Abstractions

LAST AUTHOR

Since the human genome sequence revealed that we have an unexpectedly low number of genes — around 25,000 — scientists have increasingly looked to RNA to explain the much greater number of complex biological functions that occur within us. Individual RNAs can generate and regulate the expression of many proteins, but it has been technically difficult to track RNA's biochemical fingerprint in living tissues. In 2003, Robert Darnell, a neuro-oncologist at Rockefeller University in New York City, and his colleagues tailored an *in vitro* technique to irreversibly attach RNA via its binding sites to proteins of interest in mouse brain tissue. On page 464, they describe how they modified this technique to create a genome-wide map of the sites where a neuronal protein binds to RNA. Darnell tells *Nature* more.

What led you to do this work?

We were studying a group of rare brain diseases, called paraneoplastic neurological disorders, which arise in conjunction with immune responses to common cancers that target RNA-binding proteins. We wanted to explore — mechanistically and physiologically — how RNA regulation affects both cancer and the healthy brain. To resolve that dynamic, however, we had to figure out a way to monitor RNA biochemistry in living tissue.

What "Aha!" moments drove this work?

The first occurred seven years ago when we realized that we could apply an old test-tube trick to live brains, and bind RNAs irreversibly to brain proteins. That was the basis of a technique called CLIP — crosslinking immunoprecipitation — which allowed us to sequence the bound RNAs. Next, we realized that high-throughput sequencing methods could give us the resolution needed to create a genome-wide RNA-binding map. But we also had to find a way to sort through the millions of sequences for the biologically relevant binding sites. The 'Aha!' solution rested on the assumption that biologically relevant binding should be reproducible from one mouse brain to another.

Did you find much binding that was not biologically relevant?

No, which was a big surprise. Roughly 90% of the sites where we found RNA bound to protein were consistent from animal to animal. So those sites served as big neon signs pointing to crucial points of RNA regulation. We found a large number of RNA interactions with Nova2, the neuronal RNA-binding protein we were studying. This included interactions at sites that determine how RNA can be spliced — that is, cut and pasted together in various ways — to generate different proteins, and at unexpected sites, such as in non-coding-sequence regions. ■

MAKING THE PAPER

Barry Trost

Technique cuts time and resources to make complex potential drug.

Humans have looked to the natural world for medical provisions for millennia. The ancient Egyptians and Greeks knew of the painkilling properties of the opium poppy, and for centuries inflammation, pain and fever were treated with willow-bark extract, the active ingredient of which, salicin, was later used in the synthesis of aspirin. But not all natural therapeutics are as abundant as the willow tree and opium poppy, and without efficient mass production, they would be of little use to modern medicine.

Enter the chemists. They can develop synthetic recipes to recreate complex natural structures. The ideal approach is one that is efficient in terms of time, labour and resources. Barry Trost, an organic chemist at Stanford University in Palo Alto, California, now reports a very efficient method for the complete synthesis of a structurally complex compound called bryostatin 16.

Roughly 20 bryostatins are known to exist, and are found in *Bugula neritina*, a tiny, colony-forming marine creature. The compounds have been shown to have promising antitumour properties and cognition-boosting effects; however, further development is hindered by their complexity. Compared with earlier methods used to make other bryostatins, Trost and his graduate student, Guangbin Dong, cut the total number of steps required to make bryostatin 16 almost in half.

Trost chose bryostatin 16 because it has the potential to be a pivotal intermediate from which most other bryostatins can be constructed. The common bryostatin framework is a 26-member ring, with three 6-member rings embedded within it. "Bryostatin 16 has all the core structural elements, and a few other elements can be easily introduced in the end game," he says. "You could also use it to make many unnatural bryostatins," he adds.

Initially, Trost tried to synthesize bryostatins using a reaction called 'ring-closing metathesis'. This approach can be used to synthesize rings with up to 30 members. Metathesis is so broadly applicable to generating complicated molecules that it earned its discoverers the 2005 Nobel Prize in Chemistry. However, every organic reaction has its limitations, Trost says. "For this particular target, it totally failed."

Trost and Dong turned to a method called a palladium-catalysed alkyne–alkyne coupling reaction. Trost had been studying the reaction in his lab for years, but he had never tried it with such a complicated structure. The technique generated the main bryostatin ring perfectly.

The new method is both resource-efficient — known as 'atom economy' — and time-efficient. For instance, Trost and Dong built the molecule's three pyran rings (rings comprising 5 carbons and 1 oxygen) using simple addition reactions, which avoid the generation of by-products, and so the extra steps that would be needed to remove these. Previous efforts to synthesize bryostatin analogues from scratch have 40-plus steps in the main, or linear, sequence and more than 70 steps in total. Trost's method has a linear sequence of 26 steps and a total of just 39 steps (see page 485).

The work is a major leap forward in synthetic-chemistry efficiency, says Trost. He sees fundamental chemistry research as crucial to improving the quality and diversity of drug candidates pursued by pharmaceutical companies. In drug-discovery efforts, complicated structures such as those of the bryostatins are often simplified to make them easier to make and to amend. But, Trost observes, "you don't want to compromise the structure in terms of its biological function if you don't have to". ■

FROM THE BLOGOSPHERE

How can publishers add value to conferences? James Butcher, publisher of the Nature Clinical Practice journals, reports from the American Heart Association meeting earlier this month in New Orleans, Louisiana, at In The Field (http://blogs.nature.com/news/blog/2008/11/how_can_journal_editors_help_c.html). He notes that many major papers presented at the conference were simultaneously published in journals, cutting across several publishers. For the authors, having the published paper available for the meeting presentation helps the media to report on the study accurately. And when the news breaks in the popular press, physicians can immediately read the complete work to understand what the study means for their patients.

Peer-reviewing and editing a paper accurately to tight timelines is expensive and difficult. But Butcher believes that it is worth the additional effort from authors, editors and publishers to expedite papers so that they can be released to coincide with oral presentation of data at conferences, because "everyone benefits from the end result". ■

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