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Genetic Tool Reaps Rich Harvest

In one fell swoop, scientists have increased from dozens to hundreds the number of known genes that control crucial steps in the development of many organisms from fruit flies to humans.

Using the power of a genetic technique known as RNA interference, the researchers identified some 238 potential regulatory genes in the Wnt signaling pathway. Understanding this pathway will provide researchers with new insight into the development of cancers of the liver, colon, breast, and skin, as well as other genetic diseases.

The scientists published their functional genomic analysis of the Wnt pathway in the April 8, 2005, edition of *Science Express*, which provides rapid electronic publication of selected papers in the journal *Science*. The research was conducted by lead authors Ajamete Kaykas and Ramanuj DasGupta. They work, respectively, in the laboratories of senior authors Randall Moon at the University of Washington School of Medicine and Norbert Perrimon at Harvard Medical School. Both Moon and Perrimon are Howard Hughes Medical Institute investigators.

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- **Norbert Perrimon**

The Wnt signaling pathway, which has been evolutionarily conserved in organisms, governs an array of processes central to cellular development and function. These processes include cell proliferation, differentiation of cells into specialized tissues, and establishment of distinct regions within the cell. Changes in the Wnt pathway have been implicated in a range of human disease, including cancer and Alzheimer's disease.

According to Moon, until the new analysis, identifying the genes that produce components of the Wnt pathway had been a long, hard, gene-by-gene slog. Basically, researchers would mutate a single gene in an organism and analyze whether it affected the pathway.

“At the time this screen was conducted, the total number of genes implicated in the pathway was probably on the order of forty to sixty,” said Moon. “But this was in multiple organisms, and it wasn’t really clear whether all of these components functioned in one organism or whether people were comparing different genes in different critters. There had been no systematic single-organism genome-wide screen to ask, ‘what is an approximation of the number of genes that can affect this pathway?’” he said.

Attempting to develop a more efficient approach, DasGupta and Kaykas screened fly cells for Wnt-associated genes using a technology called high-throughput RNA interference screening, developed in Perrimon’s laboratory. RNA interference (RNAi) is considered one of the most important new techniques for analyzing gene activity. It relies on the fact that a short segment of double-stranded RNA with a sequence identical to that of a specific messenger RNA—copied from a gene as a template for protein synthesis—can interfere with that messenger RNA. The interaction essentially shuts down the corresponding gene’s function. Perrimon and his colleagues have created large libraries of RNA segments that together correspond to the entire genome of the fruit fly *Drosophila*.

“Our high-throughput RNAi screening technology can probe gene activity with far greater sensitivity than has been possible before,” said Perrimon. “It can detect very subtle activity to reveal many more genes that function in signaling pathways.” In their screening analysis, DasGupta and Kaykas used a library of some 22,000 short RNA molecules that corresponded to *Drosophila* genes. They treated fly cells with each of the RNA molecules, and determined the effect of each one on the Wnt pathway. To detect the effect of shutting down a gene, they measured the telltale fluorescent glow from a protein produced by a “reporter gene” they had incorporated into the fly genome in such a way that it was switched on only when the Wnt pathway was active.

Their screening revealed 238 candidate genes that were involved in the Wnt pathway—either activating or inhibiting it. These genes coded for proteins with a wide range of functions, including controlling other genes, acting as molecular switches for enzymes, and serving as components of the cell’s protein garbage-shredding system, or proteasome.

The researchers further validated their findings by confirming in other fly cells, in human cells, and in zebrafish embryos that a selection of the genes they had identified functioned in the Wnt pathway. According to Moon, the fact that the results of the screen included many genes that had already been identified by genetics studies to be involved in the Wnt pathway was further validation of the new technique.

“I found the discovery of the involvement of this many genes in the Wnt pathway, and their diversity, to be quite stunning,” said Moon.

According to Perrimon, whose laboratory is conducting a broad range of high-throughput RNAi functional genomic screens, the findings in the Wnt pathway offer dramatic evidence of the technique's power. “We have studied the Wnt pathway for many years and used classical genetics techniques to identify most of the initial components,” said Perrimon. “RNAi screening is completely changing the way we approach these problems. Before, it would take years to identify a few genes. But with this technology, we can identify in only weeks all the genes that are functionally involved in a pathway.”

Moon said the explosive increase in the number of known Wnt-associated genes brings with it a major opportunity for understanding the role of these genes and for developing drugs to treat cancer and other diseases.

“About eighteen percent of the human genes involved in the Wnt pathway are linked to diseases, according to genomic databases,” said Moon. “I think this is an extremely high percentage and points to the value of doing rapid screens in model organisms such as the fly.

“It's possible to use the same reporter system to screen small molecules for their effect on the Wnt pathway,” said Moon. “Using our new knowledge of the genes that function in the pathway, we can pinpoint those molecules' targets. And this rapid identification sets the stage for characterizing small molecules that are potential lead compounds as treatments for cancers and other diseases involving this pathway,” he said.

Perrimon said the new technology will enable scientific insights far beyond that of individual gene function. “For the first time, we can explore these systems globally, identifying patterns of correlations among groups of genes, understanding how information flows within entire cellular signaling networks,” he said.