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Innovations

Benitec, Ltd.

Gene Silencing from Down Under

"If you could invent a tool to allow you to affect gene function and also give you a generic or universal therapeutic, you couldn't think of anything better." Ken Reed, PhD, Director of Research and Technology at Benitec, Ltd., has been researching RNAi for the past several years and still speaks with great enthusiasm and amazement when discussing the novelty of RNAi and how Benitec technology makes use of nature's own cutting-edge capabilities.

Hailed by *Science* as "2002's Breakthrough of the Year," RNA, which has always played second fiddle to DNA, is now credited as the molecule behind the gene silencing mechanism of RNA interference (RNAi), plants' natural defense mechanism against transposons and viruses, and also potentially involved in tissue differentiation in mammalian cells. While the crucial question of why RNAi emerged in mammalian cells remains unanswered, Benitec is steadily pursuing the therapeutic benefits of targeted mammalian gene suppression using specific synthetic RNAi.

In mammalian cells, small double-stranded (ds) short hairpin RNAs that result from an unknown signaling mechanism interact with the RNase-III-like enzyme named Dicer. Dicer then cleaves the hairpin dsRNA into 21–25 nt length dsRNA, called siRNA (small interfering RNA), which is then incorporated into a multisubunit protein complex called the *R*NA-*i*nduced *s*ilencing *c*omplex (RISC). The process of coupling siRNA with RISC mediates degradation of any RNA that shares a similar sequence to the siRNA, effectively silencing the respective gene.

Discovery of this phenomenon immediately spurred research into how scientists could use this mechanism to control gene suppression, i.e., silencing particular genes by introducing synthetic siRNAs into cells. Early attempts in nematodes as well as cultured mammalian cells proved successful in silencing genes of choice. However, in animal models, the same technique was not applicable because the introduction of dsRNAs longer than 30 nt elicited a cell autonomous stress response resulting in cell stasis or apoptosis. Shorter fragments of 21–25 nt siRNA were successfully introduced into animals, but these short duplexes were only effective for a limited number of days. What the drug discovery community desired and needed was continuous expression of the specific dsRNA without eliciting the cell autonomous stress response.

"It is very, very, very difficult to get bare nucleic acids into cells in a living animal," explains Dr. Reed. Less than a year ago, discussions at an RNAi conference focused heavily on how best to package and deliver RNAi. Benitec found a solution with their DNA directed RNAi (ddRNAi) technology, which first introduces a DNA construct into a cell, allowing the longer dsRNA to be transcribed from this sequence intracellularly and thus evading the cell autonomous stress response. With this approach, Benitec has successfully used lentiviruses to deliver RNAi into hippocampal neuron cells. And, unlike other viral vectors, lentiviral vectors do not need to be injected into the cell nucleus but simply into the cell itself. Furthermore, lentiviral vectors allow for permanent gene suppression, as they readily and stably integrate into the chromosome at a site directed by the vector's gene targeting sequence. For transient suppression, Benitec takes advantage of adenoviruses since these do not integrate into the chromosome but survive independently within the cell. Furthermore, adenoviruses can be manipulated to thrive only for a defined period of time, from a few weeks to a few months. More recently, Benitec has successfully used lentiviruses to introduce RNAi into mice. As observed in melanoma cell lines, in transgenic mice with induced melanoma, ddRNAi suppressed expression of the culprit oncogene.

"Can you believe that a property of cells of all multicellular organisms that is this universal, this potent, and this brilliant wasn't discovered until 1998? It's nature's best-kept secret." – Ken Reed, PhD, Director of Research and Technology, Benitec

Dr. Reed clarifies that science has seen a fast rise in RNAi's potential as a therapy compared with other more arduous gene therapies because RNAi involves shutting down the expression of a gene, as opposed to the replacement of a gene. While the ddRNAi approach will not work as well for those diseases caused by rogue genes, it works well for viral diseases and cancers, in which the disease-causing genes are not essential.

"The specificity of this is brilliant," says Reed. He explains that current drug therapies target approximately 500 proteins out of an estimated 5,000 "druggable" targets. Reed continues, "It is difficult to find a small molecule that will hit one target and nothing else." It is self-evident that an RNA sequence confers absolute specificity. A variety of promoters to be used with these DNA constructs provide control of this specific sequence in human and animal models. Promoters ensure that the DNA is robustly transcribed, providing the cell with continual dsRNA. Additionally, by using a promoter that is only active in certain cell types, another layer of control can be obtained. Reed adds that the vectors can be cell-type speci-

fic too, facilitating control of this ddRNAi technology on many levels.

One of the most fascinating aspects of RNAi is the road to its discovery. Reed exclaims, "Can you believe that a property of cells of all multicellular organisms that is this universal, this potent, and this brilliant wasn't discovered until 1998? It's nature's best-kept secret." RNAi was discovered serendipitously in plant research. In the 80s, it was noted that certain transgenes in plants were becoming less and less effective through successive generations. In 1990, Dr. Richard Jorgensen, in an effort to make "bluer" petunias, inserted an extra copy of the "blue" gene into plants. In addition to blue flowers, some of the plant progeny produced all white flowers. It was then observed that an endogenous gene could be turned off with the transformation of an extra copy of that same gene. These transgenic plants were observed to have an abundance of messenger RNA, which was subsequently destroyed. With further study in several organisms, the marvel mechanism of RNAi was subsequently revealed.

It is thought that RNAi is a significant component of the plant defense mechanism. This defense most likely evolved against transposons, which are so virulent that without a countering mechanism an organism will go extinct in a few generations. Reed explains, "Plants really do [have] it tough—they are rooted in the ground, they can't escape, they can't evade things that worry them, they don't have a systemic immune system, and yet they have to endure all the abuses that mammalians are subject to but under greater constraints." For mammals, however, the natural role of RNAi is not as clear. RNAi does not appear to have a role against viruses in mammals, but it is thought to participate in cell differentiation. Despite the differences and unknowns, it was Dr. Michael Graham who made the astute revelation that this RNAi phenomenon he first came across in plants was a general mechanism and not plant specific. Graham, who has been studying RNAi for the past seven years, is Benitec's Principal Inventor and Research Scientist.

Located in Queensland, Australia, Benitec was founded in 1997 to develop and commercialize research from Queensland's Department of Primary Industries (DPI). Since issuance of their 1998 ddRNAi patent in mid 2003, Benitec has had dominant patent position for ddRNAi gene silencing. In the fall of 2003, Benitec announced success in disabling multiple genes simultaneously using ddRNAi. The ability to silence multiple genes lowers the possibility that any one cell or virus can develop resistance to an RNA interference treatment. Most cancers and HIV, in particular, have high mutation rates. Additionally, type II diabetes, autoimmune disorders, and cardiovascular dysfunctions result from multiple gene defects. Thus, multiple gene silencing would prove highly beneficial for many conditions. At present, Benitec has been successful with simultaneously silencing three genes, and they are currently working on increasing this number. The approach to multiple gene silencing is quite similar to the single gene ddRNAi approach. The viral DNA vector encodes for several genes separated by short nonsense sequences.

In addition to therapeutic gene silencing, Benitec is interested in using ddRNAi in genomic studies to ascertain the biological function of genes. Similarly, they are using ddRNAi for disease modeling. In cultured cells and animal models, genes that are suspected to be causal to a disease can be silenced, and the result can be analyzed for future treatments. Also, target validation can be carried out with ddRNAi to determine if a known disease-associated gene is likely to result in a therapeutic effect.

Benitec is fortunate to be in the driver's seat on this emerging technology. Amazingly, this natural mechanism discovered within the last decade is now recognized worldwide as the simplest and most precise method for shutting down the activity of any specific gene. At this stage, Benitec, with its patent strength and surprisingly small work force of eight scientists, is seeking relocation to the States. Benitec is no doubt sitting on a goldmine of science discovery and entrepreneurial success.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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