPs. Through experimenting with multiple trials, the implementation of newly synthesized X and Y UBPs can be done in living organisms. This paper went in-depth about DNA replication in bacteria, especially that of *E. coli*, which is related to the cell biology course. For, a whole unit was spent on cellular replication. Another way this paper relates to the cell biology course is through understanding how nucleotides work and function. For, nucleotides are one of the fundamental components an organism needs to be considered alive. All life on Earth possesses the same four base nucleotides of A, T, G, and C, but arranged in different orders. This research will allow future researchers to experiment with different combinations of UBPs and find out if there are different types of UBPs other than X and Y to see if there are better combinations that would lead to fewer flaws in the genomes of living organisms.

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