Each fall, the University of Pittsburgh hosts a conference at which scientists from across the country have the opportunity to present impactful research they have done. As a first-year student in the Biological Sciences Ph.D. program at Duquesne University, I had the privilege of attending Science 2018 this fall alongside my classmates. One fascinating talk that I had the opportunity to learn from was “Development of CRISPR-Cas Systems for Genome Editing” by Dr. Feng Zhang, a researcher at Howard Hughes Medical Institute and a neuroscience professor at Massachusetts Institute of Technology. Dr. Zhang described the CRISPR-Cas system in terms of its origin as well as applications, including those pertaining to medical diagnostics and genome alterations, such as those in response to harmful diseases.

CRISPR-Cas is a biological system harbored by bacteria, paralleling the human immune system. That is, in the same way that the human body makes efforts to protect itself against infection, bacteria defend themselves as well. Bacteriophages, or “phages,” are viruses that invade bacterial cells. Because viruses are nonliving organisms, they rely on the mechanisms of their hosts, such as human or bacterial cells, to allow them to replicate. Due to this dependency, part of the viral infection process involves genetic material being introduced into the host cell. In the CRISPR defense system, however, a bacterial cell already containing part of the viral genome develops the ability to resist infection by viruses with those same sequences.

The abbreviation CRISPR stands for “Clustered Regularly Interspersed Short Palindromic Repeats” and refers to the repetitive nature of viral DNA sequences within the bacterial genome, where they are located in close proximity to one another. Furthermore, these sequences are palindromic; similarly to how English-language palindromes read the same forwards and backwards, biological palindromes read the same forwards as a strand of DNA paired to match them would read backwards. In addition to the repeated viral DNA segments, CRISPR-associated (Cas) proteins are also present as a part of this defense system.
A CRISPR-Cas system may be Class 1, where multiple proteins and CRISPR RNA (crRNAs) with guiding functions attack and break down invading viruses, or Class 2, where a single protein works in conjunction with crRNA. Cas13 is a protein found in some RNA-targeting Class 2 CRISPR-Cas systems. However, these systems demonstrate collateral activity and can break apart sequences aside from their target viral RNA so long as they are relatively close.

Certainly, collateral activity is helpful to bacteria in defending against many infections, but this characteristic has also been employed to develop methods of medical diagnosis. In the Zika virus test, for example, a testing antibody, a double-ended “reporter,” and a specific Cas13 called PsmCas13b are added to a solution of the diagnostic sample. If Zika virus is present, PsmCas13b becomes activated and cleaves not only Zika virus, but also the reporter molecule, which releases an antibody-binding chemical called biotin. The biotin-antibody complex that has formed then generates fluorescence that can be viewed by the scientist conducting the test, indicating a positive result.

The ease of this testing method, coupled with the specificity of PsmCas13b, being activated only by Zika virus despite its collateral capabilities, lend to its favorability as a diagnostic tool. Furthermore, the affordability of the reagents – antibodies, reporters, and the PsmCas13b system – also renders it a reasonable option in regards to financial feasibility. All in all, from the initial step of adding these reagents into the patient sample, to the final step of receiving test results, the process typically takes no longer than thirty minutes. CRISPR-Cas-based diagnostic technology has been applied to detect additional illnesses or genetic characteristics as well. For diagnosing Dengue virus, a different form of Cas13 and a slightly different reporter complex are used, but otherwise, the same general processes are used as with testing for Zika virus. Likewise, certain
plant pathogens or genetically modified (GMO) traits have been detected through CRISPR-Cas within soybean crops.

The future of this technology also offers possibilities for genome editing, including that related to disease. Many neurological diseases occur as a result of fairly small-scale genetic mutations, namely mutations of a single nucleotide within the genetic code. Even seemingly minor changes alter the structures of proteins in the body, which in turn can cause harmful health effects. However, REPAIR and RESCUE are two programmable systems that can convert a single nucleotide, such as one that has mutated and brought about disease, into another, such as what it may have been prior to mutation. These techniques have not yet been tested in animal models such as mice, let alone in humans, but reflect promising potential for future work to combat mutation-induced disease. Additional next steps in this area include the introduction of REPAIR and RESCUE technologies into animal models, altering RNA editing systems for greater specificity and efficacy, and creating human delivery systems that could be used, should further research support their ultimate use as therapeutic tools.

All in all, I was amazed by the content that Dr. Zhang presented at Science 2018. I had previously learned about the principles of CRISPR-Cas, but Dr. Zhang’s talk expanded my knowledge by elucidating the system’s broad scope of diagnostic and potentially therapeutic applications. I feel that CRISPR-Cas deserves more recognition given its vast implications for future continued scientific research and medical test development, coupled with the fascinating nature of the subject matter itself. I look forward to learning more about the diagnostic and engineering-based aspects of CRISPR-Cas as research on, and implementation of, this technology continues to progress.

References