EVALUATING TEMPLATE-SWITCH MUTATIONS (TSM) IN *E.coli* AFTER TREATMENT WITH DEXAMETHASONE, AN FDA-APPROVED ANTI-INFLAMMATORY DRUG

Honors Thesis

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ABSTRACT

This paper will provide an overview of an experiment to discover potential side-effects of current FDA-approved drugs, Dexamethasone, that can cause mutations in DNA. Mutations in DNA can change the structure and function of cells. DNA can transform into non-β form structures that stall replication and cause genomic instability. Quasi-palindromes (QP) are imperfect inverted repeats of DNA sequences which can block the DNA replication fork during DNA synthesis. If the DNA replication fork is blocked by quasi-palindrome structures, DNA polymerase can use an alternative method to continue DNA replication, called "template-switching," which results in a perfect palindrome – a perfect inverted repeat of DNA. There is limited research for template-switch mutagenesis which tests selected FDA-approved drugs to understand the effect of TSM. The goal of this research project is to investigate the cellular effects of Dexamethasone, an anti-inflammatory glucocorticoid, which prevents the release of DNA, in quasi-palindrome mutations using E. coli as the model organism. The aim is to understand the consequence of selected drugs in template-switching quasi-palindrome mutations to increase our knowledge of the potential side effects for current FDA-approved drugs.

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INTRODUCTION

Mutations in DNA can change the structure and function of cells. DNA can transform into non- β form structures that stall replication and cause genomic instability (Klaric, Perr & Lovett, 2020). Inverted DNA can assemble into hairpin and cruciform structures during DNA replication (Figure 1).

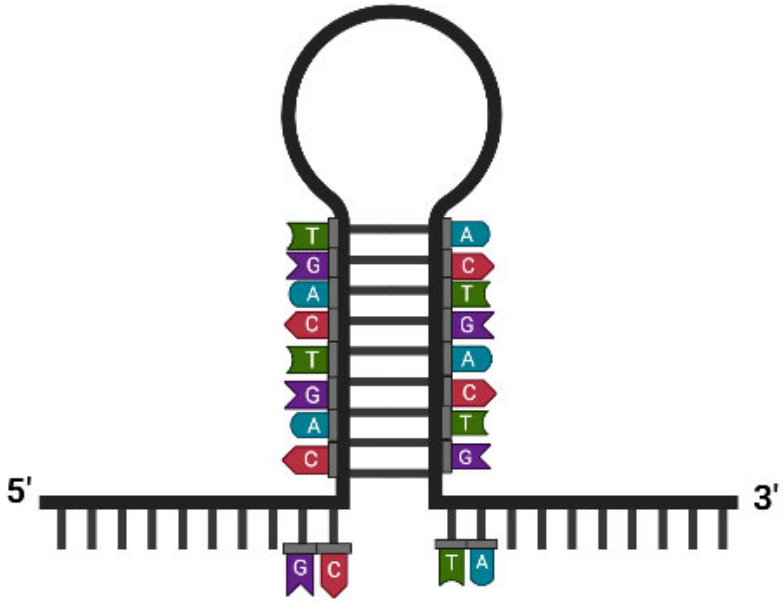

Figure 1: Inverted DNA in a hairpin structure.

Quasi-palindromes (QP) are imperfect inverted repeats of DNA sequences which can block the DNA replication folk during DNA synthesis. Quasi-palindromes are found on mutational hotspots in bacteria, yeast, and other organisms. As it was first proposed by him, Ripley states, if the DNA replication fork is blocked by quasi-palindrome structures, DNA polymerase can use an alternative method to continue DNA replication, called

"template-switching", which results in a perfect palindrome – a perfect inverted repeat of DNA (Ripley, 1982) (Figure 2).

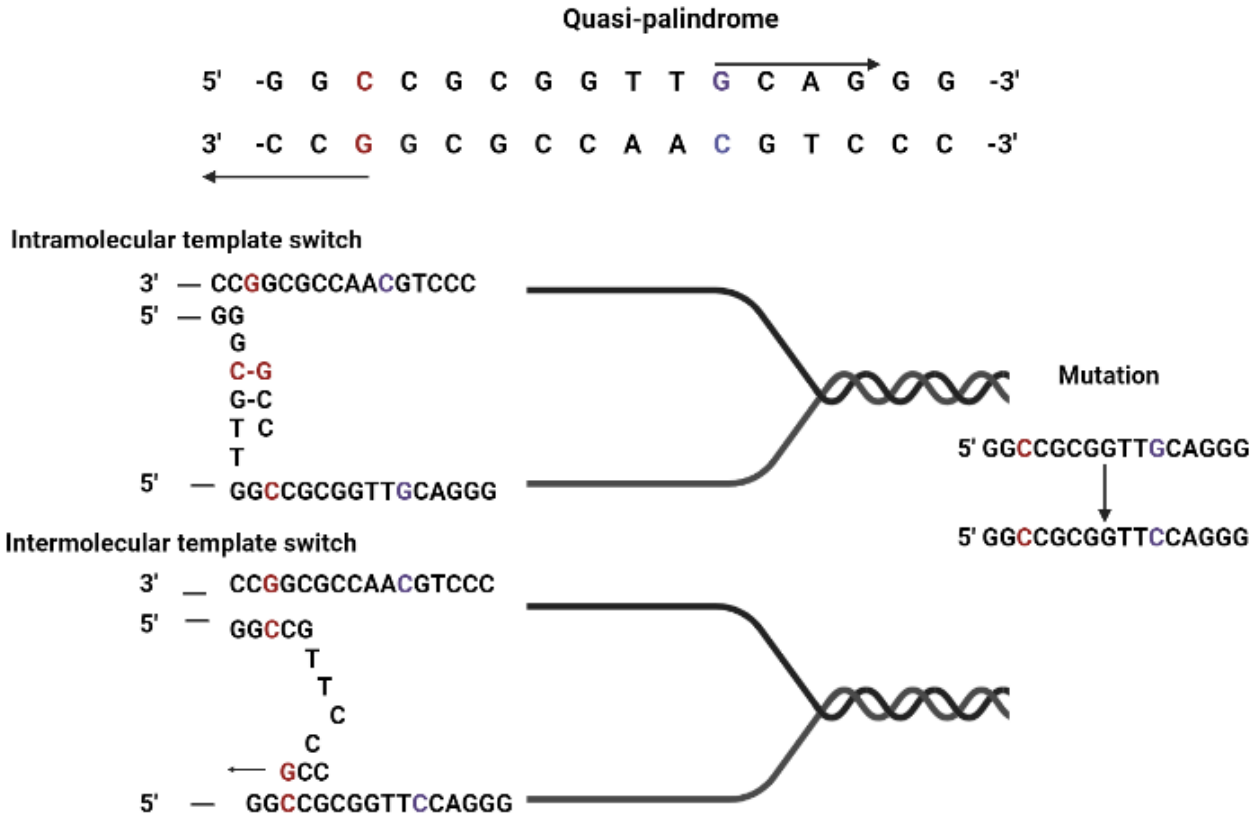

Figure 1: Quasi-palindrome template switch mechanism.

Template-switching is an efficient method; however, it is known to be mutagenic. These structures can cause mutations that have been associated with a variety of diseases such cancer, neurodegeneration, and premature aging (Laranjo, Klaric, Pearlman, & Lovett, 2019). Research on template-switch mutations have increased mutation rates of treatment from well-known drugs such as 5-Azycatidine (5-azaC), an FDA-approved chemotherapy drug, and Azidothymidine (AZT), an antiretroviral drug. There is limited research for template-switch mutagenesis which tests selected FDA-approved drugs to

understand the effect of TSM. The goal of this research project is to investigate the cellular effects of FDA-approved drugs in quasi-palindrome mutations using *E. coli* as the model organism. We aim to understand the consequence of selected drugs in template-switching quasi-palindrome mutations to increase our knowledge of the potential side effects for current FDA-approved drugs such as Dexamethasone.

DNA can naturally raise spontaneous mutations in the genome. At mutational hotspots in *E.coli*, there is a mechanism of template switching at Quasi-palindrome sites (Klaric, Perr & Lovett, 2020). Template switching at Quasi-palindrome sites has been observed to cause mutagenesis because of the mutational hotspots. These mutations at the QP sites come from mismatched nucleotides which create the buildup of secondary DNA sequences that form into a hairpin structure and degrade single-stranded DNA. Proximity of repeats within QPs makes it possible for newly synthesized DNA to mispair with the incorrect local complementary sequence through template-switching. Quasi-palindrome mutations report for about 60% of all mutations in the *thyA* gene in *E.coli* (Lovett, 2004). Quasi-palindromes are prone to mutation because the DNA structures can confuse with regular genetic function and lead to mutations which can cause disease. Ripley's team (1982) discovered QPs while examining mutations in T4, a virus that infects *E.coli* bacteria. They speculated that the changes they were observing —the completion of the QP by deleting non-matching sequences inside the palindrome—were caused by a replication mistake and a template modification. These mutational events have now been seen in a variety of taxa, including bacteria, yeast, humans, and other bacteriophages.

Dexamethasone is an anti-inflammatory glucocorticoid, which prevents the release of DNA. It is used to replace corticosteroid when the body does not make enough

of it. It can also relieve inflammation (swelling, heat, redness, and pain) and is used to treat certain forms of arthritis, intestinal disorders, autoimmune conditions, asthma, and certain types of cancer. Additionally, dexamethasone is used in chemotherapy to reduce acute toxicity in cancer patients and protect them against long-term side effects of genotoxic drugs. Current research has observed the role of dexamethasone in astrocytoma cell lines and if they damage DNA and repair it when cancer evolves (Ortega-Martinez, 2015). Lack of knowledge called for critical research to determine if dexamethasone alone might guard against cancer in these cell types and whether combining it with irradiation (IR) may help address the excessive proliferation that is inherent in the oncogenic process. Different pathways responsible for DNA damage and repair were examined when dexamethasone was delivered in conjunction with or without IR to elucidate the function of dexamethasone in these pathways. Furthermore, this study (Ortega-Martinez, 2015) observed how dexamethasone may lead to DNA damage and, as a result, apoptosis in astrocytoma cell lines. The results revealed that dexamethasone reduced normal cell proliferation when compared to Control conditions (Ortega-Martinez, 2011). Simultaneously, dexamethasone increased cell mortality, resulting in very low concentration in the cell cultures. Dexamethasone also functions by inducing apoptosis, as has been previously observed in different kinds of cell cultures (Yang, Zhang, Si-Ma, Fu, Zhao, Li, & Yang, 2011). Cell cycle data are consistent with the conclusion that dexamethasone caused cell death, implying that dexamethasone did not cause a stop in any stage of the cell cycle. It can be said that dexamethasone can cause DNA damage in astrocytoma cells on its own.

In another study, Menotta and colleagues (2012) observed Ataxia telangiectasia (AT), a rare genetic neurological disorder that is treated with glucocorticoid drugs. The results found that cells induced with dexamethasone skips upstream mutations on the ATM coding sequence and restore ATM activity in AT cells which can overcome mutations and prevent the disorder. This is significant in the type of mutation that occurs and where it is in the cell line.

These two studies experimented on different cell types and resulted in two vastly different conclusions, dexamethasone causes DNA damage, and the other that dexamethasone prevents mutations in the ATM gene in affiliated AT patients. There is little research on the type of mutation mechanism caused from using dexamethasone drugs in *E.coli*. This interests me in researching potential QP template-switch mutation mechanisms in Dexamethasone using the model organism. Various articles used different concentrations in their study to test dexamethasone. According to Lohuis, Leeuwen, Verheijden, Miert, and Brand in the 1988 paper, "Effect of Dexamethasone on Experimental *Eschericha coli* Mastitis in the Cow", and the 1989 paper by Lohuis, Leeuwen, Verheijden, Brand, and Miert, "Effect of Steriodal Anti-Inflammatory Drugs on *Escherichia coli* Endotoxin-Induced Mastitis in the Cow, the concentration used was 30 mg. However, in another research study the dexamethasone's concentration was 50μM (Ortega-Martinez, 2015). Additionally, Menotta et. al, 2012 used 100 μM of dexamethasone in their research. Due to the range of concentrations used, I tested concentrations at 0 mg, 15 mg, 30 mg, 45 mg, 60 mg, and 100 mg. This experiment found the limit of concentration at 60 mg to evaluate the effect of Template-Switch mutations in QP.

To quantify frequency of QP template-switch mutations, I performed a fluctuation assay; a fluctuation assay detects the number of mutations induced in bacteria. The number of mutations per culture and the differential growth rate between mutant and wild-type cells is needed to calculate. Estimate the mean number of mutations per culture (m) and divide by the QP total number of cells in the culture (Gillet-Markowska, Louvel and Fischer, 2015).

HYPOTHESIS

I hypothesize that the drug, Dexamethasone, will not induce template switch mutations based on previous research from Ortega-Martinez, and Menotta et al in human body cells. The DNA may be damaged, but it will not induce this specific mutation mechanism, TSM. If Dexamethasone does induce template switch mutations, then there will be an increase in the frequencies in template switch mutations in *E.coli.*

MATERIALS AND METHODS

To analyze whether the drugs of any effect on template-switch mutations, I will set up an experiment with a control which will be used in comparison to the selected FDA-approved drug, Dexamethasone. The methodology will use *lacZ* gene of *E.coli*, QP5, and qualitative methodology combined with quantitative fluctuation. Dilutions of the drug will be measured on LB plates and LacMin plates to perform quantitative analysis as shown in Figure 3. TSM in the QP reporter makes a non-functional *lacZ* gene functional and will only report QP mutations (Figure 4).

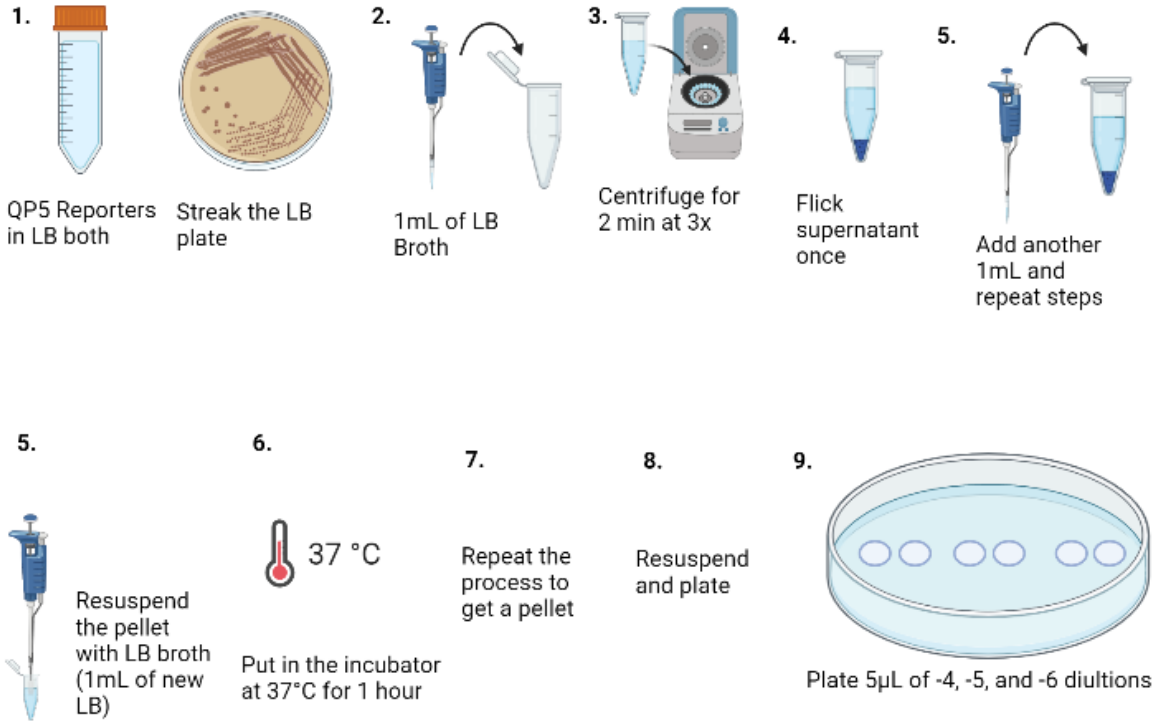

Figure 3: Experimental Approach.

QP5 reporters will grow overnight with lacZ gene of E.coli and of Luria Broth (LB) media test tube and then plated on a LB plate, which is the ideal media for bacteria. The plate will be streaked in a way that isolates the bacteria into single colonies. The single colonies will then be transferred into another LB test tube and left overnight. 1 mL of LB and 100 µL of the overnight culture will be resuspended and placed into the Eppendorf tube. The Eppendorf tube will be centrifuged until there is a supernatant and pellet. The supernatant will be disposed of, and the pellet will remain. The Eppendorf tub will then be added with another 100 µL of fresh LB solution and resuspended. The Eppendorf will be placed on the rotator/ incubator at 37 °C for 2 hours, every 15 minutes flick the tube. After the 2 hours are completed, centrifuge the tube until there is a pellet, remove the supernatant and then add 940 µL of LB and 60 µL of Dexamethasone stock solution to the cultures and label, then resuspend. Place the Eppendorf in water bath for 2 hours at 37 °C while flicking every 15 minutes. Once the time is off, centrifuge until there is a pellet and remove the supernatant. Resuspend with 200 µL of LB. Then a series of dilutions were served to dilute the concentration. On a LB media plate, measure 5µL of -4

dilution, -5 dilution, -6 dilution in teams of two. On a LacMin plate, measure 5µL of 0 dilution, -1 dilution, -2 dilution in teams of two. The LB and LacMin plates will grow overnight and be checked the following day.

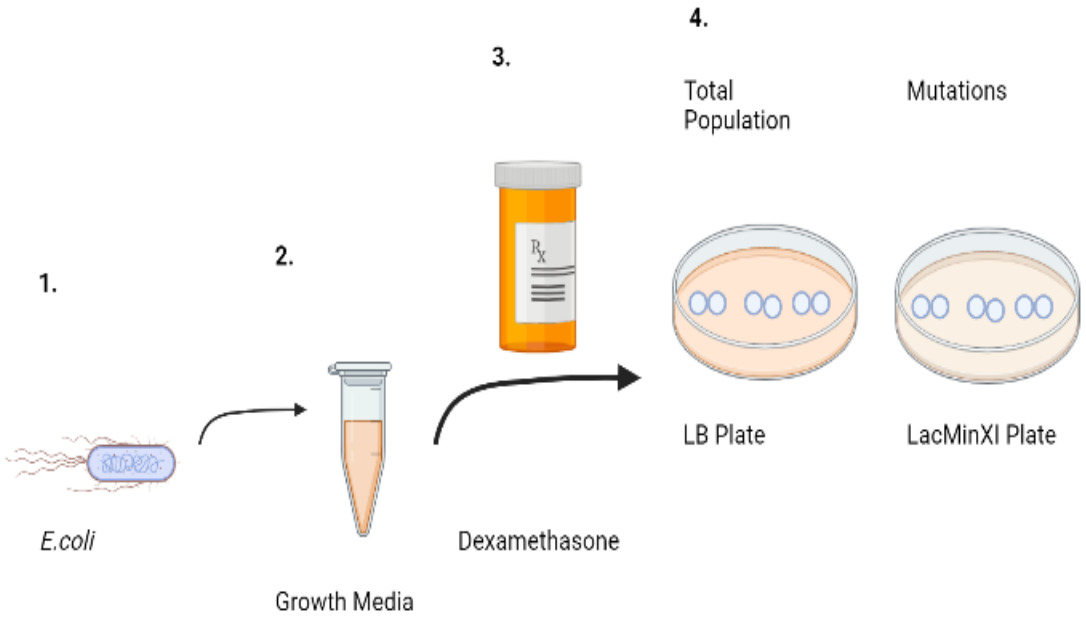

Figure 4: Plated bacteria show the rate of TSM in the *lacZ* gene.

Added *E.coli*, *lacZ* gene, QP reporter to Growth Media. Drug was added to media and incubated. After, a control and different dilutions at 60µM were plated on LB and LacMinXI plates. TSM were determined on LacMin plate.

RESULTS:

There was growth on the LB plate and on the LacMin plate (Figure 5). Doses on the LB tested bacterial growth and the LacMin plate tested mutations. Diffusion and fluctuation assays were used to evaluate mutations. After exposure to Dexamethasone at 60 μM, the average frequency of template switch mutations in *E.coli* on the leading strand were 5.69×10^{-7} and the control were 4.00×10^{-6} (Figure 5). Data suggests that exposure of the *E.coli* cells to Dexamethasone decreases the frequencies of template switch mutations in the leading stand (Figure 5).

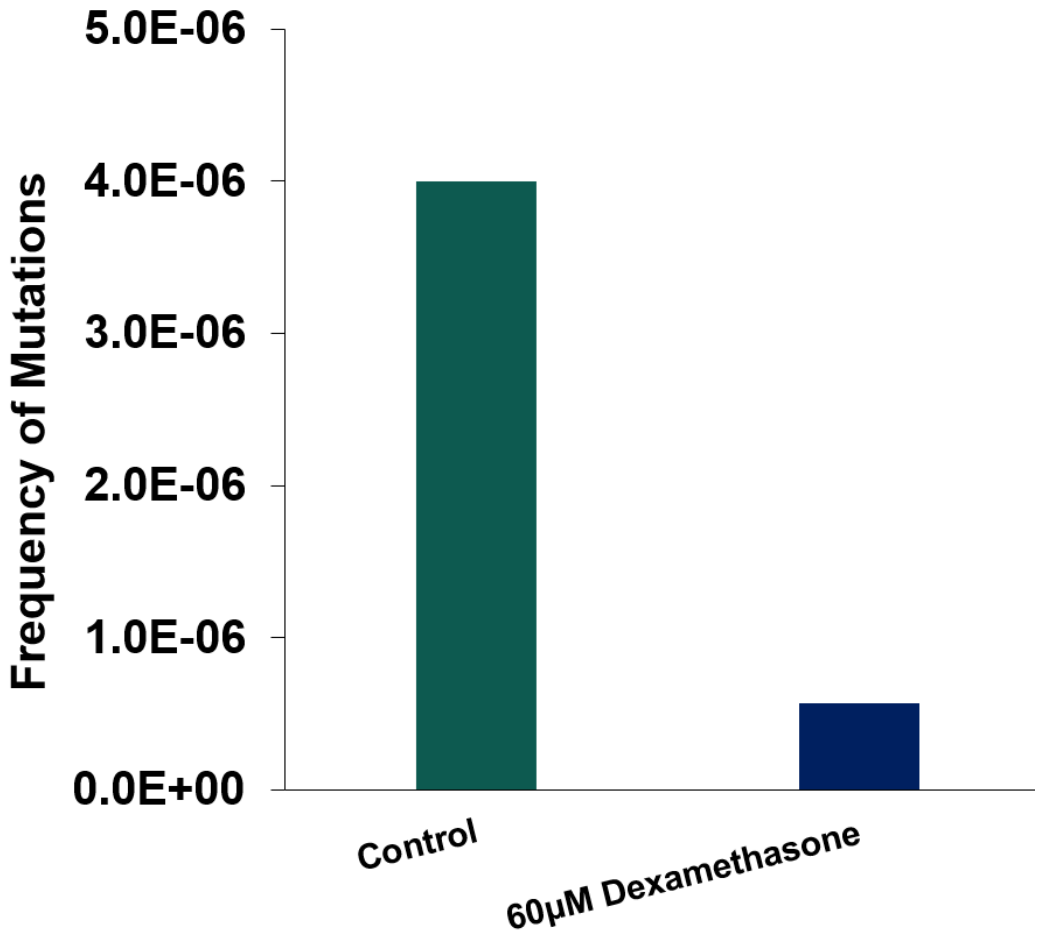

Figure 5: TSM Frequency after Dexamethasone treatment.

The number of mutations in Dexamethasone decreased compared to control.

DISCUSSION:

The data supports the hypothesis; Dexamethasone will not induce TSM. Mutations for 60μM Dexamethasone on the LacMin plate is less than the control (without the drug). This study provides critical information regarding the anti-inflammatory glucocorticoid drugs in template-switch mutation. Dexamethasone has mainly been used to treat rheumatoid arthritis and cancer, specifically in brain tumors. Dexamethasone had a lower number of mutation frequencies than the control. The leading strand of *E.coli* decreases TSM when compared to the control (Figure 4). Comparison of different concentrations of Dexamethasone is needed to compare if TSM mutations increases with increased concentration.

FURTHER RESEARCH:

In addition to the work above, I propose testing other steroid medicines such as hydrocortisone under the same conditions to evaluate if other steroid medicines are inducing TSM or not.

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