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# 'Dead' gene comes to life, puts chill on inflammation, researchers find

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A gene long presumed dead comes to life under the full moon of inflammation, Stanford University School of Medicine scientists have found.

The discovery, described in a study to be published July 23 in *eLife*, may help explain how anti-inflammatory steroid drugs work. It also could someday lead to entirely new classes of anti-inflammatory treatments without some of steroids' damaging side effects.

Chronic inflammation plays a role in cancer and in autoimmune, cardiovascular and neurodegenerative diseases, among others. Anti-inflammatory steroid drugs are widely prescribed for treating the inflammatory states that underlie or exacerbate these conditions.

"Inflammation tells your body something is wrong," said the study's senior author, Howard Chang, MD, PhD, professor of dermatology at Stanford and the recipient of an early career scientist award from the Howard Hughes Medical Institute. "But after it does its job of alerting immune cells to a viral or bacterial infection or spurring them to remove debris from a wound site, it has to get turned off before it causes harm to healthy tissue."

That appears to be what the "undead" gene does. Chang's team, which identified it, has named it Lethe, after the stream in Greek mythology that makes the deceased who cross it forget their pasts.

The master regulator of inflammation inside cells—a bulky complex of several proteins, collectively called NF-kappa-B—is a transcription factor: It can switch on hundreds or even thousands of genes in a cell's nucleus. When aroused by signals at the cell surface (typically delivered by circulating proteins or microbial components), NF-kappa-B activates pro-inflammatory genes, gearing that cell up to combat viral or bacterial assaults and respond to an injury.

Lethe, which the investigators found is activated by NF-kappa-B, subdues the master regulator's massive influence on the genome, curtailing the inflammatory response.

NF-kappa-B also plays a key role in aging. In a study published in 2007 in *Genes and Development*, Chang and his colleagues showed that old skin cells in which NF-kappa-B was temporarily inactivated began to act young. This finding has since been confirmed in other tissues and by other researchers.

To learn more about NF-kappa-B, Chang's group decided to activate it and see which genes get turned on or off. But rather than "normal" genes, which are essentially recipes for making proteins, they were curious about DNA sequences that generate long noncoding RNA molecules, or lncRNAs, which Chang helped to discover during the past decade.

RNA is best known as the intermediate material in classic protein production. Gene-reading machines in cells produce RNA transcripts, or copies, of protein-coding genes. These transcripts, known as messenger RNAs, are free to leave the cell nucleus for the cytoplasm, where they can transmit genes' instructions to the protein-making machines situated there.

But lately RNA has been shown to play an increasing number of

additional roles that have nothing to do with making proteins. The lncRNAs Chang studied are made by the same molecular machinery that protein-coding genes use to make a messenger RNA. Instead of heading for the cytoplasm to make proteins, though, lncRNAs can remain in the nucleus and directly regulate genes. More than 10,000 lncRNAs have now been discovered, although scientists are only beginning to understand what they do.

To see which lncRNAs were induced during inflammation, Chang and his colleagues exposed cultured fibroblasts from embryonic mice to TNF-alpha, an immune-signaling protein known to trigger NF-kappa-B. They found that levels of hundreds of lncRNAs inside the cells were driven either up or down by TNF-alpha stimulation.

Of those lncRNAs, a total of 54 were copied from so-called pseudogenes: DNA sequences that, while they closely resemble genes, don't code for proteins. More than 11,000 pseudogenes—one for every two protein-coding genes—have been identified in the human genome. Scientists believe pseudogenes are copies of actual genes that, during the replication of some ancestral organism's germ cell, were accidentally inserted into the genome and, redundant but harmless, came along for the evolutionary ride. Over the intervening eons, these genetic doppelgangers have roamed along the genome, mutated and decayed to the point where, it is believed, they no longer do anything at all.

"Pseudogenes have been considered to be completely silent, ignored by cells' DNA-reading machinery," Chang said. "But we got a real surprise. When a cell is subjected to an inflammatory stress signal, it's like Night of the Living Dead."

Equally surprising, Chang said, is that different signaling chemicals or microbial components (such as bits of bacterial cell walls or of viral DNA) wake up different groups of lncRNA-encoding DNA sequences,

including pseudogenes. "They're not really dead, after all. They just need very specific signals to set them in motion."

Lethe was one such pseudogene tripped off by stimulation of NF-kappa-B. Lethe directly interfered with the complex's ability to seat itself on appropriate DNA sequences, shutting down the pro-inflammatory genes the transcription factor ordinarily activates.

Several pseudogenes were activated in a selective manner. For example, TNF-alpha and another circulating signaling protein—but not microbial parts—activated Lethe.

Because some pseudogenes sit near protein-coding genes, some scientists have argued that the generation of RNA transcripts from the pseudogenes is simply an artifact of normal transcription of full-fledged protein-coding genes. "There's a tendency to assume it's some protein-coding gene that NF-kappa-B is really targeting, and to downplay the activation of a lncRNA as noise, a 'ripple effect' like the one you see when a boat goes by," Chang said.

But TNF-alpha failed to activate two nearby protein-coding genes on either side of Lethe. Reciprocally, stimuli that turned these two other genes on didn't affect Lethe. Meanwhile, two other pseudogenes that very closely resemble Lethe were not activated by TNF-alpha, as Lethe was.

Another surprising finding was that dexamethasone, a commonly prescribed anti-inflammatory steroid drug, activates Lethe. Various other steroid hormones that are not anti-inflammatory in nature, such as vitamin D or estrogen or a male steroid hormone, failed to boost Lethe levels.

"We're wondering whether there might be ways to artificially raise Lethe

levels without steroids. These drugs have potentially deleterious side effects such as elevated blood pressure and blood sugar, thinning of bones and general suppression of the immune system," Chang said.

The study results suggest that not only Lethe but other pseudogenes undergo similarly selective awakenings to generate lncRNAs in response to different external inflammatory stimuli. "From the pattern of activated lncRNAs, you can tell what the cell has encountered—a virus, a bacteria or something else," Chang said. "These patterns of activation may be able to serve as an indicator of what kind of inflammatory situation or pathogenic invasion is responsible."

A third surprise: While NF-kappa-B levels and activity within cells increase with an organism's advancing age, Lethe is dramatically downgraded with increasing age—but eightfold more so in females. Lethe levels in spleens of older mice, compared with those of young mice, dropped 20-fold in males but 160-fold in females. "This gender-specific difference is not seen in young mice," Chang said. "Could this have any implications for the increasing female-to-male ratio, with advancing age, for autoimmune diseases in humans?"

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