Maximizing Translation of mRNA Therapeutics
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Abstract
Messenger RNA (mRNA) has emerged as a promising class of nucleic acid therapeutics due to its safety profile and ability to induce robust immune responses. However, mRNA translation is inefficient and requires co-transcriptional capping with CleanCap™ to achieve high yields of Cap1 mRNAs. The recent introduction of nucleotide modifications has further expanded the repertoire of mRNA therapeutics, offering a means to fine-tune the innate immune response and improve translation efficiency. In this report, we describe the development of a novel yeast-based luciferase assay to evaluate mRNA translation using 5′ moU, a modified uridine that has been shown to improve translation. We show that the incorporation of 5′ moU into the luciferase sequence resulted in equivalent luciferase activity compared to WT luciferase when tested in wheat germ extracts. This reduces the innate immune response by reducing PKR activation.

Figure 1: Cap0, Cap1 and Cap2 Structures of 5′-Ends of mRNAs
- Eukaryotic mRNAs have a Cap0 or Cap2 structure.
- Sensing of proper cap structure is thought to be involved in self/non-self RNA recognition.
- Cap structures influence activation of PKR.

Figure 2: Capping Efficiency Assay Shows CleanCap™ Yields High Levels of Cap1
- Co-transcriptional capping with CleanCap™ (Cap1) helps evade an immune response.

Figure 3: Pseudouridine 5′-Triphosphate Derivatives
- mRNA body modifications help to evade an immune response
- Pseudouridine or 5-methyluridine/pseudouridine are current industry standard

Figure 4: U Depletion of Primary Luciferase Sequence Improves Incorporation of Bulky Pseudouridine Derivatives by T7 Polymerase
- Some pseudouridine derivatives did not incorporate well
- We depleted the Fuc sequence for Us to try and remedy this
- U depletion resulted in good incorporation
- We tested the derivatives that did incorporate for translation and activity

Figure 5: In Vitro Translation and Cell Activity of Modified Luciferase mRNAs
- U depleted sequences translated better in wheat germ extracts
- Bulker pseudouridine modifications did not translate well
- U depleted pseudouridine modifications resulted in higher activity in THP-1 cells
- We therefore continued our studies using the U depleted sequence

Figure 6: Pseudouridine Derivatives and 5moU Resulted in Lower Toxicity Compared to WT and PseudoU
- Activity in THP-1 cells

Figure 7: Slot Blot Demonstrates that HPLC Purification Depletes dsRNA
- An HPLC method depletes mRNAs of contaminating dsRNA
- This reduces the innate immune response by reducing PKR activation.

Figure 8: Cell Activity of HPLC vs. non-HPLC Luciferase mRNAs
- HPLC purification dramatically increased the activity of wt mRNA, improved the activity of PsU mRNA but did not alter the activity of 5moU mRNA
- Could this be because PKR does not bind 5moU-dsRNA?
- Could this also be true for the Pseudouridines?

Conclusions
- We have introduced a number of novel modified bases with interesting translational and immunological properties
- U depletion improved transcription quality, yield and activity
- HPLC purification to remove dsRNA reduced toxicity and interferon response and increased activity
- Interestingly, HPLC purification of 5moU mRNA did not increase activity
- One possibility is that 5moU mRNA is not efficiently recognized by PKR
- Translational activity in wheat germ extracts did not directly correlate with cell activity, which may indicate differences in immune stimulation by these mRNAs

Future Directions
- 5 moU is not recognized by PKR, then activity of 5moU should be equivalent in IFN-α and IFN-β-NK
- Measure activity, toxicity and interferon response in THP-1 cells for HPLC purified Pseudouridin derivative mRNAs

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