Evidence of Learning #5

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TED Talk

I've got to say, I was greatly awed by Dr. Liu's official bio, which I took a look at before I watched this video. He is considered to be a superb chemist and biologist, taking a PhD from Harvard and even going on to teach there. Dr. Liu has written over 180 research papers and filed over 70 patents, all of which have contributed to the numerous awards and accolades he has gained in the scientific community. Not only is his research astounding, but also the number of scientific companies he has co-founded. Going into the TED Talk, I was having high expectations.

When I finished the TED Talk, I caught my jaw being wide open. Not only was Dr. Liu a fantastic and memorable speaker, but his depiction of base editing and its future implications really filled me with hope. He gave a great deal of background information and dumbed down genetic concepts in a way the layman would understand. Originally, I knew, of course, of the great contribution CRISPR is to the field of genetics. However, I had completely misunderstood CRISPR and its origins. I never knew that CRISPR is actually a biological mechanism "stolen" from bacteria, which have been using the scissor-like search engine to fend off viruses for hundreds of millions of years. Six years ago, genetic researchers were able to refine CRISPR in a way to target and cut off specific DNA sequences in the human genome.

Since I was in middle school, I believed CRISPR was a sort of holy weapon, a shield that would lead the future of medicine. In many ways, this was completely false, and I was very shocked when the TED Talk revealed this. Now that I have dedicated much time to researching genetics, I understand the pitfalls of the CRISPR & cas9 system that Dr. Liu described much better now. Since it can only cut out specific DNA sequences, this does not fix or restore the biological functions that pathogenic point-mutations deny in genes. As a result, Dr. Liu, his colleagues, and students innovated a brilliant way to artificially rewrite base pairs using a technology called a "base editor."

These base editors are an amalgam of 2 or 3 proteins which steal the CRISPR search system that finds DNA but disables its cutting mechanism. This allowed Dr. Liu's scientists used another naturally-occurring protein that acted as a pencil, converting, or rewriting, a faulty, mutated base pair into the correct one. A third protein is used to prevent the cell itself from removing the modified base pair. One challenge was the problem of converting the

other base in the pair, since they have to be compatible with one another. Dr. Liu and his team cleverly took advantage of a cell mechanism by "nicking" the base they wanted removed. This directed, or tricked, the cell into taking away the non-matching base, solving the problem!

Another hurdle Dr. Liu's team faced when they were converting certain bases, there wasn't a naturally occurring protein they could use for the conversion. As a result, one of Dr. Liu's colleagues actually manufactured a protein to resemble the G base, allowing for the creation of a second base editor! The innovation and creativity that Dr. Liu describes in the research & development process has only drawn me more to the field I'm in, and his descriptions of all the genetic diseases this could fully treat has made me glad to enter the field of genetics, which is reaching new horizons every year. Not only could humans be able to repair children or even adults, but far more useful genetically modified plants could be created! As I mentioned before, the implications are limitless

Of course, there are setbacks. On the bright side, these base editors have worked in animal trials in the three years they have been in development. However, human clinical trials have not generally been started because there is the challenge of getting the base editors into the body and correct cells. As expected, Dr. Liu presented an unusual but curious method that scientists are working on--to manufacture viruses like the common cold to deliver base editors instead of its regular harmful package. Furthermore, they still need to work on creating base editors for all the other bases that are changed in single-point mutations.

To be frank, I cannot wait to discuss this technology with my mentor, Dr. Sullivan, with whom I already shared some thoughts on CRISPR. Now I feel a bit of a fool for not knowing about the innovations, such as base editors, in the six years since CRISPR was refined for humans. As a prospector to become a clinical geneticist, I understand that I will likely not be at the forefront of research, as I will mainly be treating patients. However, this TED Talk has inflamed my curiosity and wonder about my particular field even more!

- In any day, the cells in your body will collectively accumulate billions of these single-letter swaps, which are also called "point mutations."
- Now, most of these point mutations are harmless. But every now and then, a point mutation disrupts an important capability in a cell or causes a cell to misbehave in harmful ways.
- Grievous genetic diseases caused by point mutations are especially frustrating, because we often know the exact single-letter change that causes the disease and, in theory, could cure the disease.
- Throughout the history of medicine, we have not had a way to efficiently correct point mutations in living systems, to change that disease-causing T back into a C. Perhaps until now. Because my laboratory recently succeeded in developing such a capability, which we call "base editing."
- The story of how we developed base editing actually begins three billion years ago.
    - Bacteria evolved a defense mechanism to fight viral infection. That defense mechanism is now better known as CRISPR. And the warhead in CRISPR is this purple protein that acts like molecular scissors to cut DNA, breaking the double helix into two pieces.

    - But the most amazing feature of CRISPR is that the scissors can be programmed to search for, bind to and cut only a specific DNA sequence. So when a bacterium encounters a virus for the first time, it can store a small snippet of that virus's DNA for use as a program to direct the CRISPR scissors to cut that viral DNA sequence during a future infection. Cutting a virus's DNA messes up the function of the cut viral gene, and therefore disrupts the virus's life cycle.
- Remarkable researchers showed six years ago how CRISPR scissors could be programmed to cut DNA sequences of our choosing, including sequences in your genome, instead of the viral DNA sequences chosen by bacteria. But the outcomes are actually similar. Cutting a DNA sequence in your genome also disrupts the function of the cut gene, typically, by causing the insertion and deletion of random mixtures of DNA letters at the cut site.
- Now, disrupting genes can be very useful for some applications. But for most point mutations that cause genetic diseases, simply cutting the already-mutated gene won't benefit patients, because the function of the mutated gene needs to be restored, not further disrupted. And while we can sometimes introduce new DNA sequences into cells to replace the DNA sequences surrounding a cut site, that process, unfortunately, doesn't work in most types of cells, and the disrupted gene outcomes still predominate.
- Being a chemist, I began working with my students to develop ways on performing chemistry directly on an individual DNA base, to truly fix, rather than disrupt, the mutations that cause genetic diseases. The results of our efforts are molecular machines called "base editors." Base editors use the programmable searching mechanism of CRISPR scissors, but instead of cutting the DNA, they directly convert one base to another base without disrupting the rest of the gene.
- We engineered the first base editor, shown here, from three separate proteins that don't even come from the same organism. We started by taking CRISPR scissors and disabling the ability to cut DNA while retaining its ability to search for and bind a target DNA sequence in a programmed manner.
- In order to be stable in cells, the two strands of a DNA double helix have to form base pairs. And because C only pairs with G, and T only pairs with A, simply changing a C to a T on one DNA strand creates a mismatch, a disagreement between the two DNA strands that the cell has to resolve by deciding which strand to replace. We realized that we could further engineer this three-part protein to flag the unedited strand as the one to be replaced by nicking that strand. This little nick tricks the cell into replacing the nonedited G with an A as it remakes the nicked strand, thereby completing the conversion of what used to be a C-G base pair into a stable T-A base pair.
- Led by Nicole Gaudelli, a former postdoc in the lab, we set out to develop this second class of base editor, which, in theory, could correct up to almost half of pathogenic point mutations, including that mutation that causes the rapid-aging disease progeria.
- We realized that we could borrow, once again, the targeting mechanism of CRISPR scissors to bring the new base editor to the right site in a genome. But we quickly

encountered an incredible problem; namely, there is no protein that's known to convert A into G or T into C in DNA.

    - Given the absence of a naturally occurring protein that performs the necessary chemistry, we decided we would evolve our own protein in the laboratory to convert A into a base that behaves like G, starting from a protein that performs related chemistry on RNA. We set up a Darwinian survival-of-the-fittest selection system that explored tens of millions of protein variants and only allowed those rare variants that could perform the necessary chemistry to survive. We ended up with a protein shown here, the first that can convert A in DNA into a base that resembles G.

- While base editors are too new to have already entered human clinical trials, scientists have succeeded in achieving a critical milestone towards that goal by using base editors in animals to correct point mutations that cause human genetic diseases. Scientists recently used a virus to deliver that second base editor into a mouse with progeria, changing that disease-causing T back into a C and reversing its consequences at the DNA, RNA and protein levels.
- Base editors have also been used in animals to reverse the consequence of tyrosinemia, beta thalassemia, muscular dystrophy, phenylketonuria, a congenital deafness and a type of cardiovascular disease -- in each case, by directly correcting a point mutation that causes or contributes to the disease. In plants, base editors have been used to introduce individual single DNA letter changes that could lead to better crops.
- Additional work lies ahead before base editing can realize its full potential to improve the lives of patients with genetic diseases. Delivering molecular machines like base editors into cells in a human being can be challenging. Co-opting nature's viruses to deliver base editors instead of the molecules that give you a cold is one of several promising delivery strategies that's been successfully used. Continuing to develop new molecular machines that can make all of the remaining ways to convert one base pair to another base pair and that minimize unwanted editing at off-target locations in cells is very important. And engaging with other scientists, doctors, ethicists and governments to maximize the likelihood that base editing is applied thoughtfully, safely and ethically, remains a critical obligation.