**INTRODUCTION**

Cas9 nucleases is the key effector of type II CRISPR adaptive immune systems found in bacteria. The nucleases can be programmed by a single guide RNA (sgRNA) to cleave DNA in a sequence-specific manner. This property has led to its widespread adoption as a genome editing tool in research laboratories and holds great promise for biomedical and therapeutic applications. The general mechanistic features of catalysis by Cas9 homologs are comparable; however, a high degree of diversity exists among the protein sequences, which may result in subtle mechanistic differences. 

**RESULTS**

**S. aureus and S. pyogenes Cas9 bind respective sgRNA with comparable affinities and form active RNP**

Unlabeled Cas9 was titrated in the presence of Cy5-labeled sgRNAs and fraction bound was calculated from changes in fluorescence anisotropy.

**OVERVIEW OF CAS9 CATALYSIS**

Cas9 consists of two major lobes and is in an apo state in the absence of RNA. The fold of the single guide RNA (sgRNA) 3’-terminal −80 ribonucleotides is recognized by Cas9. Upon binding the sgRNA, Cas9 undergoes a large conformational change, marked by rotation of the RuvC domain, forming a stable ribonucleoprotein complex (RNP).

The Cas9 RNP searches the DNA for a protospacer adjacent motif (PAM), which is NGG for SpyCas9 and NNGRRT for SauCas9. Locating the PAM poises the complex to form a hybrid duplex between the “reverse” strand of the DNA and the 5’-terminal −20 ribonucleotides of the sgRNA. If the DNA is complementary, an R-loop is formed, and the DNA is cut by RuvC- and HNH-like domains. Upon DNA cleavage, S. pyogenes Cas9 is known to have extremely slow product release and may exhibit additional DNase activity.

**Comparison of S. pyogenes and S. aureus Cas9 homologs**

S. pyogenes Cas9

S. aureus Cas9

**REFERENCE**