Vladislav Ashikhmin/Alamy Stock Vector; Andrew Stelzer

Bringing RNA into the fold:

Small molecules find new targets in RNA to combat disease

By Shraddha Chakradhar

Three decades ago, Michael Pape received some surprising feedback on a paper that he had written for an undergraduate biology class. The paper focused on the then-recent discovery of ribozymes, RNA-based enzymes that could catalyze biochemical reactions in cells. But his professor thought that he was perhaps overestimating the importance of RNA. Pape was confused by this cynicism. He saw the discovery of ribozymes as evidence that RNA's role in a cell was more than just mediating between genes and proteins.

When Pape began working in the pharmaceutical industry a few years later, he faced skepticism again—this time, when he wanted to target RNA that is translated into a protein that helps to transport cholesterol. "RNA was a throwaway for the pharmaceutical industry" at the time, he says. "We were pretty much just focusing on proteins." Pape is now a cofounder of the RNA-based drug-discovery company Nymirum in Ann Arbor, Michigan. Back then, his "crazy idea," as he calls it, was to determine how RNA that is translated into apolipoprotein-A1, the main protein found in high-density lipoprotein—is degraded. He hoped to find small molecules that could inhibit this degradation to help the body to maintain high levels of this 'good' cholesterol. But the project didn't work. "We just didn't know enough about RNA, nor did we have the right tools to probe RNA," Pape says.

Over the past two decades, the idea of targeting RNA with drugs has moved into the spotlight. Although scientists have known about the secondary structures of ribosomal RNA and transfer RNA since the 1960s, seminal studies conducted in the late 1990s and early 2000s shed light on the fact that RNA, similarly to a protein, is capable of folding into complex tertiary structures. Moreover, scientists are homing in on RNA as a drug target on the basis of an expanded understanding of the different kinds of RNA that exist and have roles in disease. All of this work to better characterize RNA is now reaching a tipping point: at least half-a-dozen academic investigators and companies have programs that are exploring

how to target RNA specifically using small molecules. Most of this work is in the drug-discovery phase, and only a couple of programs have isolated any lead candidates. "It's early days still," says Robert Batey, a biochemist at the University of Colorado Boulder who researches RNA's structural properties.

The idea of targeting RNA with small molecules builds on the success of using antibiotics to target bacterial RNA. Commonly used antibiotics, such as streptomycin and tetracycline, bind to bacterial RNA to inhibit protein synthesis, but those who discovered these medicines did not know the mechanism behind the therapy. "These antibiotics targeted RNA serendipitously, so why not look for RNA targets intentionally?" asks Michael Gilman, CEO of Arrakis Therapeutics, a start-up in Waltham, Massachusetts, that is investigating small-molecule RNA targets.

Sizing down

Beyond antibiotics, other medicines that target RNA have used complementary sequences of RNA known as antisense oligonucleotides to correct diseases caused by RNA splicing errors. A few of these therapies have been approved by the US Food and Drug Administration (FDA), including, most recently, the spinal muscular atrophy (SMA) drug Spinraza (nusinersen) from Biogen and Ionis Pharmaceuticals. Although the success of antisense oligonucleotides has encouraged researchers to continue focusing on RNA as a drug target, these therapies have drawbacks. For one, antisense therapies have to be injected and so are not as convenient for patients. For reasons still unclear, antisense oligonucleotides also tend to act largely in the liver alone, making it difficult for the therapy to be adapted for use against disease in other cells outside the liver. Some therapies seem to work elsewhere in the body, however. Spinraza, for instance, is injected directly into the spinal cord and works in motor neurons. By contrast, small molecules can be administered in pill form and can be modified to have an effect on a broad range of cells in the body, and they are known to cross the blood–brain barrier. "Small molecules are going to be cheaper and easier to deliver," Batey says. "There have been inroads made by both types of approaches, but it's really about marrying the right approach to disease."

The latest efforts to target RNA using small molecules aim for a broader range of diseases than previous types of RNA-based treatments. Many new small-molecule efforts focus on cancers, given that some RNAs encode proteins that can be oncogenic. "We're starting with RNAs whose role in disease is undisputed," Gilman says. He adds that Arrakis is looking at messenger RNAs (mRNAs), such as c-Myc and Ras, which are translated into proteins implicated in many cancers. Beyond cancer, the company is exploring mRNAs that are translated into proteins involved in neurodegenerative and rare diseases. The company raised $38 million in series-A funding in February to put toward all of its drug-discovery platforms. Although the company has identified a few small molecules of interest in its areas of focus, it has not yet optimized any candidates for clinical testing. "We hope to have a candidate at the end of this funding round, which is two and a half years away," Gilman says.

Another company, Novation in Burnaby, Canada, is also interrogating mRNAs in the context of cancer, as well as chronic inflammatory conditions, cardiovascular disease and neurological disorders. "We have found that there are small molecules that can influence mRNA stability," says Dominique Cheneval, a cofounder and president of Novation. The more stable an mRNA is, the more protein it can produce over time, whereas destabilized mRNA degrades more quickly in the cytoplasm and is unable to be translated into much protein.

In the case of inflammation, which often happens as a result of injury or disease, certain mRNAs are more stabilized, says Cheneval. This leads to increased production of chemokine and cytokine proteins. Overproduction usually subsides after the injury has healed, when the stability of these mRNAs is also reduced (*Wiley Interdiscip. Rev. RNA* **1**, 60–80, 2010). However, "if the stabilization doesn't resolve itself, then you get chronic inflammation," Cheneval says. In the context of chronic inflammation, finding a small molecule that could destabilize certain kinds of mRNA could inhibit the translation of inflammatory proteins. Expanding on this idea, Novation is developing a small molecule to reduce the production of cytokines in psoriasis, and Cheneval says that this program is the furthest along. For diseases that result from lack of a specific protein, by contrast, the company is working to identify small molecules that would stabilize mRNA to facilitate protein production. At present, these ideas remain far from the clinic. "We're trying to raise funds right now, so we're still about two years away from validating a lead candidate," Cheneval says.

Meanwhile, the start-up Ribometrix in North Carolina is scouring the three-dimensional (3D), or tertiary, structures of RNA to find potential targets for small-molecule drugs. "If you're going after tertiary structures, it looks like any other kind of drug discovery," says Kevin Weeks, a biochemist at the University of North Carolina in Chapel Hill and founder of Ribometrix. "Just like with protein folding, you can find appropriate clefts and crevices within RNA to target." The company is searching for microRNAs and mRNAs associated with cancer, fibrosis, Huntington's disease and multiple sclerosis. According to Weeks, the company has identified ten targets. "What gets me excited about these small molecules is if you draw structures, they look like normal drugs," Weeks says. "They don't look like anything special." He adds that the company hopes to have multiple clinical leads by 2019.

Matthew Disney

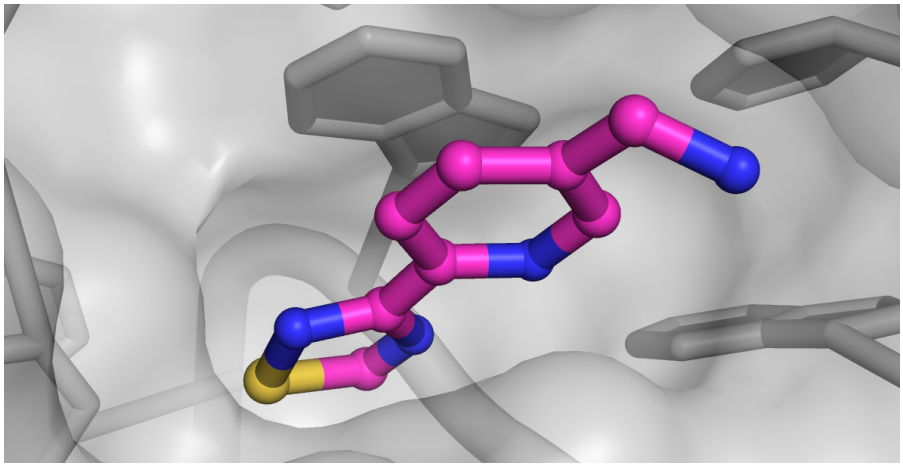
Probing structure: The Scripps Institute's Matthew Disney.

An expanded appreciation for the role of noncoding RNA and noncoding regions of mRNA has also led to drug-discovery programs that are specifically targeting these regions. Researchers now understand that noncoding RNA and noncoding regions of mRNA play important parts in regulating the mechanism and functions of mRNA. Interrupting these regulatory roles through the use of small molecules could thus be another way to target RNA.

PTC Therapeutics in South Plainfield, New Jersey, has a long history of developing various RNA-based therapeutics, including Translarna (ataluren), which is approved in the European Union for the treatment of a type of Duchenne muscular dystrophy. But now, the company also has a program dedicated to finding targets in the untranslated regions of mRNA. "We view RNA as a series of biological processes that can be modulated for benefit," says Nikolai Naryshkin, executive director for biology at PTC Therapeutics. The company is looking at sites where noncanonical base pairing occurs, which enables RNA to fold into 3D structures. The program is still in the discovery phase and has yet to identify a lead candidate in this space.

Structural solutions

The current understanding in the field is that RNA forms complex structures that resemble proteins before it unfolds to form single-stranded mRNA that can be translated into protein. Unlike proteins, RNA does not have an active binding site when it's folded, Gilman



Ribometrix

Finding a fit: How a small molecule (in color) might fit into a cleft in RNA (grey).

says. Consequently, the key to targeting RNA is to lock the molecule into a conformation that prevents it from unfolding. So, any site where a small molecule can lock RNA into its shape is considered an active binding site, Gilman explains.

Nymirum has dedicated its entire platform to identifying sites in RNA structures that are optimal for binding to small molecules. "We look for three-dimensional folds and pockets that specifically bind to small molecules," says Andrew Stelzer, vice-president of Nymirum. Because the company's sole mission is to identify RNA structures, the team doesn't have a therapeutic focus. Instead, it partners with several pharma companies to provide them with possible RNA targets, according to Stelzer. As *Nature Medicine* went to press, none of these partnerships had been made public.

In his lab, Matthew Disney developed a computational tool known as Inforna, which uses RNA sequencing to inform drug design. Specifically, the tool matches microRNA sequences to their structural counterparts. "If we got drugs that bind to specific patterns in RNA, we could then find those patterns *in vivo*," says Disney, a biochemist at the Scripps Research Institute in Jupiter, Florida. "It's like finding a key for a specific lock, and then looking for that lock in other places." Disney says that his team was able to use this method to identify a small molecule that targets microRNA to trigger apoptosis in breast cancer cells. The team is currently optimizing the drug in animal models, but it hopes to take the candidate molecule to clinical trials soon.

One surprising finding about RNA's structure is that the molecule does not statically remain in one conformation, but instead switches among many different forms. "RNA molecules move, they dance, they breathe," Pape says, describing these changes as akin to toggling between different conformations. "What Nymirum has developed is a way to see those movements." Instead of having a single structure with a set number of binding sites, scientists have the opportunity to capture the different conformations that RNA goes through. "Now," Pape says, "you don't just have one target—you can generate a variety of targets."

Scientists are particularly excited about targeting RNA because this approach opens up the possibility of targeting proteins that have been considered 'undruggable.' "Of the roughly 20,000 proteins that exist, only a tiny fraction are druggable by small molecules," Gilman says. This is because proteins often form large, complex structures with other proteins, and the activity of a small-molecule drug against a large molecule such as a complex protein structure is usually insignificant. Moreover, Cheneval says, these larger protein structures don't often have active binding sites to which small molecules can bind. "Proteins that are undruggable can then be targeted through their RNA to prevent translation or create more protein, as needed," Gilman says. Given that only about 15% of proteins are currently druggable, targeting the rest through their RNAs is "an investment worth making," says Gilman.

Renewed focus

Despite the excitement surrounding RNA, it has been a challenge to find drugs that target only specific RNA structures. Different RNA molecules can have similar binding sites, and so drugs can end up binding to RNA that is not meant to be targeted. Weeks explains that this lack of specificity is what foiled attempts in the 1990s to fight HIV by targeting the virus's RNA with small molecules. These efforts often involved targeting a portion of the HIV RNA strand known as the transactivation response (TAR) element. A TAR element is a so-called secondary structure of RNA, in which part of the chain of RNA nucleotides forms a hairpin-like bulge off of the main strand. These hairpin structures are common in human RNA, Weeks says, which, presumably, made it difficult for small molecules to distinguish HIV's TAR elements from similar hairpins in human RNA.

To circumvent any issues with specificity, each of the aforementioned companies is now focusing on tertiary structures, which have more clefts and niches for binding, rather than the secondary structures that result from base pairing. Weeks's company is using mutational profiling to find the select RNA motifs that fold into complex 3D forms. These shapes, which contain potential binding sites, are then tested against a library of small molecules to find appropriate matches. Arrakis's proprietary platform, called PEARL-seq, allows the company to apply small molecules of interest to RNA and then sequence the sample to figure out the tertiary structural component behind the binding site. The company can then characterize the binding sites of interest in detail and distinguish them from other off-target sites to which the molecule could bind. "We know that [the small molecule] is selected for that specific RNA and not other RNAs," Gilman says. Ultimately, researchers think that any issues with selectivity in targeting RNA are no more complicated than those facing other drug-discovery programs.

In the end, the scientists who are mining through small-molecule libraries to target RNA are encouraged by the growing interest in RNA-based therapies. Disney has championed the idea of small-molecule targets for RNA for more than a decade, and has observed a change in attitude toward the field. "My first few grant applications got torched," Disney says. "But people now have a greater appreciation for the role of RNA in biology."

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Correction

In the December 2016 issue, the piece "Sex on the brain: Unraveling the differences between women and men in neurodegenerative disease" (*Nat. Med.* **22**, 1370–1372, 2016), the explanation of the US National Institutes of Health's (NIH's) policy on sex differences in animal research is incorrect. The policy does not require that researchers include equal numbers of male and female animals, but rather that they account for sex as a biological variable in their research design. The error has been corrected in the HTML and PDF versions of the article.