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## Researchers Adapt RNA Interference to Study Gene Function on a Large Scale

A method for determining the function of large numbers of genes is being developed and piloted by Howard Hughes Medical Institute (HHMI) researchers at Harvard Medical School. In a trial of the technique, the researchers characterized the role in growth and viability of nearly all the genes in the genome of the fruit fly *Drosophila*.

Although the fruit fly genome was chosen for the first study, the researchers are confident that their technique can be applied to any organism, including humans. "A major challenge now that many genome sequences have been determined, is to extract meaningful functional information from those projects," said HHMI researcher Norbert Perrimon, who directed the study. "While there are a number of analytical approaches that can measure the level of gene expression or the interaction between proteins, ours is really the first high-throughput, full-genome screening method that allows a systematic interrogation of the function of every gene."

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The research team, which included Perrimon and colleagues at Harvard Medical School, the University of Heidelberg and the Max Planck Institute for Molecular Genetics in Germany, described its technique in the February 6, 2004, issue of the journal *Science*.

The screening technique developed by Perrimon and his colleagues builds on methods developed in one of the hottest areas of biology, RNA interference (RNAi) research. In RNAi, double-stranded RNA (dsRNA) that matches the messenger RNA produced by a given gene degrades that messenger RNA—in effect wiping out the function of that gene in a cell. RNAi is widely used as a research tool to selectively erase the cellular contributions of

individual genes to study their function.

In their mass screening technique, Perrimon and his colleagues first created a library of 21,000 dsRNA that corresponded to each of the more than 16,000 genes in the *Drosophila* genome. They then applied each of these dsRNA molecules to cultures of *Drosophila* cells and assayed how knocking down the function of a targeted gene affected cell numbers in the cultures. This basic measure, said Perrimon, revealed genes that are not only involved in general cell growth, but also in the cell cycle, cell survival and other such functions.

The researchers then selected 438 genes for further characterization. The degradation of these genes produced profound affects on cell number. “Out of this subset, we found many that produced proteins involved in general metabolic processes such as the ribosomes that are components of the protein synthesis machinery,” said Perrimon. “But we also found genes that are more specific to cell survival.”

According to Perrimon, only 20 percent of the genes that were identified had corresponding mutations — an important characteristic for studying gene function. “The classic approach to studying gene function is to identify mutations in genes and select those that produce interesting phenotypes that yield insight into function,” said Perrimon. “But this approach has never really given us access to the full repertoire of genes. With this high-throughput technology, however, we can study the function of a complete set of genes. We can systematically identify all the genes involving one process.”

The researchers also found that a large proportion of the genes identified in the genome screen do not code for a known protein, “which means that there are a great number of proteins that remain to be identified,” said Perrimon.

Perrimon emphasized that “while in this paper we describe applying this technique only to one specific assay—the effect on cell number—we are already applying the methodology to determine the roles of genes in many other aspects of signal transduction and cell biology. We are using the technique to study gene function in pathways involved in communication between cells and those associated with cancer; as well as aspects of cell biology such as cell shape or cytoskeletal organization.”

Once researchers amass data on gene function from many such assays, said Perrimon, they can begin to group genes according to the signatures of their response in such assays. Such groupings will offer a guide to further biological studies to map the functional cellular protein machinery that the genes produce in living organisms.

“The idea is that with this information we might be able to connect a number of proteins together, implying that they may be working either in the same pathway, or they may be part of the same molecular machine in the cell,” said Perrimon.

The RNAi assay will contribute to the screening of new drugs, he said. “One exciting aspect of this approach is that we can combine our assay with screening of potential therapeutic compounds,” he said. “One of the big problems in the pharmaceutical industry is that researchers may discover pharmacologically active compounds but have no idea what their targets are in the cell. However, it would be possible to perform coordinated screens—one for compounds that interfere with a target pathway and an RNA interference screen for genes that act in that pathway. This correlation would allow you to match the compounds with the proteins they affect in a much more useful way.”

Similarly, said Perrimon, researchers can use RNAi to selectively target genes in cells infected with pathogenic bacteria, to determine which ones affect the bacteria's ability to infect cells. Such a screen could yield key targets for pathogen-specific antibacterial drugs, he said.