

RNA interference works with double-stranded small non-messenger RNA molecules, which cleave mRNA that is homologous to their own sequence. Another knockdown tool is the application of antisense molecules. These oligonucleotides inhibit the translation of RNA by binding to a complementary sequence on that RNA molecule. Finally, ribozymes can be employed. These molecular scissors cut RNAs at specific positions.

Companies usually only use one of these approaches to study signalling cascades. However, the target validation company Atugen (<http://www.atugen.com>) uses all three of them [1,2]. 'If we get the same phenotype with all three methods, we can be sure it is not an artefact,' explains Atugen's CSO Klaus Giese. And in more recent months, Atugen have also employed those three knockdown tools to discover novel targets, namely in the PI3 kinase pathway.

A tale of discovery

One of the reasons why PI3 kinase can become overactivated is because tumour

suppressors, such as PTEN (phosphatase and tensin homologue deleted on chromosome ten), lose their function. Atugen scientists mimic this by knocking down the expression of PTEN mRNA. They then study changes in gene and protein expression over time using gene chips and 2D protein gels. 'All in all, we have identified 300 targets that play a role in oncology,' says Giese. 'Some 150 of those have been described before. The other 150 targets have not yet been linked to tumour development and invasion.'

However, the crucial question is whether these targets really have a causative role in cancer. To determine this, Atugen's scientists are currently running the 150 new candidate genes through their target validation programme: they are systematically silencing those 150 new candidate genes and testing them in cell-based proliferation and invasion assays, using prostate and breast cancer cell lines, as well as samples from cancer patients. The most promising candidates (23 so far, according to Giese) are being assessed in orthotopic mouse models.

Giese reveals that, so far, they have found four new targets that have been validated

in vivo. Two of those are kinases, and Giese expects that they can be inhibited with small-molecule drugs. The other two targets are considered non-druggable because they are lacking a domain that can be easily blocked with small-molecule drugs. But Giese hints that they are involved in protein-protein interactions. 'One could try to find their binding partners, which in turn may be druggable,' he suggests.

At present, Atugen do not have their own drug discovery platform and, therefore, rely on partners in the pharmaceutical industry to develop inhibitors of their target molecules. But having made the move from target validation to target discovery, their ambitions do not stop there. Giese says they are now migrating into the drug discovery business as well.

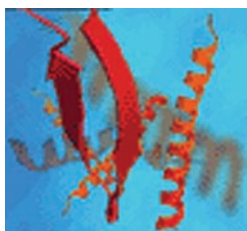
References

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New high-throughput NMR

Thomas S. May, freelance writer

Proteins are the 'stuff of life' and discovering their structures can be a significant step towards the development of



new drugs. However, determining protein structures is a difficult task that requires a great deal of expertise, sophisticated instruments – and a lot of time.

Now, a new method of analyzing data acquired by nuclear magnetic resonance (NMR) instrumentation promises to cut

down – dramatically – the amount of time needed to identify protein structures. As a result, the new, high-throughput method devised by scientists at the University at Buffalo (<http://www.buffalo.edu>) will make drug development considerably faster, the researchers claim [1].

Determining protein structures

So far, two methods have been used primarily to determine protein structures: NMR spectroscopy and X-ray crystallography. The vast majority of protein structures have been solved using X-ray crystallography,

a method that enables scientists to explore the details of protein structures but requires crystallization of the protein being studied.

NMR does not entail crystallization but is usually slower than crystallography and is limited to solving the structures of small and medium-sized molecules. NMR uses powerful magnets to determine the chemical shifts of the atoms that make up the protein molecule. The technique involves conducting a series of 'multi-dimensional' experiments, which measure the resonance frequencies of – and the distances between – the nuclei of the atoms.

Because a large number of experiments are typically needed to characterize proteins, it can take a long time (several weeks to years) to solve a single protein structure using NMR spectroscopy. However, a new method developed by Thomas Szyperski of the University at Buffalo and Seho Kim (Rutgers University; <http://www.rutgers.edu>) could shorten the amount of time required to determine protein structures by several orders of magnitude [1].

Faster speed, higher precision

This technique – GFT NMR – has the potential to enable scientists to take full advantage of the newest, highest-field NMR machines and the newly available cryogenic probes, Szyperski says. ‘With this new method, we’ve increased data collection speed by orders of magnitude,’ he states.

The new technique uses G-matrices (a system of linear equations) and Fourier transforms to calculate resonance frequencies in NMR experiments, hence the name GFT NMR. ‘We record larger numbers of low-dimensional NMR spectra, and using the G-matrix we can linearly combine them to retain the information of the high-dimensional experiment,’ Szyperski explains. ‘This way, we can sample spectra much more rapidly and get not [only] the resonance frequencies themselves, but multiple sums and differences of them, which gives us higher precision,’ he adds.

The advent of GFT NMR is a breakthrough in NMR method development, according to Volker Doetsch, a pharmaceutical chemist at the University of California San Francisco (<http://www.ucsf.edu>). ‘It will dramatically reduce the measurement time that is currently necessary for recording multi-dimensional NMR spectra,’ he said.

The new GFT NMR method, combined with cryogenic probe technology, promises to revolutionize NMR technology by enabling researchers to conduct multi-dimensional NMR experiments in minutes or hours, as compared to days or weeks, Doetsch contends. ‘The biggest impact of the new technology will be on the currently ongoing structural genomic projects, which aim to determine as many protein structures as possible,’ he notes.

Doetsch suggests that a fully automated and robust data analysis method, in combination with the fast GFT method, will make NMR a fast and efficient tool for protein structure determination. ‘Since many drug development projects rely on structural information, the new technique will also have an impact on future drug development projects,’ he predicts.

Low sensitivity still a problem

Not all experts are convinced that the new GFT NMR technique will dramatically reduce the time required to determine protein structures. Some claim that NMR is a notoriously insensitive method, and that

this will remain a hindrance even when using the new cryogenic probes and higher-field NMR machines. The reason for this is that cryogenic probes have only yielded a factor of three in sensitivity, and higher fields only increased sensitivity by about a factor of two.

Usually, for studies conducted in drug discovery, limitations are sample availability, low solubility of proteins and large line widths. These problems lead to severe losses in sensitivity, such that NMR experiments are usually conducted at the detection limit, some experts argue.

Because of the low sensitivity, GFT spectra will be useful only for ‘well-behaved proteins,’ which are small and highly soluble, says Gottfried Otting of the Australian National University (<http://www.anu.edu.au>). ‘Unfortunately, typical drug targets are bigger and less soluble than one would like,’ he adds.

The skeptics also point out that, so far, GFT NMR has only been tested using one small molecule, where the chemical shift assignments were already known. Therefore, they say that it remains to be seen whether this new technique will indeed dramatically speed up the characterization of larger protein molecules and make the process of drug discovery significantly faster.

Reference

- 1 Szyperski, T. and Kim, S. (2003) GFT NMR, a new approach to rapidly obtain precise high-dimensional NMR spectral information. *J. Am. Chem. Soc.* 125, 1385–1393

Nematode model for obesity

Vida Foubister, freelance writer

The search for genes that are involved in fat accumulation and storage has unearthed a new model organism – worms. After years of studying other animals, most commonly mice, two research groups turned to *Caenorhabditis elegans* and found genes that are involved in fat regulation and which appear to be conserved in mammalian models. This raises the possibility that

further research into the genetics of fat storage in *C. elegans* could hasten the identification of therapeutic targets for obesity, diabetes and other metabolic diseases.

An invertebrate model

The appeal of *C. elegans* lies in its simplicity. It has a four-day generation time and

produces 300 progeny, and it is small – about 10,000 worms fit on a 6 cm petri plate. In essence, worms enable scientists to perform relatively cheaply and efficiently what would be considered ‘big science’ using a mouse model.

Worms, however, are farther removed from humans and thus all findings require translation and verification. ‘You trade the