THOUSAND WORDS ABOUT...

JMS Journal of Medical Science

The pivotal role of uridine modifications in the development of mRNA technology

Piotr Rzymski

Department of Environmental Medicine, Poznan University of Medical Sciences, Poland

https://orcid.org/0000-0002-4713-0801

Corresponding author: rzymskipiotr@ump.edu.pl

Received 2023-10-05
Accepted 2023-11-05
Published 2023-12-07

How to Cite: Rzymski P. The pivotal role of uridine modifications in the development of mRNA technology. Journal of Medical Science. 2024 March;93(1):e938. doi:10.20883/medical.e938

doi: https://doi.org/10.20883/medical.e938

Keywords: mRNA vaccines, infectious diseases, cancer treatment, pseudouridine, Nobel Prize

ABSTRACT

In 2023, Katalin Karikó and Drew Weissman were awarded the Nobel Prize in Physiology or Medicine for their nucleoside base modifications research that later enabled mRNA vaccine development against COVID-19. This paper briefly reviews these achievements in the context of the development of mRNA technology and its enormous potential for medicine in the prevention of various infectious diseases and cancer treatment, including personalised therapies. It is beyond any doubt that discoveries made by Karikó and Weissman were pivotal in overcoming one of the major hurdles in the practical application of mRNA molecules, i.e., the recognition of exogenous mRNAs by endosomal Toll-like receptors and downstream innate immune response, ultimately leading to the decreased translational activity of delivered mRNA and its degradation. Although the Nobel Prize for Karikó and Weissman is fully justified, it must be stressed that mRNA technology would never unfold its potential for public health without a collective scientific effort encompassing over 40 years of research.

Introduction

On October 2, 2023, the Nobel Assembly at Karolinska Institute awarded Katalin Karikó and Drew Weissman the Nobel Prize in Physiology or Medicine "*for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19*" [1]. However, this achievement will likely have a broader impact on contemporary medicine, both within and outside the prevention of infectious diseases. This article discusses the accomplishments made by Karikó and Weissman in the context of the development, achievements, and future of mRNA technology.

Brief history of practical use of mRNA molecules

To understand the impact of research conducted by Karikó and Weissman, one should first comprehend the history of mRNA technology. The mRNA molecules and their regulatory role in the synthesis of proteins in cells were described in 1961. The first attempt to introduce mRNA molecules into cells to induce the translation of the desired protein dates back to 1976 when duck globin mRNA was microinjected into human and avian cells [2]. In 1978, the rabbit globin mRNA was introduced into mouse lymphocytes using liposomes as vehicles [3]. Almost a decade lat-

er, in 1989, the efficient and reproducible method for RNA transfection, based on cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride, incorporated into a liposome, was developed as shown by *in vitro* introduction of *Photinus pyralic* luciferase mRNA, synthesized *in vitro*, into variety cell types, including human, that resulted in increased enzyme activity [4]. One year later, mRNAs encoding chloramphenicol acetyltransferase, luciferase, and beta-galactosidase were injected into mouse skeletal *in vivo*, leading to detectable protein expression [5]. Soon, this approach was attempted for immunization (e.g., against influenza) and led to the induction of humoral and cellular immunity in mice [6, 7].

Revolutionary nucleosides modifications

However, significant challenges arose: (1) the vehicles used for mRNA had unfavorable safety profiles, (2) the use of naked mRNA was prone to immune recognition and degradation by RNAse, and (3) using dendritic cells transfected with mRNA *ex vivo*, offered as the potential solution to issues described in point 1 and 2, was impossible to be implemented in mass vaccinology [8]. Works by Karikó and Weissman provided a solution to the issue described in point 2, i.e., sensing of exogenous RNA by endosomal Toll-like receptors (specifically, TLR3, TLR7, and TLR8), ultimately leading to the production of pro-inflammatory cytokines and type I interferons, which activate RNA degradation [9]. However, as shown in 2005, the incorporation of various modified nucleosides ablated this response to different extents, resulting in higher translational activity of mRNA. Specifically, using N^6-Methyladenosine and ^{2}-Thiouridine suppressed the ability of RNA to stimulate TLR3, whereas N^6-Methyladenosine, ^{5}-Methylcytidine, ^{5}-Methyluridine, ^{2}-Thiouridine, and pseudouridine (Ψ) modifications blocked stimulation of TLR7 and TLR8. The immune stimulation was also suppressed proportionally with the number of modified nucleosides incorporated in RNA, but even a few modifications were superior compared to unmodified RNAs [10]. Substituting uridine with Ψ (see **Figure 1**) was eventually evidenced to significantly increase the activity of exogenous mRNA introduced into cells by reducing their recognition by innate immunity and increasing the stability of the RNA molecule [11, 12]. Realization of this was pivotal for the further development of the mRNA platform. As postulated, the altered secondary structures in modified mRNAs cannot be recognized effectively by RNA-dependent protein kinase, which correlates with attenuated IF2α phosphorylation [13]. As shown later by other authors, the substitution of uridine by N^1-Methyl-pseudouridine ($m^1\Psi$) (see **Figure 1**) revealed an even better performance than the use of Ψ because, in addition to TLR7 and TLR8, it also decreased the activation of TLR3 (14). As suggested, this superb translation activity of $m^1\Psi$-containing mRNA could result from increased ribosome density resulting from the deceleration of elongation [13]. Broader evasion of Toll-like receptors and downstream innate immune signalling improved mRNA's cellular viability and significantly increased translation [14].

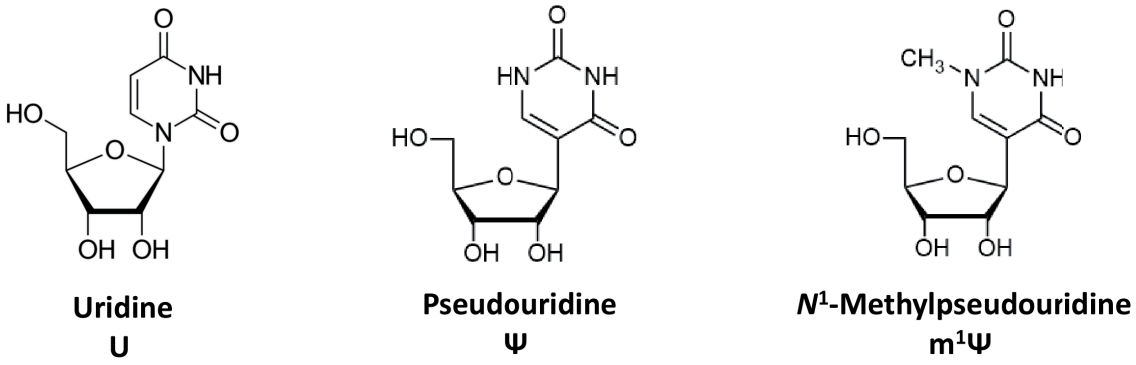

Figure 1. The uridine is a natural constituent of mRNA molecules. Pseudouridine is one of the modified nucleosides discovered by Katalin Karikó and Drew Weissman, 2023 Nobel Prize laureates in Physiology or Medicine, to evade Toll-like receptors recognition of exogenous mRNAs and increase the translational activity of these molecules. N^1-Methylpseudouridine was later demonstrated to be even more superior in this regard and was eventually used in mRNA vaccines against COVID-19.

Achievements of mRNA vaccines

Both authorized mRNA vaccines against COVID-19, i.e., BNT162b2 (BioNTech/Pfizer) and mRNA-1273 (Moderna), employed $m^1\Psi$ substituting each uridine [15]. Their use has been evidenced to be a life-saving intervention. COVID-19 vaccines, including mRNA vaccines given at over 2.5 billion doses, have averted an estimated 19.8 million deaths in the first year of the global COVID-19 vaccination campaign. The number of deaths averted per administered dose was more significant in high-income countries, and this phenomenon was attributed to better access to more immunogenic and efficacious mRNA vaccines [16]. In other words, vaccine equity, postulated numerous times throughout the COVID-19 pandemic, would save even more lives [16–19]. A Polish retrospective study also evidenced the high effectiveness of the mRNA vaccine, BNT162b2, in preventing COVID-19 deaths, with an estimated 61,803 deaths averted by vaccination in 2021 in Poland [20].

Future of mRNA technology

Beyond any doubt, such public health benefits would not be possible without previous discoveries made by Karikó and Weissman. However, their significance was not fully realized for years. The success of mRNA vaccines against COVID-19 led to continuous interest in further applications of the mRNA platform. As discussed recently, this technology provides various advantages, bypassing numerous issues that had long been slowing the progress of vaccine candidates when employing more traditional approaches [17]. As a result, various candidates developed using mRNA technology, i.e., against influenza viruses (including universal mRNA influenza vaccine), human immunodeficiency virus 1, respiratory syncytial virus, Nipah virus, Zika virus, human cytomegalovirus, and Epstein-Barr virus are currently on different stages of testing, including clinical studies [17].

Moreover, the mRNA platform is employed to develop novel cancer therapeutics with encouraging results from early clinical trials employing mRNA as monotherapy and in combination with checkpoint inhibitors [21]. The flexibility of mRNA technology allows the mRNA sequence to be quickly optimized to specific tumour-associated neoantigens that can vary widely between individuals, ultimately allowing the direction of the immune system in a highly personalized treatment approach [22]. Its potential has been recently shown in the phase 1 clinical trial of personalized mRNA neoantigen vaccine BNT122, expressing up to 20 neoantigens, for treating pancreatic ductal adenocarcinoma, a highly malignant form of cancer [23].

The collective research effort

The Nobel Prize in Physiology or Medicine for the achievements of Karikó and Weissman is fully justified. However, one should note that the mRNA technology would not unfold its potential for public health without a collective effort encompassing over 40 years of research. Pivotal discovery also included the development of nanoparticle carriers (formulated with PEGylated lipids, cholesterol, ionizable lipid, and phospholipids), which are characterized by an improved safety profile compared to cationic lipids used initially and enhance the cellular delivery of mRNA molecules [24]. Moreover, modifications of the 5′ cap and 3′ poly-A tail of mRNAs and selection of particular 5′UTR and 3′UTR also significantly stabilize mRNA molecules and increase their translational efficiency [25–27]. The critics highlight that the way the Nobel Prizes recognize individuals does not reflect the collaborative nature of modern research [28]. As Richard Feynman, a 1965 Nobel Laureate in Physics, once said, when asked about the meaning of this award: "*I don't like honours. I've already got the prize. The prize is the pleasure of finding the thing out, the kick in the discovery, the observation that other people use it. Those are the real things*". In the case of mRNA technology, the real thing is human health that has already been saved and can be saved in the future.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
The author discloses consultation fees, lecture honoraria, and grants from Moderna and Pfizer.

References

1. The Nobel Prize in Physiology or Medicine 2023 [Internet]. Nobelprize.org. [cited 2023 Oct 5]. Available from: https://www.nobelprize.org/prizes/medicine/2023/press-release.
2. Stacey DW, Allfrey VG. Microinjection studies of duck globin messenger RNA translation in human and avian cells. Cell. 1976;9:725–732. doi: https://doi.org/10.1016/0092-8674(76)90136-7.
3. Dimitriadis GJ. Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes. Nature. 1978;274:923–924. doi: https://doi.org/10.1038/274923a0.
4. Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci U S A. 1989;86:6077–6081. doi: https://doi.org/10.1073/pnas.86.16.6077.
5. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, et al. Direct gene transfer into mouse muscle in vivo. Science. 1990;247:1465–1468. doi: https://doi.org/10.1126/science.1690918.
6. Martinon F, Krishnan S, Lenzen G, Magné R, Gomard E, Guillet JG, et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur J Immunol. 1993;23:1719–1722. doi: https://doi.org/10.1002/eji.1830230749.
7. Conry RM, LoBuglio AF, Wright M, Sumerel L, Pike MJ, Johanning F, et al. Characterization of a messenger RNA polynucleotide vaccine vector. Cancer Res. 1995;55:1397–1400.
8. Heiser A, Coleman D, Dannull J, Yancey D, Maurice MA, Lallas CD, et al. Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. J Clin Invest. 2002;109:409–417. doi: https://doi.org/10.1172/JCI0214364.
9. Liu A, Wang X. The pivotal role of chemical modifications in mRNA therapeutics. Front Cell Dev Biol. 2022;10: 901510. doi: https://doi.org/10.3389/fcell.2022.901510.
10. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005;23:165–175. doi: https://doi.org/10.1016/j.immuni.2005.06.008.
11. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16:1833–1840. doi: https://doi.org/10.1038/mt.2008.200.
12. Anderson BR, Muramatsu H, Nallagatla SR, Bevilacqua PC, Sansing LH, Weissman D, et al. Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res. 2010;38:5884–5892. doi: https://doi.org/10.1093/nar/gkq347.
13. Svitkin YV, Cheng YM, Chakraborty T, Presnyak V, John M, Sonenberg N. N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. Nucleic Acids Res. 2017;45:6023–6036. doi: https://doi.org/10.1093/nar/gkx135.
14. Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. J Control Release. 2015;217:337–344. doi: https://doi.org/10.1016/j.jconrel.2015.08.051.
15. Nance KD, Meier JL. Modifications in an emergency: The role of N1-methylpseudouridine in COVID-19 vaccines. ACS Cent Sci. 2021;7:748–756. doi: https://doi.org/10.1021/acscentsci.1c00197.
16. Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Lancet Infect Dis. 2022;22:1293–1302. doi: https://doi.org/10.1016/S1473-3099(22)00320-6.
17. Rzymski P, Szuster-Ciesielska A, Dzieciątkowski T, Gwenzi W, Fal A. mRNA vaccines: The future of prevention of viral infections? J Med Virol. 2023;95: e28572 doi: https://doi.org/10.1002/jmv.28572.
18. Rzymski P, Camargo CA, Fal A, Flisiak R, Gwenzi W, Kelishadi R, et al. COVID-19 Vaccine Boosters: The Good, the Bad, and the Ugly. Vaccines. 2021;9:1299. doi: https://doi.org/10.3390/vaccines9111299.
19. Rzymski P, Szuster-Ciesielska A. The COVID-19 vaccination still matters: Omicron variant is a final wake-up call for the rich to help the poor. Vaccines. 2022;10:1070. doi: https://doi.org/10.3390/vaccines10071070.
20. Pietrzak Ł, Polok K, Halik R, Szuster-Ciesielska A, Szczeklik W. Effectiveness of BNT162b2 vaccination in preventing COVID-19–associated death in Poland. Pol Arch Med Wewn. 2023;133:1-8. doi: https://doi.org/10.20452/pamw.16575.
21. Lorentzen CL, Haanen JB, Met Ö, Svane IM. Clinical advances and ongoing trials of mRNA vaccines for cancer treatment. Lancet Oncol. 2022;23:450–458. doi: https://doi.org/10.1016/S1470-2045(22)00372-2.
22. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. Signal Transduct Target Ther. 2023;8:9. doi: https://doi.org/10.1038/s41392-022-01270-x.
23. Rojas LA, Sethna Z, Soares KC, Olcese C, Pang N, Patterson E, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. Nature. 2023;618:144–150. doi: https://doi.org/10.1038/s41586-023-06063-y.
24. Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022;188:114416. doi: https://doi.org/10.1016/j.addr.2022.114416.
25. Grudzien-Nogalska E, Jemielity J, Kowalska J, Darzynkiewicz E, Rhoads RE. Phosphorothioate cap analogs stabilize mRNA and increase translational efficiency in mammalian cells. RNA. 2007 Oct;13(10):1745–55. doi: https://doi.org/10.1261/rna.701307.
26. Sikorski PJ, Warminski M, Kubacka D, Ratajczak T, Nowis D, Kowalska J, et al. The identity and meth-

ylation status of the first transcribed nucleotide in eukaryotic mRNA 5′ cap modulates protein expression in living cells. Nucleic Acids Res. 2020 Feb 28;48(4):1607–26. doi: https://doi.org/10.1093/nar/gkaa032.
27. Orlandini von Niessen AG, Poleganov MA, Rechner C, Plaschke A, Kranz LM, Fesser S, et al. Improving mRNA-based therapeutic gene delivery by expression-augmenting 3′ UTRs identified by cellular library screening. Mol Ther. 2019 Apr 10;27(4):824–36. doi: https://doi.org/10.1016/j.ymthe.2018.12.011.
28. Graham F. Daily briefing: 'Elitist but essential' – what our readers think about the Nobel Prizes. Nature [Internet]. 2023 Sep 29 [cited 2023 Oct 5]; Available from: https://www.nature.com/articles/d41586-023-03110-6 doi: https://doi.org/10.1038/d41586-023-03110-6.