Introducing Favorable Mutations of Polymerase Orthologs in Human DNA Polymerase

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Abstract:

This paper explores the possibilities of introducing mutations and features from the T4 bacteriophage's DNA polymerase into human DNA. It first starts off with questions about the effectiveness of polymerase, a key protein in all cells, and looks for ways to improve it. It then discusses the L412M mutation, found in the finger domain of the polymerase. This mutation increases the processivity of the polymerase, an important trait vital to the protein's efficiency. L412M also affects fidelity, which is the property of the polymerase that measures accuracy. It pushes the accuracy rate much higher and creates less chance for the development of mutations. The paper also discusses CasPlus, which involves the use of T4 polymerase in conjunction with CRISPR, a common gene editing tool, in order to reduce on-target activity and improve accuracy. The addition of the polymerase decreases the chance that larger deletions will occur. It also prevents chromosomes from moving while the editing process occurs. This would mean that if humans can produce polymerases similar to those in bacteriophages, it would be able to accept future edits with less risk, and therefore, reduce one of the risks in somatic gene editing. Finally, the paper moves on to the process of implementation of the features, as well as discussing the ethics of the overall issue. It cautions a stance that is forward-looking, yet concerned with the moral and societal impact of the solution and promoting equality while pushing the treatment.

Introduction:

DNA polymerase is a vital protein in the nucleus of every cell, and acts by adding nucleotides to the split strands of DNA during replication. Specifically, the four nucleotides it can add are adenine, guanine, cytosine, and thiamine. The order of these nucleotides tells us how genes are expressed or what characteristics an organism will have and tells us about much of the significant activity in the replication cycle. While DNA Polymerase makes only about one error for every billion nucleotides it adds (due to the presence of exonuclease, a subunit of the polymerase that checks for the proper base), even that minute error rate accumulates. Overall, there is an enormous number of bases and genes in the human body resulting in multiple errors created with each cell replication. Those errors result in mutations, or a variation of the DNA sequence. Mutations can lead to the formation of proteins with altered structures or lacking expression entirely, which will directly affect our bodies and produce harmful effects, such as sickle cell disease.

The greatest issue with DNA polymerase is that it will halt and stop the connecting of base pairs for the DNA strand at difficult sequences when replicating. This can lead to the incorrect translation of genetic information. If you look at other organisms, there are numerous different naturally occurring types of DNA polymerase. Some of these have differences or other characteristics that get around this issue. None of the naturally occurring polymerases have been considered for use in humans, theoretically or otherwise. Researchers have, however, been able to produce polymerases in the laboratory, generating more variants. This review will

cover the feasibility of utilizing bacteriophage polymerase properties in human bodies, and more particularly within the scope of their beneficial properties.

Section 1: T4 DNA Polymerase L421M Mutation and Applications

T4 DNA polymerase is a bacteriophage enzyme that was isolated from the T4 bacteriophage, which is a virus that infects *Escherichia coli* bacteria. It serves a critical function in DNA replication and repair in the bacteriophage, with the highest fidelity in DNA synthesis due to its nature of hijacking other organisms for replication.(Laos, Thomson, and Ellington 2014) T4 DNA polymerase is also renowned for possessing a robust proofreading activity, which allows it to remove incorrectly incorporated nucleotides, reducing replication errors. This enzyme is highly characterized and utilized in molecular biology, particularly in methods such as DNA sequencing, mutagenesis, and polymerase chain reaction (PCR), due to its accuracy in DNA synthesis.(Laos, Thomson, and Ellington 2014) A specific mutation, L412M, within the finger domain of T4 DNA polymerase alters its activity. This mutation substitutes a leucine for a methionine at position 412 in the sequence, changing the proofreading and polymerization activity of the enzyme.(Figure 1) Mutation L412M was found to enhance polymerase processivity by copying longer strands of DNA without falling off the strand and also changing fidelity.(Laos, Thomson, and Ellington 2014)

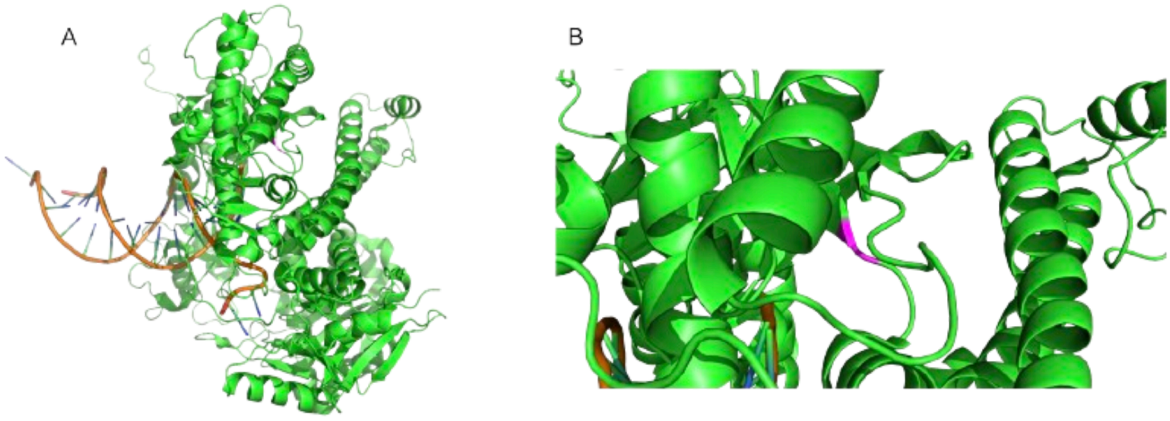

Figure 1: *The T4 DNA Polymerase protein is shown, with the highlighted region in pink displaying the would-be affected region by the mutation, L412M, and the orange strand showing the DNA. The second section (B) shows more detail at a closer look.*

The L412M mutation of T4 DNA polymerase can be translated to human medicine and biotechnology as increased fidelity and stability of DNA replication under stressful conditions. (Laos, Thomson, and Ellington 2014) The mutation can be used to develop highly processive and inhibitor-resistant DNA polymerases for next-generation sequencing, gene editing, or molecular diagnostics applications, which require high processivity and inhibitor resistance.(Laos, Thomson, and Ellington 2014) In the therapeutic space, for instance in cancer or genetic disease treatment, the engineering of human polymerases with the same mutations

would be capable of enhancing the fidelity of DNA repair enzymes or furthering synthetic gene therapies. Further, studies of the L412M mutation would be capable of uncovering molecular mechanisms of DNA replication that would be useful in the development of methods for minimizing replication errors accountable for human disease.(Laos, Thomson, and Ellington 2014)

Section 2: T4 DNA Polymerase Use in CRISPR

T4 bacteriophage polymerases were also found to possess many other inherent properties which can be useful, for instance, on gene editing activities. CRISPR genome editing has greatly transformed genetic and therapeutic studies, but there are still many side effects of this technology, for instance, off-target effects and undesired on-target chromosomal modifications, for instance, extensive deletions and translocations.

New research in *The EMBO Journal* describes CasPlus, a refined CRISPR process with the addition of T4 DNA polymerase to avoid such off-target effects.(Sun et al. 2024) The researchers found that by adding T4 DNA polymerase, large deletions are greatly restricted but small insertions with precision are favored, thus making the gene editing process more accurate.(Figure 2) This technique performed optimally in human cardiomyocytes to repair Duchenne muscular dystrophy (DMD) frameshift mutations, with its efficiency surpassing the conventional CRISPR/Cas9.(Sun et al. 2024)

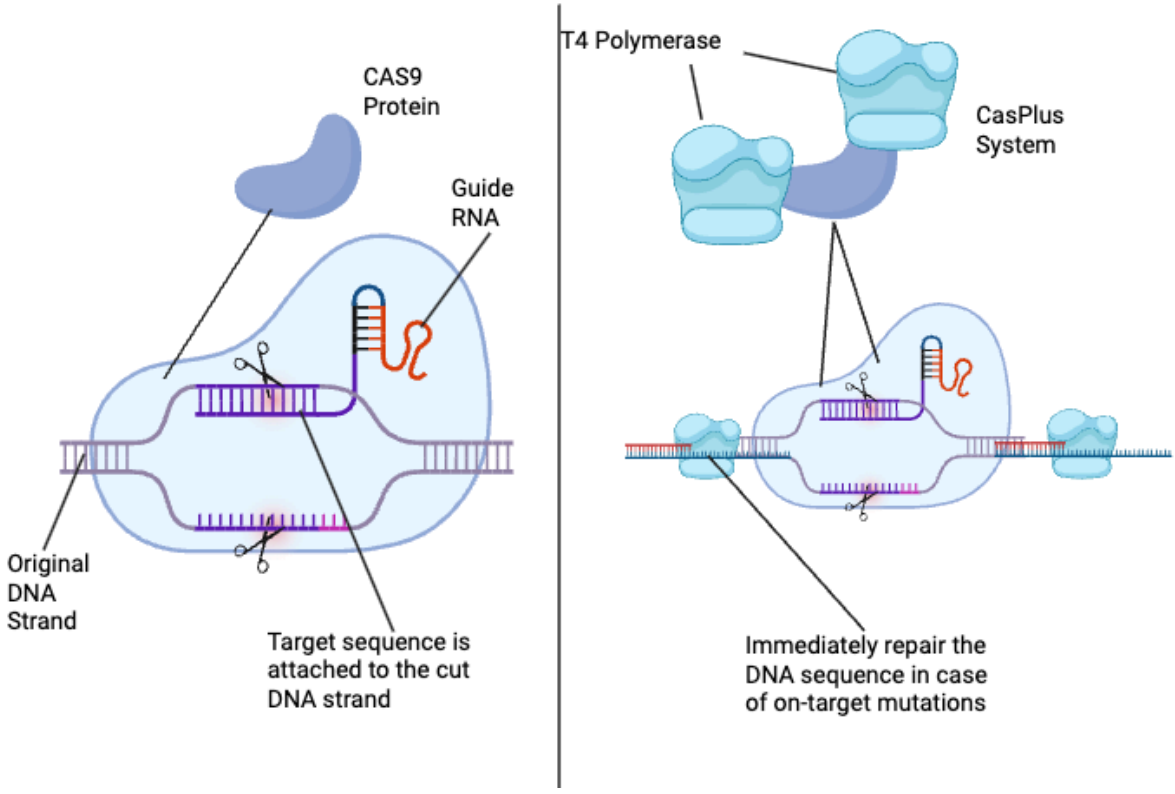

Figure 2: *The first section shows a diagram displaying the basic functions of the CRISPR method. The second section displays the intricacies and additions of the CasPlus process, with special attention shown to the polymerases.*

Aside from disease correction, CasPlus also showed added benefits of inhibiting chromosomal translocations when multiplex guide RNAs are used in primary human T cells.(Sun et al. 2024) This renders CasPlus highly promising for use in developing less toxic gene-editing therapies, especially for diseases requiring multiple genetic manipulations. The fact that it has the ability to limit unwanted genome editing without compromising and even boosting editing efficiency makes the method a powerful reagent for gene therapy and cell therapy, including regenerative medicine and personalized medicine.(Sun et al. 2024) Not only will the introduction and incorporation of T4 DNA polymerase into human bodies be a quantum leap in the knowledge of interspecies mutation, but a quantum leap in genetic engineering in general.(Sun et al. 2024)

With this procedure, study subjects would be primed in the future to accept future edits with less likelihood of mutations or off-target activity. If the insertion of this piece of CasPlus can be inserted into the polymerases that our bodies utilize, then it will be a safer world for genetic engineering and one fewer hurdle in the path of genetic engineering in humans because it averts the major risks of utilizing CRISPR/Cas9 editing. Thus, the utilization of T4 DNA polymerase in CRISPR/Cas9 editing is a step towards more precise and safer genome editing.(Sun et al. 2024)

As gene therapies are moving into the clinical translation phase, off-target mutations must be minimized to an absolute minimum for regulatory approval and patient safety. Future studies must take this analysis to additional cell types and disease models to make its widespread applicability certain. Successful application of this cutting-edge editing technology can propel the progress of CRISPR-based therapy, while mitigating concerns of genomic instability, to safely bring gene therapy to the threshold of mainstream medicine.

Section 3: How Do We Insert Different Mutations into Human Polymerases?

One might think there are only benefits to inserting T4 bacteriophage mutations into human polymerases. However, introduction of new mutations into the human body is a dangerous task that should be approached gradually and with cautious planning. Even omitting ethical concerns from the equation – something that should never be done – adding such characteristics as the L412M mutation, or the CasPlus mutation, will involve planning to determine the sequence to be changed and changing the polymerase gene to include the new forms in.(Sun et al. 2024)

The process utilized in changing the genes can also carry dangers of its own. The current conventional way of editing genes is by utilizing clustered regularly interspaced short palindromic repeats (CRISPR). Off-target activity is always going to be a worry when utilizing CRISPR/Cas9 due to the non-specific nature of its effects, and on-target activity such as deletions already described can actually hinder this experiment rather than advance it.(Sun et al. 2024) This should be avoided with careful planning and exploration of every potential avenue of error. DNA polymerase of the human body takes on an anthropomorphic hand shape with

areas of which are meant to be traced over.(Laos, Thomson, and Ellington 2014) If someone were to try to reproduce the L412M mutation in human body cells, the final target of the polymerase edit is in the so-called "palm" area of the polymerase, where the mutation causes a loop.(Laos, Thomson, and Ellington 2014) The exact sequence in which to write this loop will have to be determined.

However, somatic editing of humans as a therapeutic does bring with it serious ethical issues. Among them is the potential for unintended biological effects, such as off-target mutations, immune responses, or new pathologies. While somatic modifications are not inherited, the irreversibility of some of the changes heightens the risk for individual patients. Moreover, unequal access to somatic gene-editing technologies would further entrench current social disparities in favor of those with access to expensive treatments and to the disadvantage of already disadvantaged populations. Ethical regulation should therefore extend beyond assurance of safety and efficacy of somatic editing to include broader social implications of its use.

A second essential ethical question concerns the scope and aim of somatic editing. Despite being originally argued on treatment grounds, there is indeed a risk of mission creep into non-therapeutic enhancement, such as physical or mental enhancement traits. The risk raises essential questions of what it is to be human, justice, and social pressure for conformity to new genetic norms. Informed consent is also challenging, as the novelty and complexity of gene-editing technology could confuse patients and affect their understanding of the hazards and boundaries. It is the job of researchers, clinicians, and policymakers to put rigorous standards of informed consent, equitable access, and wise regulation so that the use of somatic editing preserves human dignity and public good before going and editing accordingly.

Conclusion:

T4 DNA polymerases that are designed to harbor mutations like L412M have considerably improved enzymatic properties that are reflected in enhanced gene-editing efficiency. *In vitro* studies demonstrate that the L412M motif-A substitution substantially improves T4 polymerase processivity and replication efficiency with very little loss in fidelity.(Laos, Thomson, and Ellington 2014) This makes T4 polymerase an attractive enzyme for genome engineering.

Indeed, a recent study demonstrated that the inclusion of an engineered T4 polymerase with CRISPR/Cas9 (the "CasPlus" system) significantly improves editing accuracy. For example, CasPlus strongly suppressed unwanted large deletions and chromosomal translocations while significantly increasing the efficiency of precise small insertions and gene restorations. In addition, in DMD disease models, CasPlus accomplished more efficient editing of frameshift mutations and higher dystrophin expression than Cas9 alone did.

These results underscore the therapeutic promise of exploiting high-fidelity, highly processive T4 polymerase variants for gene editing to cure disease: with enhanced accuracy and minimized genomic damage, they can significantly improve the prospects for precise disease correction therapies. In contrast, the exploitation of such powerful tools for human somatic gene therapy presents fundamental ethical challenges. Even with improved enzymes,

off-target edits or unforeseen genomic consequences remain a critical safety concern. Comprehensive preclinical testing and careful surveillance are therefore required to minimize these risks.

Furthermore, somatic gene therapies also present patient autonomy and justice concerns. Notably, obtaining truly informed consent for novel and complex risks and benefits is of the utmost importance, and equitable access to these potentially life-transformative treatments must be actively assured. As experts have noted, CRISPR-based therapies stand to increase disparity in healthcare unless special efforts are made to reach and benefit diverse, historically underserved populations. Lastly, the path forward entails a balancing act between innovation and responsibility. Guarded optimism is warranted and continued research with technological innovation should proceed hand-in-hand with rigorous ethical review and policy development. It is only through this balanced approach that we can benefit from engineered T4 polymerases for human therapy while respecting safety, equity, and patients' rights.

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(Reha-Krantz, Woodgate, and Goodman 2014)