ALS SCIENCE HIGHLIGHT

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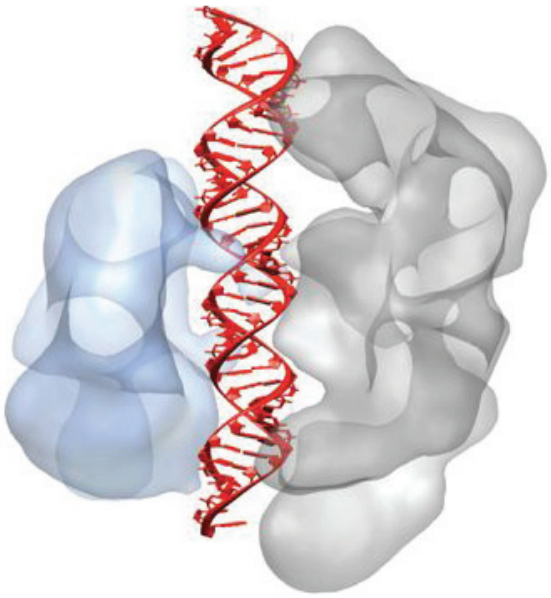
Intriguing DNA Editor Has a Structural Trigger

A powerful new tool for genome editing and gene regulation has emerged in the form of a family of enzymes known as Cas9. Cas9 could become an even more valuable tool with the creation of the first detailed picture of its three-dimensional shape. An international collaboration used x-ray crystallography to produce high-resolution structures of two major types of Cas9 enzymes. Combined with electron microscopy, the results point the way to the rational design of new and improved versions of Cas9 enzymes for basic research and genetic engineering.



The Cas9 enzyme wraps around target DNA.

Cas9 enzymes play a key role in bacterial immune systems. Bacteria face a never-ending onslaught from viruses and invading strands of nucleic acid known as plasmids. To survive, they deploy a variety of defense mechanisms, including an adaptive immune system involving sequences in their DNA known as CRISPR (clustered regularly interspaced short palindromic repeats). A combination of CRISPR and squads of CRISPR-associated (Cas) proteins utilize small, customized RNA molecules (guide RNA) to target and cleave critical portions of an invader's double-stranded DNA and confer immunity from similar invasions in the future.

The potential is there for bacteria and other microbes to be genetically engineered to perform valuable services, from the production of safer, more effective medicines and sustainable fuels, to the clean-up and restoration of our air, water, and land. Cells from more advanced organisms can also be modified for research or to fight disease. To achieve these and other worthy goals, the ability to precisely edit the instructions contained within a target's genome is a must. Genetic engineers have already begun harnessing Cas9 for genome editing and gene regulation. However, despite successes to date, the technology has yet to reach its full potential because the structural basis for guide-RNA recognition and DNA targeting by Cas9 had been unknown.

Now, researchers have addressed this knowledge deficit by first solving the three-dimensional crystal structures of two Cas9 proteins, representing large and small versions, from *Streptococcus pyogenes* (SpyCas) and *Actinomyces naeslundii* (AnaCas9) respectively. Using protein crystallography Beamlines 8.2.2 and 8.3.1 at the ALS and PXI and PXIII at the Swiss

Publication about this research: M. Jinek, F. Jiang, D.W. Taylor, S.H. Sternberg, E. Kaya, E. Ma, C. Anders, M. Hauer, K. Zhou, S. Lin, M. Kaplan, A.T. Iavarone, E. Charpentier, E. Nogales, and J.A. Doudna, "Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation," *Science* **343**, 1215 (2014).

Research conducted by: M. Jinek and C. Anders (University of Zurich); F. Jiang, S.H. Sternberg, E. Kaya, E. Ma, M. Hauer, and A.T. Iavarone (Univ. of California, Berkeley); D.W. Taylor, K. Zhou, and S. Lin (Howard Hughes Medical Institute and UC Berkeley); M. Kaplan (HHMI); E. Charpentier (Umeå University, Sweden; Helmholtz Centre for Infection Research, Germany; and Hannover Medical School, Germany); and E. Nogales and J.A. Doudna (UC Berkeley, HHMI, and Berkeley Lab).

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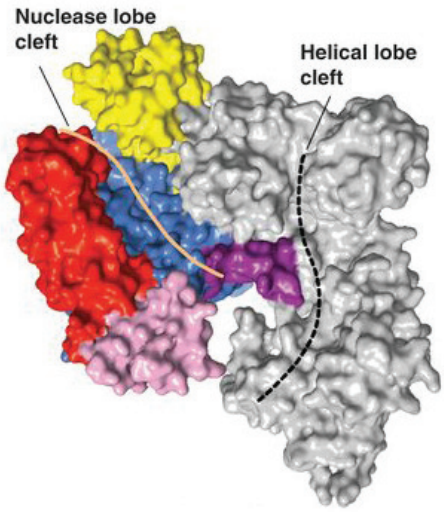
Genetic Plug-Ins

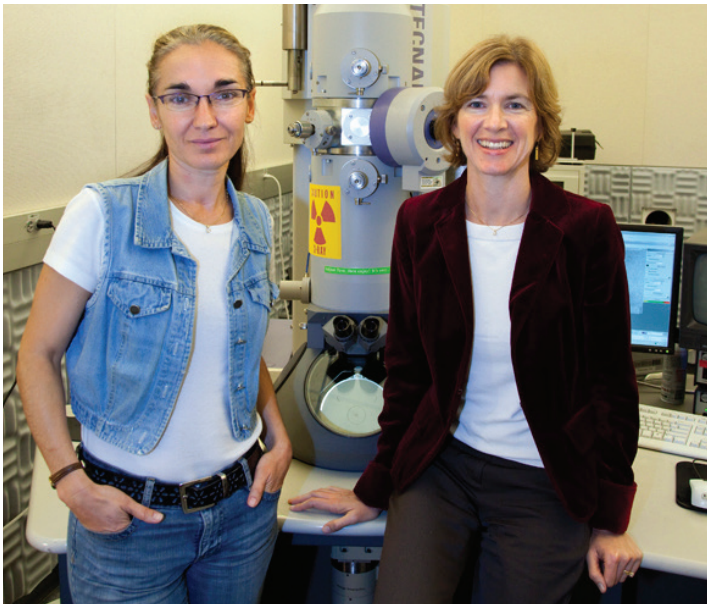
Software plug-ins make it relatively simple to extend and customize programmable devices. Imagine how tedious it would be if, every time you wanted to play a new online game or add a new widget to your computer, you had to dig deep into the code and reprogram it line by line, or worse, build a whole new computer from scratch!

In biology, DNA is the code that runs the hardware, and scientists would like to "plug in" very specific snippets of DNA coding for a variety of reasons. For example, microbes could be engineered to consume certain environmental toxins or to produce cleaner biofuels or better medicines. Genomic surgery—correcting mutations that cause disease—is another goal. At the most fundamental level, quick and efficient genome editing is essential to future advances in biology and health.

Cas9 is now generating a lot of excitement because, unlike earlier genome-editing tools, its basic architecture doesn't have to be rebuilt for each DNA site targeted; researchers simply have to "reprogram" it with the appropriate snippet of guide RNA. It promises to remove a major bottleneck in the field, transforming what used to be a complicated and expensive process into something like applying a genetic plug-in: more routine and, therefore, more powerful.



The structure of SpyCas9 features a nuclease domain lobe (colored) and an alpha-helical lobe (gray) each with a nucleic-acid-binding cleft that becomes functionalized when Cas9 binds to guide RNA.



Eva Nogales and Jennifer Doudna.

Light Source, the researchers discovered that despite significant differences outside of their catalytic domains, all members of the Cas9 family share the same structural core. The high-resolution (2.6- and 2.2-Å) images showed this core to feature a clam-shaped architecture with two major lobes—a nuclease domain lobe and an alpha-helical lobe. Both contain conserved clefts that become functional in nucleic-acid binding.

The researchers also employed electron microscopy to visualize the Cas9 bound to either guide RNA, or both RNA and target DNA. The images revealed that the guide RNA structurally activates Cas9 by creating, between the two main lobes, a channel that functions as the DNA-binding interface. The results underline that, in addition to sequence complementarity, other features of the guide RNA must be considered when employing this technology. The Cas9 protein, on its own, exists in an inactive state, but upon binding to the guide RNA, the Cas9 protein undergoes a radical change in its three-dimensional structure that enables it to engage with the target DNA.

With these high-resolution structures of the two major types of Cas9 proteins, researchers can start to see how this family of bacterial enzymes has evolved. The two structures are quite different from each other outside of their catalytic domains, suggesting an interesting structural plasticity that could explain how Cas9 is able to use different kinds of guide RNAs. Also, the differences in the two structures suggest that it may be possible to engineer smaller Cas9 variants and still retain function, an important goal for some genome-engineering applications.