THE REAGENTS BEHIND MUCH OF MOLECULAR BIOLOGY

THE ABILITY TO AMPLIFY, MODIFY AND FABRICATE DNA is fundamental to modern molecular biology. Why do so many researchers take their constituent parts for granted?

When an unidentified respiratory disease began circulating the world in early 2020, molecular biology researchers suddenly found that their work was the world's top story.

Efforts to identify the mystery pathogen, to develop diagnostic tools, and ultimately, to vaccinate people against it, all depended upon molecular diagnostics techniques centered on the manipulation and amplification of genetic code.

The spike in demand for the many reagents required for such work has been unprecedented, says Edvinas Paliulis, product manager for molecular biology at Thermo Fisher Scientific in Vilnius, Lithuania. But few were more sought after than deoxyribonucleotide triphosphates (dNTPs), from which DNA is assembled.

dNTPs are one of molecular biology's most fundamental building blocks. In ordinary times, few molecular biologists give them much thought, taking their availability for granted. Running out of a staple laboratory reagent, however, can inspire a newfound appreciation for it.

MARINA LITVINOVA/SHUTTERSTOCK

▲ In biomedical and clinical research, access to deoxyribonucleotide triphosphates (dNTPs) is vital for sequencing work and certain diagnostics.

dNTPs then until now

The replication and amplification of DNA, using dNTPs, is a cornerstone of modern molecular biology, and has been so for more than 50 years. The methods have helped researchers reveal countless insights into molecular processes and disease; they enabled the Human Genome Project, and they were vital in the global response to the SARS-CoV-2 pandemic.

The story of dNTPs goes back quite a bit further.

In 1869, Swiss chemist, Friedrich Miescher, isolated an unexpected, phosphorus-rich substance from human white blood cells. It was DNA, and insights into its chemical nature gradually followed. In 1919, Russian biochemist Phoebus Levene correctly proposed that DNA was built from sequences of subunits called deoxyribonucleotides, each of which consisted of a deoxyribose sugar, a phosphate, and one of four possible nitrogen-containing bases.

When James Watson and Francis Crick solved the question of DNA's structure, in 1953, they demonstrated that DNA is composed of two complementary strands of deoxyribonucleotides, which are held together by hydrogen bonds between specific pairs of nucleobases. Each DNA strand is a template for the construction of its partner. That insight formed the basis by which DNA can be copied—whether in an organism or at the lab bench.

"dNTPs have been widely used in research ever since," says Paliulis. "Whenever we need DNA, dNTPs are used to produce those molecules."

Demand from dNTP detectives

During the pandemic, the need for DNA synthesis was acute. "When the virus emerged, sequencing its genome was the first step to understanding what it is and how we can deal with it," Paliulis says. The enabling technology that underpins much of molecular biology, including genome sequencing, is the polymerase chain reaction (PCR). The technique uses heat to separate double-stranded DNA and combinations of enzymes, dNTPs, and primers—also made from dNTPs—to make copies. As the process cycles, the original sample DNA is amplified.

"Sequencing is basically a more refined and convoluted DNA copying process," Paliulis says. It typically uses modified, fluorescently tagged nucleotides to enable the genetic sequence to be read.

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In the case of SARS-CoV-2, researchers initiated a massive sequencing effort, with dozens of labs around the world working both independently and together to map the viral genome. In less than two months, researchers had a sequence in hand.

While the surge in sequencing early in the pandemic probably drove a slight increase in dNTP demand, the true spike arrived shortly after, with the need for accurate SARS-CoV-2 diagnostics. "The most reliable method to detect a disease—because it looks at areas of the genome unique to that virus—is a PCR method called quantitative PCR (qPCR)," Paliulis says.

Also based on DNA amplification, qPCR adds specific primers to bind the target DNA and fluorescent tags that can light up the sample if the target is present. The technique is highly sensitive and accurate. "qPCR was so important for the pandemic, to establish how many cases we have, and to treat those infected people," Paliulis says.

As the pandemic spread, so did the demand for diagnostics, putting immense pressure on the supply of dNTPs. At Thermo Fisher alone, production increased to hundreds of litres per month. "That might not seem like a lot," Paliulis says, "but for one qPCR test we need only a few microlitres of dNTPs; 100 litres could test roughly 100 million people."

Synthesis and manufacture of dNTPs can be done in a variety of ways, from the chemical or enzymatic phosphorylation of the deoxynucleoside or corresponding monophosphate to enzymatic reduction of the ribonucleotide triphosphate. Thermo Fisher Scientific uses multi-step chemical synthesis to manufacture dNTPs.

"The enzymatic approaches are fast and easy, but the main disadvantage is many times they leave contaminant traces," Paliulis says. "Chemical synthesis has purification steps too, but enzymatic synthesis leaves more impurity traces, and purification is far harder. In the molecular diagnostics field, especially for tightly regulated processes like in vitro diagnostics, we need as little contamination as possible."

Rapid development

Fittingly for the substance that has been the quiet but critical enabler of modern molecular biology, dNTPs also played a background role supporting the advent of RNA vaccines, which were first approved for use against SARS-CoV-2. As DNA is a much more robust substance than RNA, reference RNA is typically stored as 'copy DNA', which is fabricated from dNTPs.

"You can store the copy DNA at -20°C, and revert it back to RNA whenever you want," Paliulis says. Without it, the development of RNA vaccines would have been much more difficult, if not impossible.

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As with any well-funded effort in science, Paliulis sees further advancements growing from it, particularly further refinements of the molecular diagnostics techniques that employ dNTPs. "With government focus and investment, a lot of new businesses sprang up, new ideas were generated, and the sector seems more prolific than ever," Paliulis says. "One trend is to try to develop qPCR testing methods that are much faster or require less equipment, or can skip some purification steps."

However developments unfold, one fact remains certain: dNTPs will remain at the heart of molecular biology for decades to come, faithfully clipped together one by one to form new DNA. ■

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