Meeting report

RNAi for research and therapy

Michael A Goldman

Address: Department of Biology, San Francisco State University, San Francisco, CA 94132, USA. E-mail: goldman@sfsu.edu

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A report on the RNAi symposium at the Cambridge Healthtech Institute 'Beyond Genome' Conference, San Francisco, USA, 21-24 June 2004.

We have come so far so fast in understanding RNA interference (RNAi) and its central role in biology. Rarely has a novel mechanism in molecular genetics had such broad implications, ranging from gene therapy and drug discovery to our very understanding of what the word 'gene' means. Every major pharmaceutical company has a substantial effort now in RNAi technology, and among the smaller biotechnology companies RNAi is the mainstay of several, with catchy names like Sirna Therapeutics. RNAi is the current buzzword in academic and corporate circles, and it certainly captured the imagination at the RNAi symposium of Cambridge Healthtech Institute's 'Beyond Genome' conference. This report focuses on work presented at the meeting that aims to elucidate the natural cellular role of small interfering RNA (siRNA) molecules and microRNAs (miRNAs), and some of the very exciting advances in the use of RNAi technology in drug discovery and therapeutics.

It is becoming ever clearer that siRNA molecules of various sorts play roles in normal development in a wide variety of organisms. The first of this class of molecules to be discovered, *lin-4* in *Caenorhabditis elegans*, is transcribed as a 61 nucleotide precursor to a 22 nucleotide miRNA that has a role in the regulation of developmental timing. Pre-miRNAs are processed by two different members of the RNase III family, Drosha and Dicer, explained John Rossi (City of Hope National Medical Center, Duarte, USA). An miRNA achieves its functional state as part of a nucleoprotein complex called RISC (RNA-induced silencing complex). Although miRNA molecules are thought to interfere with mRNAs, there is evidence that some of them, and other non-coding RNA molecules, are involved in transcriptional silencing and heterochromatin formation as well.

The concept of RNAi has revised our thinking in many arenas. First, post-transcriptional regulation has always played second fiddle to transcriptional regulation for students of metazoan gene expression. Now we know that interfering RNAs are not just the sledge-hammers we can use to inhibit expression in an experimental system, but are in fact an integral part of the fine-tuning of gene expression in normal cells. The class of miRNA genes, which produce short-hairpin RNA (shRNA) precursors of interfering RNA molecules, probably numbers in the thousands, representing a category of *trans*-acting regulatory genes never before imagined. RNAi has become the tool of choice in many knock-out or knock-down gene-expression experiments, now nearly as ubiquitous in laboratories as PCR. Using RNAi as a tool shows great promise in the discovery of new drug targets, and interfering RNAs or vectors producing precursor RNA molecules are already in testing as therapeutic agents.

Several presentations at the meeting help reinforce the idea that miRNAs may have a prominent role in development. Kenneth Kosik (Harvard Medical School, Boston, USA) has identified many miRNAs in neural tissue. A subset of these was predominantly expressed in or confined to neural cells in mammals, and some of these miRNAs were developmental-stage-specific. Evidence that key developmental gene clusters contain and are regulated by miRNAs was presented by I-Hung Shih (Massachusetts Institute of Technology (MIT) and Whitehead Institute, Cambridge, USA), who described the occurrence of miRNA genes (including *miR-196* and *miR-10*) within insect and mammalian homeobox (*HOX*) gene clusters. Several of the *HOX* genes are down regulated in response to expression of *miR-196* in cell culture. In what is apparently the first direct validation of miRNA-mediated repression *in vivo* in mammalian systems, Shih and colleagues subsequently showed that *miR-196* mediates cleavage of its target, *HOXB*, in mouse embryos. Shih believes that miRNA-regulated targets in mammals are involved in a broad range of functions, including signaling and cell growth, with about 20% of the targets being transcription factors; Shih therefore

contends that miRNAs will become increasingly important in our understanding of developmental events.

According to Markus Stoffel (Rockefeller University, New York, USA), pancreatic beta cells use RNAi in the production of insulin. This finding is especially relevant today, when the incidence of type II diabetes and of obesity is nearing epidemic proportions in industrialized nations, with type II diabetes affecting nearly 12 million people in the USA. Stoffel found novel miRNAs whose expression is confined to pancreatic beta cells, and showed that one of them (mi208) inhibits glucose-stimulated insulin secretion. This is not only a fascinating example of the role of endogenous interfering RNA molecules in development and physiology, but it also suggests novel means of therapeutic intervention.

Complex diseases like cancer and diabetes may be difficult to study in traditional experimental systems. Luk Van Parijs (MIT, Cambridge, USA) thinks RNAi is the solution to these problems. NOD mice are commonly used to study type I diabetes; in these animals, an autoimmune response rapidly destroys pancreatic beta cells. Van Parijs used RNAi to knock down expression of the immune cell receptor CD8 and observed a marked reduction in the occurrence of diabetes in NOD mice. This finding suggests that inhibition of the expression of candidate disease-susceptibility genes using RNAi can provide useful data. Van Parijs has extended his approach to the study of cancer-susceptibility genes. The typical output from a microarray experiment is a list of genes whose expression levels differ significantly between normal and cancer tissue, but this cannot give any indication of a causal relationship. In order to separate cause from effect, Van Parijs has used RNAi sequentially on a set of genes whose expression is altered with the aim of determining whether or not the disease state is abrogated by repression of these genes. For instance, if expression of a gene is increased in non-Hodgkin's lymphoma, and if that increase is of a causal rather than a correlative nature, then a knock-down in expression with RNAi should result in an abrogation of the phenotype. The rapidity of RNAi experiments allows the study of single-gene and multi-gene expression changes. To facilitate this work, Van Parijs has developed a bifunctional lentiviral vector which expresses both Myc and a particular shRNA. The shRNAs were obtained from an RNAi library focusing on known or putative cytokine-regulated genes, with four shRNAs tested for each gene. The results begin to delineate a network of genes that regulate c-Myc-induced transformation and might provide novel therapeutic targets.

William Pardridge (University of California, Los Angeles, USA) used intravenous injection of RNAi as an astoundingly successful therapy for brain cancer in mice. The epidermal growth factor receptor (EGFR) plays an oncogenic role in 90% of primary brain cancers and in a substantial fraction of metastases. Pardridge's group introduced an RNA to interfere with EGFR via a nonviral vector in immunoliposomes with cell-surface receptors that target them to brain. Thus overcoming the blood-brain barrier, the group achieved an 88% increase in survival time in mice, and a 95% knockdown in EGFR expression in cell culture. Recently published work has drawn attention to Iressa, a drug targeting EGFR, as an example in which pharamacogenomic analysis is useful, as the drug is efficacious in patients with certain EGFR mutations but not others. Pardridge contends that, unlike Iressa, RNAi-based gene therapies can be designed for both normal EGFR and the mutant forms of EGFR found in many tumors.

Continuing the theme of RNAi-based therapies, Beverly Davidson (University of Iowa, Iowa City, USA) presented a novel and exciting approach to therapy for neurodegenerative disease. Spinocerebellar ataxia type 1 (SCA1) results from the expansion of a trinucleotide repeat (CAG) in the coding region of the *ataxin-1* gene, resulting in a lengthened polyglutamine (polyQ) tract in the protein itself. Over time, this leads to the accumulation of intranuclear protein aggregates and progressive neural degeneration. Davidson worked with a transgenic mouse model of SCA1 in which expression of the mutant human *ataxin-1* gene is confined to Purkinje cells of the cerebellum. The mice demonstrate both typical brain pathology and ataxia in a 'rotarod' test that requires them to cling on to a rod while it rotates. Davidson and her colleagues prepared an adeno-associated virus (AAV) vector containing an shRNA recognizing the mutant *ataxin-1* transgene. This was then delivered by intracerebellar injection directly to the brain region affected. Treated animals showed a significant improvement in performance on the rotarod test, and brain sections revealed improved histology. *Ataxin-1* inclusions in the Purkinje cell nuclei were also completely resolved. The findings are especially promising because several additional ataxias, including Huntington disease, are also caused by polyQ tracts, and RNAi may prove an effective means to treat them. Davidson cautioned, however, that some interfering RNAs were not successful at inhibiting *ataxin-1*, and considerable trial-and-error may be required en route to a successful therapy.

The key message from the conference is that we have by no means seen the last of RNAi as an intriguing biological phenomenon, a tool in basic research, and a new molecular approach for the pharmaceutical industry. Its rapid rise from startling new phenomenon to canon leaves little room for caution. But Aimee Jackson (Rosetta Inpharmatics, Seattle, USA) and Kevin Fitzgerald (Bristol-Myers-Squibb, Princeton, USA) noted that RNAi is not the magic bullet it once seemed; off-target effects are common and considerable optimization is necessary. Fitzgerald further emphasized that while typical small molecular drugs inhibit a particular function of a protein, leaving the rest of the protein and all of its interactions intact, RNAi can completely eliminate the target protein, abolishing all of its complex interactions at the same time. While RNAi plays a key role in basic research in genome biology and in drug-target validation and

therapeutics, the other symposia at the conference dealt with the fields of bioinformatics, proteomics and systems biology. These latter areas, taken together, are becoming increasingly important as the drivers of new information, and of new ways of looking at the vast data available to us now. Despite the rapid advances in RNAi and in the ability to characterize cellular proteins on a large scale (proteomics), informatics approaches are needed to speed up an otherwise excruciatingly slow and costly process of drug development. Systems approaches promise to model cellular processes in much the same way that aeronautical engineers model planes before building and flying them. *In silico* testing prior to animal and human studies, advocated by the Institute for Systems Biology (Seattle, USA) and companies like Entelos (Foster City, USA), is part of the US Food and Drug Administration's new 'critical path' initiative to move more novel drugs to market more efficiently and less expensively.