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Complexities Surrounding N-1-Methylpseudouridine (m1Ψ) in COVID-19 Synthetic Gene Therapies

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N-1-methylpseudouridine (m1Ψ) was introduced into modified-mRNA vaccines to reduce immunogenicity and increase mRNA stability, but its presence can indeed affect translation in several ways. (1) Uridine in mRNA pairs with adenosine in tRNA. However, m1Ψ, although structurally similar to uridine, can alter base-pairing dynamics. The dynamics of mRNA and tRNA interaction are affected by anything that alters the temporal, structural, or biochemical nature of the assembly and its apparatus. (2) This may lead to reduced efficiency of codon recognition, as the tRNA's anticodon may not pair as efficiently with m1Ψ as it would with uridine. This potentially leads to misreading. If the modification affects base-pairing enough, it might increase the chance of a tRNA with a noncognate anticodon binding, leading to amino acid misincorporation. (3) This concern is significant. The alteration of one amino acid can have significant consequences, as is the case in sickle cell anemia. At the 6 position, a glutamic acid (hydrophilic) is traded for a valine (hydrophobic), which alters the tertiary structure of the protein such that it causes profound disease. If ribosomal movement is disrupted, it might allow for the misreading of near-cognate stop codons or increase the chance of the ribosome dissociating from the mRNA before reaching the natural stop codon. This dynamic potentially leads to the issue of ribosomal slippage, stalling, or both. (4) The ribosome might pause at sites containing m1Ψ due to altered codon-anticodon interactions or because m1Ψ can change the mRNA's secondary structure, affecting how the ribosome moves along the mRNA. Ribosomal pausing or altered codon-anticodon interactions can increase the chance of +1 or -1 frameshifts due to ribosomal slippage, especially at slippery sequences or if the mRNA structure is altered by m1Ψ, affecting the reading frame for all subsequent codons until a stop codon is encountered or the mRNA ends. (5)

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Example:

Original mRNA sequence: AUG GCU ΨGG UAA GCA CCC UGU UAG

Slippage at Ψ sequence: AUG GCU GGU AAG CAC CCU GUU AG . . .

The Pfizer-BioNTech (BNT162b2) spike protein coding sequence in this vaccine has 728 uridines replaced by m1Ψ. (6) Similarly, the Moderna vaccine's spike protein mRNA also has its uridines replaced with m1Ψ. (7) Although exact counts might slightly differ based on specific codon optimization or slight sequence variations, the number is in the same range. Ignoring all other possible concerns above (misincorporation, dissociation, etc.), let us focus solely on the possible aberrant proteins created by this positive or negative frameshift:

Each m1Ψ can cause a frameshift: We'll consider that any one of the 728 m1Ψ sites could independently lead to either a +1 or -1 frameshift. Therefore, each m1Ψ site can result in three outcomes: no change, +1 frameshift, or -1 frameshift. This means the potential number of distinct proteins possibly created is:

$3^{728} = 2.67 \times 10^{346}$

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For reference, there are ~10^{82} atoms in the known universe. (8)

This represents the theoretical maximum of unique protein sequences from frameshift mutations at every m1Ψ site. The actual diversity of proteins produced would be constrained by cellular mechanisms like nonsense-mediated decay, no-go decay, and ribosome-associated quality control. Further, it is statistically improbable for the majority of these scenarios. However, even with these constraints, the potential for variation is significant, illustrating the complexity of mRNA translation when considering modifications like m1Ψ.

There are a host of potential concerns surrounding this issue of aberrant protein production. First, an unintended protein sequence could be recognized by the immune system as foreign, potentially leading to an inflammatory response. (9) If these proteins share homologous sequences with human proteins, there's a risk of triggering autoimmunity where the immune system attacks the body's own tissues. (10) Additionally, this could lead to misfolded proteins, which can aggregate, leading to cellular stress, toxicity, or contributing to neurodegenerative disorders like prion diseases. (11) Also, an influx of misfolded or aberrant proteins might overwhelm cellular degradation pathways like the ubiquitin-proteasome system or autophagy, leading to cellular dysfunction. Likewise, this could overwhelm cellular energetics and cause mitochondrial dysfunction. (12) While unlikely, functional proteins could be resultant of this process, with unknown/unintended consequences. For example, if these proteins could interact with chromatin or affect gene expression, they could lead to epigenetic modifications with long-term implications for cell function or lineage, or if they can interact with the cell cycle, they could promote tumorigenesis. (13) The long-term presence of these proteins could have drastic implications, especially if they interfere with normal cellular processes. This is a very limited discussion of the topic, and is by no means exhaustive or comprehensive, and I hope it serves to invite further and very necessary discussion.

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