Researchers solve piece of large-scale gene silencing mystery

December 4 2008

A team led by Craig Pikaard, Ph.D., WUSTL professor of biology in Arts & Sciences, has made a breakthrough in understanding the phenomenon of nucleolar dominance, the silencing of an entire parental set of ribosomal RNA genes in a hybrid plant or animal.

Since the machinery involved in nucleolar dominance is some of the same machinery that can go haywire in diseases such as cancer, Pikaard and his collaborators' research may have important implications for applied medical research.

Nucleolar dominance occurs when nucleoli, protein-rich, dense regions of RNA within the nucleus, form on the chromosomes inherited from one parent, but not on the chromosomes inherited from the other parent. Expression of ribosomal RNA genes drives the formation of these nucleoli. The hybrid, a result of a cross-breeding of two different species, always "chooses" to express the ribosomal RNA genes of one particular parental species, regardless of whether that species happens to be the maternal or paternal parent.

Ribosomal RNAs, or rRNAs, are a major component of the ribosomes, the protein manufacturers of the cell. Because rRNA genes are highly redundant, cells use nucleolar dominance to control the dosage of ribosomes in an organism.

According to Pikaard, if researchers could harness the silencing machinery involved in nucleolar dominance to limit the expression of

rRNA genes, they could potentially slow the growth rate of tumor cells and thereby slow the progression of diseases like cancer.

In cancer cells, nucleoli are conspicuously large because of a dramatic increase in the transcription of rRNAs, which in turn leads to an increase in the production of ribosomes. This escalation in ribosome activity means that the cell can synthesize proteins at an alarmingly rapid rate, which contributes to the out-of-control cell proliferation that is the disease's trademark.

Completely silencing all ribosomal genes would not be a viable therapeutic approach for cancer patients because ribosomes are necessary for survival. But Pikaard and his collaborators' research suggests that small interfering RNAs (siRNAs) can direct silencing agendas that are much more sophisticated than an all or nothing approach.

"Dr. Pikaard's study demonstrates the potential of a plant model system to yield important molecular details on how cells silence large clusters of genes," said Anthony Carter, Ph.D., who oversees gene regulation grants at the National Institutes of Health's National Institute of General Medical Sciences, which partially supported the research. "His findings on the control of a major class of RNA found in all cells offer new insights into gene silencing mechanisms."

Pikaard and his collaborators' work, which was published in Molecular Cell on Dec. 4, is also one of the first to demonstrate how siRNAs can play a role in controlling the dosage of vital genes. The research was supported by the National Institutes of Health and the National Science Foundation.

The weird and the wacky

Nucleolar dominance is considered an "epigenetic" phenomenon. Epigenetics refers to heritable changes in gene expression that arise from changes in the "packaging" of DNA rather than modification of the underlying DNA sequence itself. Because these changes do not follow the normal rules of genetics, Pikaard refers to them as the "X-files of biology," unusual events that are not easily explained nor predicted.

Although biologists have been studying nucleolar dominance since the 1920s, this phenomenon remained largely unresolved until recently, when Pikaard's lab reversed an old dogma. Up until this point, researchers had presumed that nucleolar dominance was all about turning on one set of parental ribosomal genes. In 1997, Pikaard and his colleagues made headlines with an experiment that used chemicals to inhibit the two-pronged method cells employ to silence genes -- DNA methylation, which adds chemical flags to genes, and histone modification, which alters the proteins that act as spools for DNA. The chemical inhibitors of silencing turned on the previously unexpressed set of parental genes, thereby demonstrated that the underlying mechanism of nucleolar dominance turns genes off, not on.

Since then, Pikaard and his collaborators have been working to disentangle the complex machinery behind this epigenetic on-off switch.

Using RNA to fight RNA

To determine the pathway regulating nucleolar dominance, Pikaard's team exploited a naturally occurring cellular mechanism known as RNA interference (RNAi).

Pikaard likens RNAi to a "search and destroy mission." Fragments of RNA known as small interfering RNAs (siRNAs) prevent specific genes from being expressed by guiding cleavage of matching RNA strands. Once these RNA strands are cut into smaller pieces, they can no longer

be translated into proteins. RNAi has high specificity because the target RNA strand must have a genetic code that is complementary to the siRNA's nucleotide sequence.

In nature, cells use RNAi to silence "junk DNA," noncoding regions of the DNA, and "selfish DNA" such as virus-derived retrotransposons (jumping genes) that can be detrimental if activated.

In the lab, Pikaard and his collaborators use RNAi to "knockdown" expression of target genes.

Using a hybrid of two species of Arabidopsis, the plant version of a lab rat, Pikaard's team knocked down expression of genes coding for products that prior research had suggested might be involved in silencing. By knocking these suspects down one by one and assessing whether nucleolar dominance had been disrupted after each knockdown, Pikaard and his collaborators were able to determine which proteins and RNAs were necessary to keep the silenced parental genes off.

New clues

The RNAi knockdowns identified several new players necessary for the silencing machinery in nucleolar dominance to function, and also highlighted the key role of siRNA.

First in the pathway is RNA-dependent RNA Polymerase 2 (RDR2), which prepares a stretch of RNA for DICER-LIKE 3 (DCL3), an enzyme that chops up RNA transcripts into smaller segments. These smaller fragments of RNA become siRNAs, which then guide the de novo cytosine methyltransferase , DRM2, to the targeted genes. DRM2 is required to put a methyl group, a chemical flag that signals for silencing, on ribosomal genes that had been active in the parental genome. MBD6 and MBD10, methylcytosine binding proteins, then

adhere to the segments of DNA that have been methylated by DRM2. At the same time, HDA6, a histone deacetylase, modifies the proteins that act as spools for the DNA.

The end result of this convergent, siRNA-mediated pathway is the large-scale silencing of hundreds of clustered rRNA genes that span millions of basepairs of DNA.

Nucleolar dominance occurs on a scale second only to X-chromosome inactivation, a process by which one of the two copies of the X-chromosome present in female mammals is randomly inactivated. Although nucleolar dominance is on a sub-chromosomal scale, it is, at least to date, "the largest scale gene silencing phenomenon that clearly seems to involve siRNAs," says Pikaard.

Pikaard explains, "siRNAs are not just regulating the selfish DNA or the junk DNA, but they're regulating the really essential genes too."

He believes that siRNAs might be the key to understanding the choice mechanism underlying which parental genes get switched off and which get left on, and he and his collaborators plan to investigate this possibility in future research.

"The truth is out there," says Pikaard.

Source: Washington University in St. Louis