Structural basis of Cas1-2/3 integrase recruitment to the CRISPR leader-repeat boundary

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Bacteria and archaea have evolved many immune systems to combat the 1023 phage infections that occur every second. One of these systesms is the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) adaptive immune system. To adapt and acquire immunity to new phages, CRISPR-associated proteins (Cas1 and Cas2) integrate fragments of phage DNA ("spacers") at the "leader-end" of the CRISPR locus, near the transcription start site. Therefore, creating a chronological molecular record of previously encountered foreign DNA. Polarized integration is crucial because spacers at the leader-end of the CRISPR provide greater immunity against recently encountered phages. We previously identified several DNA sequence motifs that facilitate integration at the first CRISPR repeat. However, these motifs are up to 0 bp upstream from the CRISPR locus. A structural understanding of the role of these distant DNA motifs (up to 440 Å away) in CRISPR adaptation is lacking. Here we use cryo-electron microscopy to determine the ~3.4 Å-resolution structure of the type I-F Cas1-2/3 CRISPR integrase complex during the integration of foreign DNA into the CRISPR locus. The binding of the prespacer to Cas1-2/3 constrains the flexible linker between the naturally fused Cas2 and Cas3 domains, which may facilitate subsequent binding of CRISPR leader motifs. Two IHF (Integration Host Factor) heterodimers cooperate with the hetero-hexameric Cas1-2/3 integrase to fold and compact the CRISPR repeat and leader DNA. IHF binding to a distal site brings two inverted repeats of a conserved leader motif together to form two anti-parallel DNA pillars bound by the Cas2 homodimer at the center of the I-F Cas1-2/3 integrase complex. These DNA pillars form an arch over the prespacer bound to Cas2, suggesting that Cas1-2/3 may bind to the prespacer before engaging the CRISPR leader. These findings reveal how bacteria fold DNA to regulate the formation of DNA memories necessary for CRISPR adaptive immunity.