Editas Medicine Co-founder Publishes High-Resolution Structures of MajorCas9 Enzymes in Science

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Findings Suggest Guide RNA-Dependent Mechanism for DNA Binding and Activation of Cas9

Cambridge, Mass., February 7, 2014 – Editas Medicine, a transformative genome editing company, today announced new published research from company co- founder Jennifer A. Doudna, Ph.D., presenting the high-resolution structures of two Cas9 enzymes, extensive single particle electron microscopy (EM) analysis and biochemical data. The researchers present a model in which guide RNA (gRNA) binding triggers Cas9 activation through conformational rearrangement, priming the complex for target DNA binding. The paper was published in the current online edition of *Science*[1] and co-authored by Dr. Doudna, Howard Hughes Medical Institute investigator and professor of biochemistry, biophysics and structural biology at the University of California, Berkeley.

"We are harnessing the biochemical properties of Cas9-guide RNA complexes in genome editing applications and this research provides critical structure-function insights into Cas9 nucleic acid binding and nuclease activity," said Kevin Bitterman, Ph.D., interim president, Editas Medicine. "We believe these findings will help guide efforts to develop improved versions of Cas9 and further enhance the specificity of our CRISPR/Cas9 genome editing technology."

The high-resolution structures of two major subtypes of Cas9, *Streptococcus pyogenes* (SpyCas) and *Actinomyces naeslundii* (AnaCas9) define a structural core architecture of the Cas9 enzyme family. The conserved core encompasses two nuclease domains responsible for DNA cleavage, as well as structurally divergent regions, including the protospacer adjacent motif (PAM) recognition loops. Photo crosslinking experiments show that the PAM in target DNA is engaged by two tryptophan-containing flexible loops, and mutations of both loops impair target DNA binding and cleavage. Additionally, the EM reconstructions from multiple states of assembly with gRNA and DNA substrates reveal that gRNA binding results in a conformational rearrangement and formation of a central channel for target DNA binding.

The data presented in the paper suggest Cas9 enzymes adopt a catalytically inactive conformation in the apo state, necessitating gRNA-mediated structural activation for DNA recognition and cleavage. Combined with the structural definition, these data provide insights into the function, regulation and evolution of the Cas9 enzyme family, which may inform ongoing refinements to genome editing technology.

About Genome Editing

Following an explosion of high-profile publications on CRISPR/Cas9 and TALENs, genome editing has emerged as one of the most exciting new areas of scientific research. These recent advances have made it possible to modify, in a targeted way, almost any gene in the human body with the ability to directly turn on, turn off or edit disease-causing genes. Editas Medicine's five founders have published much of the foundational work that has elevated genome editing technology to a level where it can now be optimized and developed for therapeutic use.

CRISPR (clustered, regularly interspaced short palindromic repeats)/Cas9 (CRISPR- associated protein 9) and TALENs (transcription activator-like effector nucleases) comprise novel gene editing methods that overcome the challenges associated with previous technologies. Early published research on CRISPR/Cas9, coupled with a growing body of work on TALENs, suggests the potential to pursue therapeutic indications that have previously been intractable to traditional gene therapy, gene knock-down or other genome modification techniques. The CRISPR/Cas9 system, the most recent and exciting approach to emerge, acts by a mechanism in which the Cas9 protein binds to specific RNA molecules. The RNA molecules then guide the Cas9 complex to the exact location in the genome that requires repair. CRISPR/Cas9 uniquely enables highly efficient knock-out, knock-down or selective editing of defective genes in the context of their natural promoters, unlocking the ability to treat the root cause of a broad range of diseases.

About Editas Medicine

Editas Medicine is a transformative genome editing company founded by five world leaders in the fields of genome editing, protein engineering, and molecular and structural biology, with specific expertise in CRISPR/Cas9 and TALENs technologies. The company's mission is to translate its genome editing technology into a novel class of human therapeutics that enable precise and corrective molecular modification to treat the underlying cause of a broad range of diseases at the genetic level. Editas Medicine was launched in November 2013 with funding from Flagship Ventures, Polaris Partners and Third Rock Ventures with participation from Partners Innovation Fund. For more information, visit www.editasmedicine.com.

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1. Jinek, Jiang, Taylor & Sternberg, *et al*. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science*, Feb. 6, 2014. DOI: 10.1126/science.1247997. [Epub ahead of print]