ADVERTISEMENT FEATURE

CIRCULAR RNAS: FROM ODDITY TO THERAPEUTIC HOPE

After decades of neglect, circular RNAs are gaining attention through REFINED ANALYTICS, opening doors to their vast potential.

When researchers first observed the presence of circular RNAs in the 1970s, they were an inexplicable biological oddity — and they remained so for many years afterward. The field of RNA biology was in its infancy, and these molecules were largely written off as accidental byproducts of the splicing process that gives rise to linear messenger RNAs.

But advances in analytical tools and a growing recognition of therapeutic opportunities has led to a surge of interest in circular RNA (circRNA). "It took close to 50 years to actually gain traction — which is not unheard of in science. It's just sad — because they're very difficult to study," says Paulius Palaima, product manager at Thermo Fisher Scientific.

Despite circRNAs making up just a small fraction[1,2] of total RNA in cells — probably less than 0.05% — researchers have developed precise methods to isolate them, by eliminating other RNA types, using techniques like exonuclease treatments and advanced sequencing. In the characterization stage, gel electrophoresis has become a useful tool for quickly distinguishing circular from linear RNAs, significantly simplifying a previously labour-intensive process.

The past decade of research has revealed distinctive features of circRNAs that could turn them into powerful clinical tools. "Linear RNA can be degraded very easily by intracellular exonucleases," says Vaida Šeputienė, R&D manager at Thermo Fisher. In contrast, she notes that circRNA has considerably higher resistance to these RNA-destroying enzymes. "There are plenty of studies which show that circRNA could persist in cells for a longer time."

So, how have these techniques evolved, and how can scientists exploit this increased stability?

> **"I THINK FOR CANCER TREATMENT, THIS IS GOING TO BE BEYOND REVOLUTIONARY."**

GOING CIRCULAR

Since the 1970s, the characterization of RNA has undergone significant advancements, evolving from labour-intensive methods like polyacrylamide gel electrophoresis to more precise techniques such as high-performance liquid chromatography and capillary electrophoresis. The rise of high-throughput sequencing technologies has further revolutionized RNA analysis, enabling comprehensive profiling of RNA species.

But the differentiation between circular and linear RNAs has remained a challenge, often requiring costly and slow methods. Until recently, the isolation and characterization of circRNAs posed significant difficulties, but researchers have now made progress using new techniques. For example, a process called exonuclease treatment can break down linear RNA, which helps increase the proportion of circRNA available for study. Additionally, scientists can deplete ribosomal RNA, allowing them to get more circRNA from the total RNA samples collected.

Characterizing the resulting circRNAs has historically relied on expensive methods. Surprisingly, agarose gel electrophoresis has emerged as a valuable tool.

In fact, a 2022 Molecular Cell paper demonstrated that Thermo Fisher's Invitrogen E-Gel agarose precast gel can enable simple discrimination of circular and linear RNA sequences with a single, quick electrophoresis-based analysis[3]. This method significantly simplifies what was once a labour-intensive process. The innovation in gel electrophoresis has enhanced its usability, helping make it safer and more accessible. Precast gels eliminate the need for hazardous chemicals and extensive preparation, enabling researchers to achieve accurate results within minutes instead of hours. Palaima notes that in other electrophoresis workflows developed for circRNA analysis, "if you make them yourself, you must deal with formaldehyde and other really nasty chemicals because you have to prevent RNases from degrading the RNA. And in the end, this traditional agarose gel is still unable to differentiate circRNA form linear RNA." In contrast, E-Gels are precast and ready to run, sparing users from having to work with dangerous substances and can resolve successfully circularized RNA molecules within minutes instead of hours.

To take advantage of the exciting potential of circRNA, researchers also need the capability to design and produce it on demand. Thermo Fisher offers a full range of enzymes and other molecular biology tools to assist with these initial steps of the development process. "We offer an extensive portfolio of solutions, ranging from plasmid cloning to our Invitrogen GeneArt Gene synthesis service, which allows customers to design, order, and receive custom plasmids that are ready to use," says Šeputienė. She also highlights a broad range of RNA polymerases, RNA ligases, reverse transcriptases for circular RNA synthesis and analysis.

CLINICAL APPLICATIONS

CircRNA could have a major impact on drug development and delivery. For example, synthetically-generated circRNAs could be used as drugs to reprogramme dysfunctional cells to express normal levels of protein since they produce up to eight times as much protein relative to their linear RNA counterparts[4].

▲ Circular RNA is a non-coding RNA that plays a role in many biological processes. Unlike linear RNA, its circular structure makes it resistant to degradation.

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Further, circRNA could also improve drug delivery, as researchers at the University of California, San Diego, recently demonstrated that delivery of CRISPR-Cas9 editing components as circRNA enabled more efficient cellular engineering[5]. Drugs based on linear RNAs can also be misrecognized as a viral infection and elicit an immune response in patients. However, circRNAs significantly reduce this risk in animal models[6].

There is also at least one biotechnology company whose entire business model is predicated on developing therapies based on circRNAs. Some of the therapeutic programmes in development include genome-editing strategies that could reverse genetic disorders like sickle-cell disease, or using circRNAs to directly reprogramme patient immune cells into targeted cancer killers that recognize tumour-specific antigens.

Palaima is particularly enthusiastic about the latter approach, which involves the direct modification of T cells within the patient's body. This contrasts with the laborious process currently used to produce T cell-based immunotherapies, which entails extracting patient immune cells and then cultivating and modifying them in the lab. "Some of the success with CAR-T cells have been astonishing, but to scale that up for all patients is going to cost a fortune," says Palaima. "Plus, with circRNAs you also gain more modularity." This means clinicians could potentially adjust treatment in patients over time with circular RNAs against alternative tumour antigens if an initial immunotherapy regimen falls short. Palaima says, "I think for cancer treatment, this is going to be beyond revolutionary."

There are still ample opportunities to accelerate progress with circRNA-based therapies. For example, Palaima suggests that future advancements may allow for the direct synthesis of molecules through chemical processes, eliminating the need for enzymes and plasmids. "This seems like a natural go-to — you have your sequence, you use the computer to instruct the machine to generate it for you, and the machine generates it for you," he says. But current synthesis technology peaks at lengths of less than 100 nucleotides, and Palaima says that most circular RNAs are thousands of nucleotides long. Many technical challenges remain here, but he is enthusiastic about the transformative potential of on-demand circular RNA synthesis. "For me, this seems like the future." ■

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