Unnatural Base Pairing Compounds

 Recently, in 2014 a team of researchers lead by Dr. Floyd E. Romesberg has discovered a nucleotide base structure that can work in conjunction with natural nitrogenous base pairs; cytosine, guanine, adenine and thymine/uracil, the standard purines and pyrimidines. They were able to observe the unnatural base pairs, X (d5SICS) and Y (dNaMTP), proceed normally through a natural base pair organism of E.coli, and was successfully able to replicate, maintain, transcribe and translate the DNA/RNA without any observable problems. There are some problems with the unnatural base pairs (UBP)’s because they tend to misalign their helix conformation of the natural shape, and other problems due to the hydrophobic nature of the UBP’s.

 To begin however, the central dogma of biology is DNA->RNA-> protein, and this all begins during the process of replication. When a cell is doing its normal cell processes within the G1 cycle, it enters the S (synthesis) phase, where replication occurs. During this time, the DNA of a bacteria, or a eukaryotic cell copies its own DNA and attempts to make a perfect copy of itself in order to divide, and undergo mitosis. The sister chromatid would be a copy of the mother cell. Replication is a process of division, while the process of transcription and translation are more for the synthesis of metabolic activities within the cell. The formation of polymeric organelles or other protein structures within the cell begin with transcription, where an mRNA takes the DNA template strand and forms a copy of the DNA structure into an RNA structure (using uracil in place of thymine). After the RNA is formed, the mRNA goes into the process of translation, with the help of tRNA and rRNA – it is able to create an the codons, which are the sequences of three nucleotides used to form the polypeptide chain to form the final protein structure. This occurs normally through the healthy natural cell, of all life forms.

 The nitrogenous bases that Dr. Romesberg discovered were shaped differently, were neither purines or pyrimidines, where there would be a 5 carbon aromatic ring attached to a 6 carbon aromatic ring, instead both the d5SICS and dNaMTP bases only contained either a sulfur and nitrogen, or only a single oxygen, with two 6 carbon aromatic rings attached to each other. Each of the nitrogenous bases are of course aligned with the sugar-phosphate backbone. These two new nucleotide bases therefore are different due to their carbon primary structures, but also they are hydrophobic in nature, and are therefore more difficult to form hydrogen bonds.

However, it should be noted that d5SICS AND dNaMTP have the capability to form S-O-H hydrogen bonds, where sulfur and the oxygen form a type of hydrogen bond. Sulfur itself as unique capabilities, such as oxygen, nitrogen and fluorine to form strong hydrogen bonds, but sulfur is a moderately good hydrogen bond donor, and a weak hydrogen bond acceptor.(Zhou,2009). This may account for the ability for the X and Y nucleotides to form hydrogen bonds together, but also it may be the external hydrophilic forces pushing the nucleotides together, as shown recently by Chalmers University of technology. They mention that the environment is hydrophilic, and the DNA molecules’ nitrogen bases are now hydrophobic and constrict away from the water or polar molecules that coats the DNA, forcing them to stick together. (Bobo,2019) These new discoveries could help explain why the X and Y UBPs are able to stick together without the same level of hydrogen bonds. Some issues exist currently however, with the UBP’s that is due to the hydrophobic nature of the molecules. These molecules mess with the alignment of the helix structure from the naturally formed double helix of the DNA, due to the changing external and internal forces, so it is required to have the UBPs paired in a mixture with many natural base pairs to maintain the integrity of the DNA structure. (Hettinger,2017) Noteworthy to mention is that most nitrogenous bases, are embedded with nitrogen, (clearly in the name) and most nitrogen’s are very stable as they display resonance, and electron delocalization frequently to where the nucleophilic attack sites are exposed, making them at unreactive most of the time to chemical reactions. If the new X and Y UBP’s are integrated within the DNA, it is possible that the chemical reaction sites would be more susceptible to nucleophilic attack and break apart some of the structure, or integrate foreign material.

The reason why the double benzene structures don’t actually push each other apart in between, causing uneven spacing among the existing nitrogenous bases in between each strand of the double helix has to do with the DNA polymerase enzyme activation which stabilize both nucleotides during their intercalation and reduce the affinity with which the triphosphate binding occurs, so primer active sites basically help catalyze bond conformation into the double helix.(Betz,K.,2013)

So how exactly did the team or researchers enable the passage of these nitrogenous bases into the *E.coli* cell and observe whether or not the UBP were excised or kept within the genome? The method used was by observing the transfer of plasmids containing indicators, which also housed the foreign DNA, as well as observing the phosphate counts and observing the loss of the phosphate groups as they degrade from tri-phosphate, to di-phosphate to a single phosphate. The researches ultimately were able to get the UBPs directly into the cells via the plasmids (which are DNA loops which are not the chromosomal genome of a cell but can integrate periodically). Through the use of nucleotide triphosphate transporters (NTTs) and found the most suited was named *Pt*NTT2. The first issue that the team reached when trying to us NTTs was that the d5SICS and dNAM nucleotides were decomposing too quickly (essentially losing their phosphate groups) which they assumed was mediated by phosphatase enzymes. In order to keep the stability of the phosphates in the media, the researchers added 50mM of potassium phosphate (KPi) into the growth medium. After this they used a transporter protein called isopropyl-β-D-thiogalactoside (IPTG) which resulted in the UBP’s presumed uptake into the *E.coli* bacterium cell body. Luckily, the team found out that the addition of KPi also increased the extracellular stability of the UBPs as their half-lives lasted 9 hours, as opposed to less than an hour prior. In figure 1, the histograms express the difference between guanine and cytosine vs the two complementary UBPs. It shows the amount of triphosphate, 3P, diphosphate 2P, monophosphate 1P, and the free base nucleoside 0P. the results show that the monophosphate and diphosphates were stable, and not degrading at 9 hours. This is important because the amount of active time sets the stage for the uptake and eventual replication of the UBPs within the living bacterial cell and the triphosphate versions are necessary in order for them to be used and replicated.

Figure 1

At this point the researchers needed to prove that the bacterium was able to integrate the plasmid containing the nucleoside phosphates. What they did was created a plasmid named pINF (information) which housed the UBP, and another plasmid named pACS (accessory) to act as a control agent to ensure replication actually took place and it housed the *Pt*NTT2 transporters. The used two useful plasmids which house antibiotic resistance genes, such as ampicillin or streptomycin resistance in order to test if the plasmids were successfully integrated into the cell, by subjecting them to the ampicillin/streptomycin for example, after the process of putting in the UBP and seeing which one survived. If it can grow in the environment of ampicillin or streptomycin, than they successfully integrated the DNA plasmid. After the process, the team found out that the cells integrated the genes, using the process above. The researchers also proved that replication took place through the process of electroporation. They tracked the pACS’s NTT’s level of production over time, and found out that the numbers increased over time, indicating that the replication had occurred. The team also used OD600 (optical density) to discern how cloudy the solution was, which indicated how many bacterium’s exist. The researchers found a 24 doublings occurring, or a 2\*107 fold growth over a 15 hour period.

 The last part worthy to note is that the DNA did cause a normal amount of problems to the cell. It seems that no damage was caused via the single UBP that was integrated and replicated. The standard amount of replication errors *in vivo* occurred at 99.4% correctness, with a corresponding error rate of approximately 10-3, meaning that it is the same error rate of the intrinsic error rates of polymerases with natural DNA after 15 hours, which suggests that it is not efficiently excised by the DNA repair pathways or mechanisms in place. Even more interesting, was that if the UBPs were actually lost they were wholesomely replaced with adenine and thymine nucleoside base pairs, so even if they are lost, the cell’s machinery is able to replace it with a natural base pair, which would hopefully not cause any major mutations (most likely a missense instead of a deletion). Finally, when there is no more UBPs the last UBP is removed by replication-mediated mispairing, not the DNA repair pathway. The synthetic DNA vanished due to the above mispairing mechanism, and lasted for 3 to 6 days at 45% to 15% retention of the UBPs using the optical density method.(Malyshev, 2014)

References Cited:

Zhou P, Tian F, Lv F, Shang Z. (2009) Geometric characteristics of hydrogen bonds involving sulfur atoms in proteins. Proteins. 76(1):151-63. doi: 10.1002/prot.22327. PMID: 19089987.

Bobo F. (2019) A New Insight Into How Dna Is Held Together , Chalmers University,

<https://www.chalmers.se/en/departments/chem/news/Pages/DNA-held-together.aspx>

Thomas P. Hettinger, (2017) Helix instability and self-pairing

Proceedings of the National Academy of Sciences, 114 (32) E6476-E6477; DOI: 10.1073/pnas.1703423114

Karin Betz, Denis A. Malyshev, Thomas Lavergne, Wolfram Welte, Kay Diederichs, Floyd E. Romesberg, Andreas Marx, (2013), Structural Insights into DNA Replication without Hydrogen Bonds, Journal of the American Chemical Society, 135, 18637-18643

<https://www.biologie.uni-konstanz.de/typo3temp/secure_downloads/82815/0/2b10c9e6f9a28129e1b119d21aeeab217c918bb1/Betz2013_JACS.pdf>

Denis A. Malyshev, Kirandeep Dhami, Thomas Lavergne, Tingjian Chen, Nan Dai, Jeremy M. Foster, Ivan R. Correa Jr, Floyd E. Romesberg, (2014), A semi-synthetic organism with an expanded genetic alphabet, *Nature* **509,**385–388 (2014). https://doi.org/10.1038/nature13314