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Living view in animals shows how cells decide to make proteins

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Scientists at Duke University Medical Center have visualized in a living animal how cells use a critical biological process to dice and splice genetic material to create unique and varied proteins.

The scientists say the findings, made in mice, help explain a key wonder of human biology: how the same genes found in every cell of an individual's body can produce different proteins in different tissues and organs. These varied proteins, in turn, dictate the function of each tissue or organ.

The findings also may offer insight into a number of diseases, including cancer, in which the genetic process -- called alternative splicing -- goes awry and produces the wrong proteins, the scientists said.

The scientists published the findings in the Dec. 1, 2006, issue of the journal RNA. The study was funded by the National Institutes of Health.

Scientists previously have examined alternative splicing in cells and tissues in test tubes, but this study marks its first successful visualization in a living mammal, said senior investigator Mariano Garcia-Blanco, M.D., Ph.D., a professor of molecular genetics and microbiology.

"We were able to watch alternative splicing as it occurred in different tissues," he said. "It's an excellent example of how experiments in living organisms provide a much more complete picture of how genes and proteins behave than do experiments using cells in culture."

Until 20 years ago, scientists believed that a single gene made a single protein. With the discovery of alternative splicing, it became clear that one gene can produce multiple proteins.

In alternative splicing, microscopic "scissors" in a gene chop the genetic material RNA into bits called "exons" and then reassemble the bits in a different order to form a new RNA molecule. In the process, some of the exons are retained while others are excluded. The exons that are retained in the final RNA determine which proteins the RNA produces within the cell.

The scissors that do the genetic chopping are, in most cells, proteins called splicing silencers and splicing enhancers.

In the current study, Garcia-Blanco's team sought to identify which silencers chop out an important segment of RNA in a gene called fibroblast growth receptor 2 (FGFR2). This gene plays a critical role in normal mouse and human development, and the order in which its RNA is assembled can alter an animal's development.

As a model system to study, the scientists genetically created a "glowing" mouse. The mouse carried in its FGFR2 gene a green fluorescent tag that would glow when a common type of silencer, called an "intronic silencer," chopped out a specific exon, called IIIb.

In this way, the scientists could track whether intronic silencers were chopping out the IIIb exon -- and if so, in which tissues and organs -- or whether other types of silencers or helper proteins were involved.

By tracking the green glow, the team found that cells in most tissues made the same decision to silence exon IIIb, but the cells used a variety of silencers and helper proteins to accomplish this task, said Vivian I. Bonano, a graduate student in the University Program in Genetics and

Genomics and lead author of the journal report.

"Identifying which silencers are active in a given tissue or organ will ultimately help scientists understand how exons are erroneously included or excluded in various disease processes," Bonano said.

For example, a cell's decision to include exon IIIb is critical because the exon's presence or absence determines which variant of the FGFR2 protein is produced, she said. Such subtle variations in proteins can alter the cell's behavior, just as switching ingredients in a favorite recipe can change the food's flavor, according to the scientists.

"Viewing these decisions is most relevant in a living animal, because cells behave differently in their natural environment versus an artificially created environment such as a laboratory tissue culture," Garcia-Blanco said. "The complexity of alternative splicing necessitates its visualization as the decisions are occurring, because taking a cell out of its context shows only its current status and not how it arrived at that place."

For instance, the splicing process can change even from day to day as an animal develops, he said, adding that extracting cells and watching them in a culture cannot convey all of these transient changes.

Moreover, different cell types within the brain or other organs can exhibit different splicing decisions, Garcia-Blanco said. For example, neurons reside next to glial cells in the brain, yet they express different proteins in different amounts, and detecting such differences in cell cultures can be exceedingly difficult, he said.

"This is a powerful tool to apply to mouse genetics to learn when and where in the animals' bodies alternative splicing decisions are made and, eventually, to learn what factors are critical in making these decisions," Garcia-Blanco said.

"Given the importance of alternative splicing in health and disease," he added, "this anatomic mapping of splicing decisions may give us considerable insight into the many human diseases associated with improper regulation of splicing."

Source: Duke University Medical Center