PERSPECTIVE

A layperson encounter, on the "modified" RNA world

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A chance conversation with a nonscientist about the mRNA-COVID vaccines, conveyed here, reminded the author of our enduring responsibility to accurately portray science to the public.

reaching the public | COVID | RNA biology

Earlier this year, as COVID-19 vaccinations were getting underway in the United States, a nonscientist friend said he was uneasy because had heard that the RNA in them had been "doped." Leaning in, I asked "How?" (I knew of course, as explained in the next paragraph). "With some chemical," he replied.

This struck me as a perfect storm of an educated, reasonably informed nonscientist being led astray by how the media often doesn't get it quite right, though we all recognize that too much detail can be narcoleptic. The art is to convey the science in just the right dose of detail, as Lewis Thomas and Carl Sagan did for example (1). I told my friend what the doping was, using lay terms. He listened thoughtfully and then I came in with my final shot: nature is full of RNA that is "doped," and even DNA is as well. These chemical modifications are not done by mad scientists but by the very biological systems in which these RNAs and DNAs reside, using their own enzymes.

He left somewhat convinced and hopefully is now vaccinated. This encounter gave me the thought that I, and my readers, should take a step back and think about all the "modified" RNAs out there.

For transfer RNAs alone, there are 120 known base modifications, with their prevalence as high as 13 of the 76 nucleotides in human cytosolic $tRNA^{tyr}$ (2). N^6-adenosine methylation of messenger RNA (3, 4) is a current area of wide interest. And as to my friend's angst, the accounts he had seen in the media were based on the fact that in both mRNA-based vaccines, all the uracil positions are replaced by N^1-methylpseudouridine (Fig. 1).

Discovered in transfer RNA (5), pseudouridine is thought to be a stabilizer in which its N^1 hydrogen can bond to the hemiacetal oxygen in the ribose of a paired adenosine. Thus, the triple helix of poly(A):-poly$(U)_2$ has a lower melting temperature than one in which the U strands are all pseudouridine (6). Elegant structural work on pseudouridine in RNA has been complemented by other studies that have, among other things, raised the possibility that the cancer chemotherapeutic action of 5- fluorouracil, the nucleic acid base analog pioneered by Gertrude Elion and George Hitchings, might not be limited to its substitution for thymine in DNA, but also its impairment with the biosynthesis of pseudouridine-containing RNA (e.g., ref. 7).

Why do the Moderna and Pfizer vaccines have N^1-methylpseudouridine substituted all throughout where uridines would otherwise be? This came from two groups, working independently, who found that inserting pseudouridine into RNA caused it to outwit the machinery that normally degrades exogenous RNAs in mammalian cells (8, 9). It was subsequently found that substitution of uridines by N^1-methylpseudouridine conferred even more resistance to degradation and also enhanced translational activity (10), and thus it was chosen by both Pfizer and Moderna for their COVID-19 vaccines. Ironically as to my friend's concern, N^1-methylpseudouridine also occurs naturally (11), a final element in my "case" to him.

But there is more. Okay, so cells modify their RNAs and now companies do so too, for good therapeutic reason. What about DNA? The first base modification of DNA was discovered in 1948 (12)

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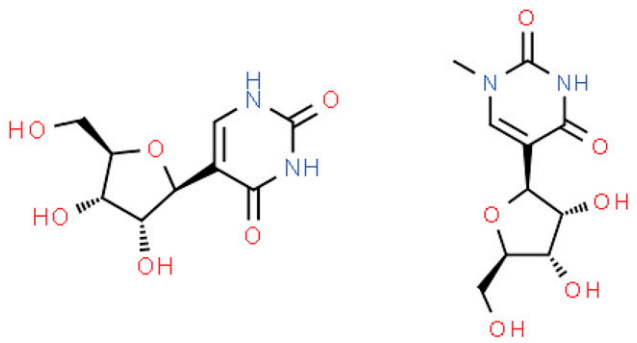

Fig. 1. Pseudouridine (*Left*) and N^1-methylpseudouridine (*Right*).

and later it was recognized that these are, in bacteria, part of a host-restriction mechanism against bacteriophages (13). Later, one DNA modification—methylation of the C5 in cytosine—was found to be part of an epigenetic process in which genes can be programmed in the mammalian life cycle (14).

In 1978 the idea of using DNA as a drug arose, specifically in the form of short single-stranded molecules that could attenuate protein synthesis by hybridizing with a targeted RNA (15). It soon became apparent that these synthetic oligodeoxynucleotides would need modification to persist long enough in cells to achieve the intended effect, and the first step in this direction was to synthesize them with phosphorothioate internucleoside bonds, in which a sulfur atom replaces the nonbridging oxygen in each internucleoside bond. Many other modifications were later developed as the antisense DNA therapeutics field evolved (16), including the strategic placement of phosphorothioates at certain positions to trigger degradation of the mRNA by RNase H, but with them flanked by different internucleoside modifications to achieve even higher resistance of the oligonucleotide to degradation (17).

Returning to my friend's query about the mRNA vaccines and how his angst may have lessened upon learning that the modifications occur naturally, I want to close on a stunning point: phosphorothioate-substituted DNA occurs in nature and plays a role in phage host-restriction and bacterial epigenetic mechanisms (18–20). And unlike all else we have discussed here so far, these are not base modifications but rather sequence-directed modifications of the internucleoside linkage itself; and if that were not enough, they are also stereo-specific. The enzymes that accomplish this have been identified and this is a new frontier in prokaryotic evolution and biology.

For RNA, it seems likely that almost all of the modifications have been discovered, although a few more may show up as RNAs from extremophiles continue to be studied. As for DNA, some years ago a group reported a bacterium that lives in Lake Mono, California, in which arsenate internucleoside linkages were claimed to occur (21). Arsenate and phosphate share tetrahedral chemical anatomy and electronic features, so this seemed plausible at first blush, given the high concentration of arsenic in this lake. (It is a rare one in having no effluent, thus accreting very high dissolved mineral concentrations over its 750,000-y geological history.) Alas, subsequent work revealed that it was not the case, but this did get everyone thinking about the notion, not only like natural phosphorothioate linkages, but others in which at the origin of life or later chemical opportunities might have led to an atom other than phosphorus. But phosphorous "checks all the boxes," as a superb essay once articulated (22). Meanwhile, we remain ready to be excited by all that awaits us, the innocent children that we are, riding on the endless frontier.

All these thoughts floated back to me, with gratitude, from one brief, appreciated encounter about a vaccine. Pasteur spoke to his public in Paris salons (1), but we scientists have even more opportunities, both in conversation and with the pen. Let us take advantage of them whenever we can.

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