Mechanistic Insights into CRISPR-Cas Genome Editors

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In bacteria, the CRISPR-Cas system functions as an adaptive system to provide resistance against molecular invaders such as viruses and other mobile genetic elements. RNA-guided effector nucleases associated with CRISPR-Cas systems have been repurposed as powerful tools for precision genome editing in eukaryotic cells and organisms. Our current work focuses on studying the molecular mechanisms of Cas9 and other CRISPR-associated nucleases using a combination of structural, biochemical and biophysical approaches. To this end, we initially determined the three-dimensional structures of Cas9 in complex to a guide RNA and target DNA, revealing the atomic interactions underpinning the recognition of a short motif in the substrate DNA (the protospacer adjacent motif, PAM), which is necessary to facilitate strand separation in the DNA and guide RNA hybridization. These studies have established a structural framework for engineering novel Cas9 variants with altered PAM specificities. More recently, we have focused on Cas12a (Cpf1), another RNA-guided nuclease enzyme that has emerged as a complementary genome editing tool to Cas9. The crystal structure of Cas12a bound to a guide RNA shows that, like Cas9, Cas12a structurally preorganizes the seed sequence of the guide RNA to facilitate target DNA recognition. In turn, structures of Cas12a bound to a guide RNA and a double-stranded DNA target capture nuclease in a pre-cleavage state, revealing the mechanism of R-loop formation. Together with supporting biochemical experiments, the structures reveal that Cas12a contains a single nuclease active site that sequentially cleaves both strands of the target DNA in a defined sequential order. Collectively, our studies provide a mechanistic foundation for understanding the molecular function of CRISPR-based genome editor nucleases and for the ongoing development of CRISPR-Cas genetic engineering for biotechnological and therapeutic applications.