Scientists identify wide variety of genetic splicing in embryonic stem cells

March 1 2010

Like homing in to an elusive radio frequency in a busy city, human embryonic stem cells must sort through a seemingly endless number of options to settle on the specific genetic message, or station, that instructs them to become more-specialized cells in the body (Easy Listening, maybe, for skin cells, and Techno for neurons?). Now researchers at the Stanford University School of Medicine have shown that this tuning process is accomplished in part by restricting the number of messages, called transcripts, produced from each gene.

Most genes can yield a variety of transcripts through a process called splicing. Variations in the ways a gene is spliced can change the form and function of the final protein product. Nearly all our genes can be spliced in more than one way. This research is the first time, however, that splicing variety has been linked to the unprecedented developmental flexibility, or pluripotency, exhibited by embryonic stem cells.

"The embryonic stem cells are loaded with many splicing variants for each gene," said Michael Snyder, PhD, chair of Stanford's genetics department. "But as the cells differentiate and become more specialized, the number of types of transcripts decreases."

Snyder and his colleagues studied the changes in RNA transcript levels occurring as the embryonic stem cells were induced in a laboratory dish to differentiate into neural cells. (The creation of RNA transcripts is an intermediate step in the generation of proteins from DNA.) In the process they generated a unique "dictionary" of neural-specific splicing

variants, or isoforms.

"We've identified an extremely comprehensive suite of neural-specific transcripts that will be very powerful," said Snyder. "We can begin to study neural differentiation with a degree of precision that's never been dreamed of before."

Snyder is the Stanford W. Ascherman, MD, FACS, Professor in Genetics and a member of Stanford's Cancer Center. He is the senior author of the research, which will be published online March 1 in the *Proceedings of the National Academy of Sciences*. The study's first author is postdoctoral scholar Jia Qian Wu, PhD.

One way to understand gene splicing is to think of it like this: Genes are made up of several "words" of DNA called exons. These exons are separated from one another on the cell's raw genetic material by intervening bits of unexpressed DNA. By changing the way the exons are joined, or spliced, together in the final RNA transcript, the cell can generate several related, yet distinct, protein products, or "sentences" from each gene. These RNA variants are called RNA isoforms — and they're important in many biological processes, from generating antibodies to detoxifying drugs.

Snyder and Wu used a method of RNA sequencing Snyder invented while at Yale University called RNA-Seq to track the many RNA isoforms found at varying levels in human embryonic stem cells. The technique can identify a much greater range of RNA transcript levels and is much more sensitive than more traditional methods like DNA microarray analysis. That means it's possible to more reliably detect rare isoforms, and, as a result, more accurately plumb the secret transcriptional life of an embryonic stem cell — which turns out to be richer than previously imagined.

"The average human gene is known to have four or five transcripts," said Snyder. "But that number will likely go much higher now with this new technology. We are measuring these with a degree of specificity that's never been possible before." Choosing which genes to express, and then how to splice those genes, adds a layer of complexity that allows a cell to fine-tune its final protein profile.

The researchers chose to study neural differentiation in a laboratory dish, rather than in the brain, because it's possible to start with and follow populations of purified cells. They monitored the variety of RNA isoforms found in the human embryonic stem cells and compared them to those found in the cells as they were coaxed through three stages of differentiation into neural cells called glia. At each stage, they found, the variety of isoforms in the cells decreased — a phenomenon they termed "isoform specialization" — as they settled into their chosen station.

When the researchers looked more closely, they saw that the isoforms remaining were involved in key neural signaling pathways or cellular receptors. Furthermore, at the earliest stages of their differentiation, the nascent glial cells contain isoforms for receptors found on many other types of neural cells — suggesting they could be induced down several other developmental pathways.

Finally, the value of the researcher's transcript "dictionary" is hinted at by the finding that the timing of expression of two genes important in neural differentiation — SOX1 and PAX6 — in humans is different than that observed in mice.

Provided by Stanford University Medical Center

genetic-splicing.html