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New RNA Libraries Can Inactivate Human Genes Selectively

Researchers have produced vast libraries of short segments of ribonucleic acid (RNA) that can be used to turn off individual human and mouse genes to study their function.

The libraries will be made widely available to laboratories studying human biology and disease. The researchers are optimistic that the libraries will become a powerful research tool for gene analysis and discovery.

"The library works efficiently as a screening tool."

— Stephen J. Elledge

Two independent research groups reported on their respective RNA interference (RNAi) libraries in the March 25, 2004, issue of the journal *Nature*. Gregory Hannon of the Cold Spring Harbor Laboratory and Howard Hughes Medical Institute investigator Stephen J. Elledge at Harvard Medical School and Brigham and Women's Hospital led the first group. The joint lead authors were Patrick Paddison, Jose Silva and Douglas Conklin in Hannon's laboratory. René Bernards of The Netherlands Cancer Institute led a second group.

Commenting on the significance of the studies in the journal *Nature*, Andrew Fraser at the Wellcome Trust Sanger Institute wrote: "As no single laboratory can specialize in every aspect of gene function, the general availability of these [short hairpin RNA] libraries as a communal resource is a major step forward, harnessing the screening expertise of the entire mammalian-cell research community."

RNA interference is a technique used with much success by researchers to switch off genes in lower organisms, including the fruit fly *Drosophila* and the roundworm *C. elegans*. Researchers stumbled upon this powerful tool for gene analysis when they discovered that introduced sequences of double-stranded RNA identical to a target messenger RNA actually triggered degradation of the messenger RNA.

Messenger RNA molecules are the genetic templates for proteins. In constructing proteins, the mRNA template is transcribed from DNA genes

and transported to the ribosomes—the cell's protein “factories” that are large complexes of protein and RNA. RNA interference is a technique that essentially shuts down the activity of the gene under study.

“But RNAi didn't work in the vast majority of human or mouse cells because there are additional antiviral responses that recognize double-stranded RNA,” said Elledge. “While the machinery to do RNAi is in mammalian cells, the antiviral machinery makes the introduced RNA toxic, and the cells die.”

Researchers subsequently discovered that short segments of interfering RNA could be introduced into mammalian cells and remain unnoticed by the antiviral machinery, said Elledge. Furthermore, they discovered that the cell itself could be engineered to make interfering RNAs by introducing the gene for short hairpin RNA molecules that fold back on themselves to create a small RNA.

To construct a library of mammalian genes for short hairpin RNA molecules, Hannon and his colleagues first had to settle on an optimal design for a short-hairpin-RNA molecule. “We tested a lot of different things—for example, the length of the hairpin, the loop structure, the structure of the transcript and what promoters to use,” said Hannon. “And we arrived at an optimal structure for this phase of the science.”

Hannon emphasized, however, “that set of parameters is something that is going to evolve continuously. There have been many advances over the last year in understanding of the biochemistry of RNAi. So, we are now constructing even more effective structures and even more effective delivery vehicles which will be built into future generations of this library.”

Once an optimized basic design of the short hairpin RNA molecule was finished, the researchers then produced a library of genes for short hairpin RNAs that could target 9,610 human genes and 5,563 mouse genes. The genes chosen were those that were likely to be involved in human disease, or to be key molecular switches in the cell.

The library of genes was integrated into a retroviral vector that was capable of shuttling the genes into other cell types. The researchers also incorporated a DNA “bar-coding” system, by which each RNA molecule can be tagged with a unique DNA sequence.

By determining the sequence of a given bar code for a short hairpin RNA, researchers using the library to screen for genes affecting a specific cellular process can identify which RNA molecule among the thousands in the library is switching off the activity of a particular gene.

But the retroviral vectors used for shuttling the short hairpin RNAs into cells only went so far. They were not efficient for getting genetic short hairpin RNAs into all cell types. That's where an innovative technique developed by Elledge and his colleagues came in handy. This technique, called “mating-assisted genetically integrated cloning” (MAGIC), greatly assisted the transfer of the short hairpin RNA library into all cell types via bacterial

mating.

In order to validate that the library worked in human cells, the researchers tested it in a genetic screen designed to report defects in human proteasome function. The proteasome is a key component of the machinery by which the cell breaks down unwanted proteins. “This was a thorough test of the system because there are a great number of different genes whose loss could interfere with proteasome function,” said Elledge. “We found quite a few genes, and concluded that the library had worked quite efficiently as a screening tool.”

Current efforts are aimed at increasing the number of human genes targeted by the library, said the researchers. They emphasized that the current and future libraries will be made available to the research community at a nominal cost through Open Biosystems, Inc., in Huntsville, AL.

“For the first time, this gives us the opportunity to do a version of forward genetics in mammalian cells—where we can look at hypomorphic mutations, ranging from mild to severe, and their consequences on phenotypes, on what will eventually evolve to a genome-wide scale,” said Hannon. “Thus, these libraries will evolve into an important resource for the research community.”