The Low-Down on Gene Editing: CRISPR and Gene Regulation

If you're into the biosciences, you've heard about CRISPR. **C**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats (CRISPR) and its Cas9 nucleases were discovered as part of bacterial immune systems in the 1970s; only in 2012 were they refined into a powerful gene-editing technology.

CRISPR/Cas9 is the new kid on the block, in company with a small list of gene editing techniques such as zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENS) which have been around since 1998 and 2009, respectively. What makes CRISPR special is that it's more highly targeted, cheaper, and computer-friendly. You can order a guide RNA (gRNA), the crucial code snippet for gene targeting, online.

While the technology works well for unicellular bacteria, it is not very effective in complex mammalian cells. By tweaking the software and hardware of this technology, scientists hope to make gains in controlled gene-editing.

Bacterial and Human Cells

Long before complex multi-cellular organisms like humans were in the picture, single-celled bacteria were our planet's dominant form of life. These bacteria competed and formed alliances with each other, and fought to protect themselves from viral invaders 1/100th their size - and bacteria are microscopic to begin with.

These viral invaders are short snippets of genetic material that cannot replicate on their own. They need a host to survive, so they sneak through the cell walls of bacteria and inject themselves into its genetic code, creating more of itself. Pretty smart.

CRISPR came from nature's own toolbox. Eventually, bacteria developed an immune system. It could absorb and remember the invader's genetic code, so that the next time the virus came around, the bacteria could send a nuclease after it, cutting the virus into useless bits. Even smarter.

CRISPR Trick #1: Cut-and-Paste DNA

"It's easy to reprogram [CRISPR/Cas] to go anywhere you want," says Oliver Medvedik, director of the Maurice Kanbar Center for Biomedical Research in New York City.

Using a 20 nucleotide gRNA, the CRISPR/Cas system can easily target and lock onto a specific portion of our 3-billion-nucleotide-long genome.

Once locked on, it chops the DNA. Since cut-up DNA is about as useful as a cut-up tight rope, the body immediately works to repair the break. Human and mammal cells use two techniques for repair: non-homologous end joining (NHEJ) and homology directed repair (HDR). With CRISPR/Cas9, scientists can deliver a homology, or gene sequence, to insert into the cut site.

NHEJ is the default solution but is highly error-prone. The DNA repair systems quickly stick the DNA back together with just about any nucleotides they can find that fit. Usually this knocks out the gene by making it unreadable. In the worse case, it results in a functional mutation that is harmful to the host organism.

HDR is the preferred solution but occurs with less than 1% efficiency in human stem cells, for example, compared to 95% efficiency in unicellular yeast cells. Medvedik is working on methods to repress the NHEJ response and tweak the Cas enzyme for more efficient HDR repair.

CRISPR Trick #2: Turn Off Disease Like a Light Switch

Scientists discovered that certain variations of the Cas nuclease, in combination with inhibitor proteins, could sit and lock on to DNA, silencing a selected gene. Modified versions go the other way, and remove inhibitor proteins, turning the genes back on. The technique, referred to as CRISPR interference (CRISPRi), is 95% effective in silencing genes whereas cutting them using Cas9 resulted in only 60-70% suppression and introduced greater risk.

CRISPR and Computers: Meatspace and Cyberspace

Previous gene-editing techniques required customized proteins for each gene sequence, an expensive and error-prone process. For CRISPR, changing a 20-nucleotide gRNA sequence is "ridiculously simple" and can be done online, says Medvedik.

He caveats, though, that "meatspace is different from cyberspace." Computers have drastically lower error rates and can run experiments in seconds, while bioengineering techniques run at high error with many months between the question and answer parts of the experiment.

Up Next: Business in Genetic Engineering

Next week, we'll discuss the ongoing patent dispute deciding who profits from CRISPR use in the marketplace.

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"If a flap of a butterfly's wing can be instrumental in generating a tornado, it can equally well be instrumental in preventing a tornado." – Lorenz, 1972