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Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation

Martin Jinek *et al.*

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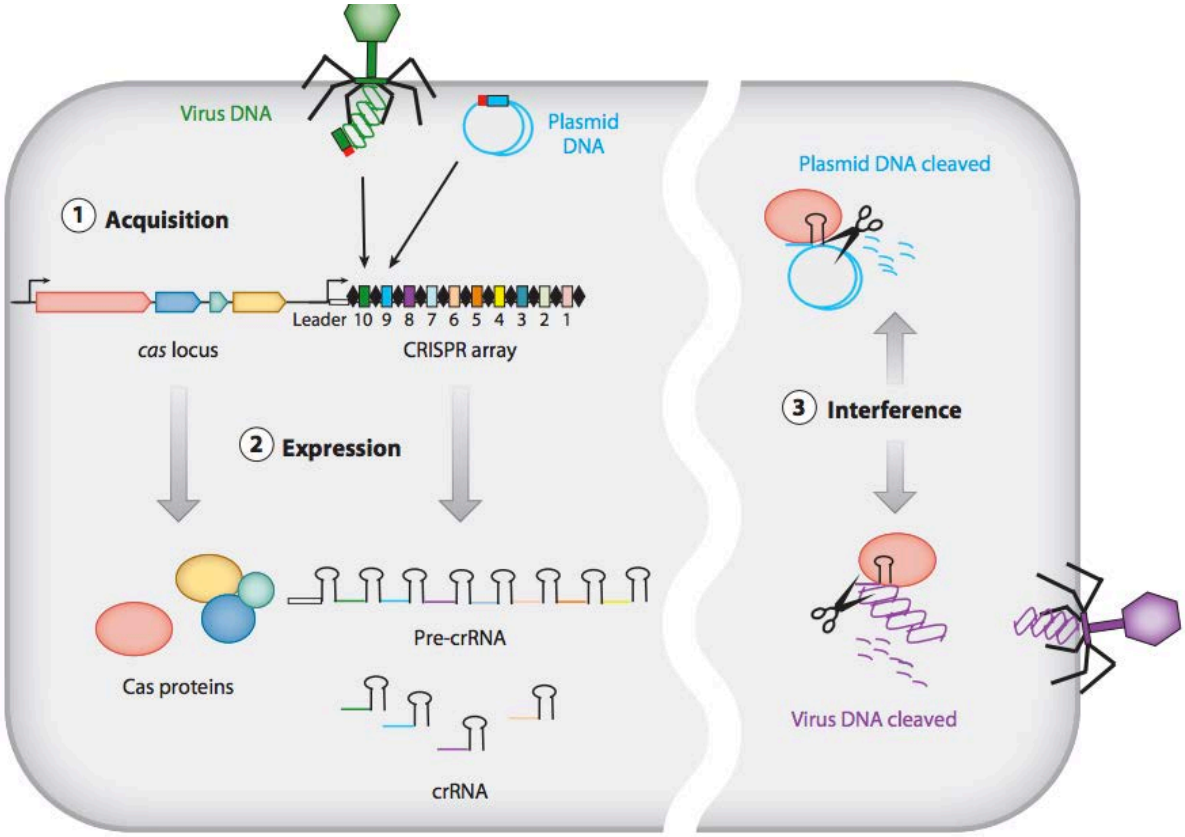
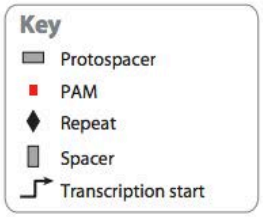
Why I Think You Should Know About This Work

- CRISPR-Cas9 system is powerful tool for genome editing-gene modifications within 1-2 weeks, modified clonal cell lines within 2-3 weeks;
- The basis for target DNA recognition and cleavage is still not clear;
- Though structure information is limited (apo only, low resolution of EM), multiple methods are combined by this paper to reveal more features of this system.

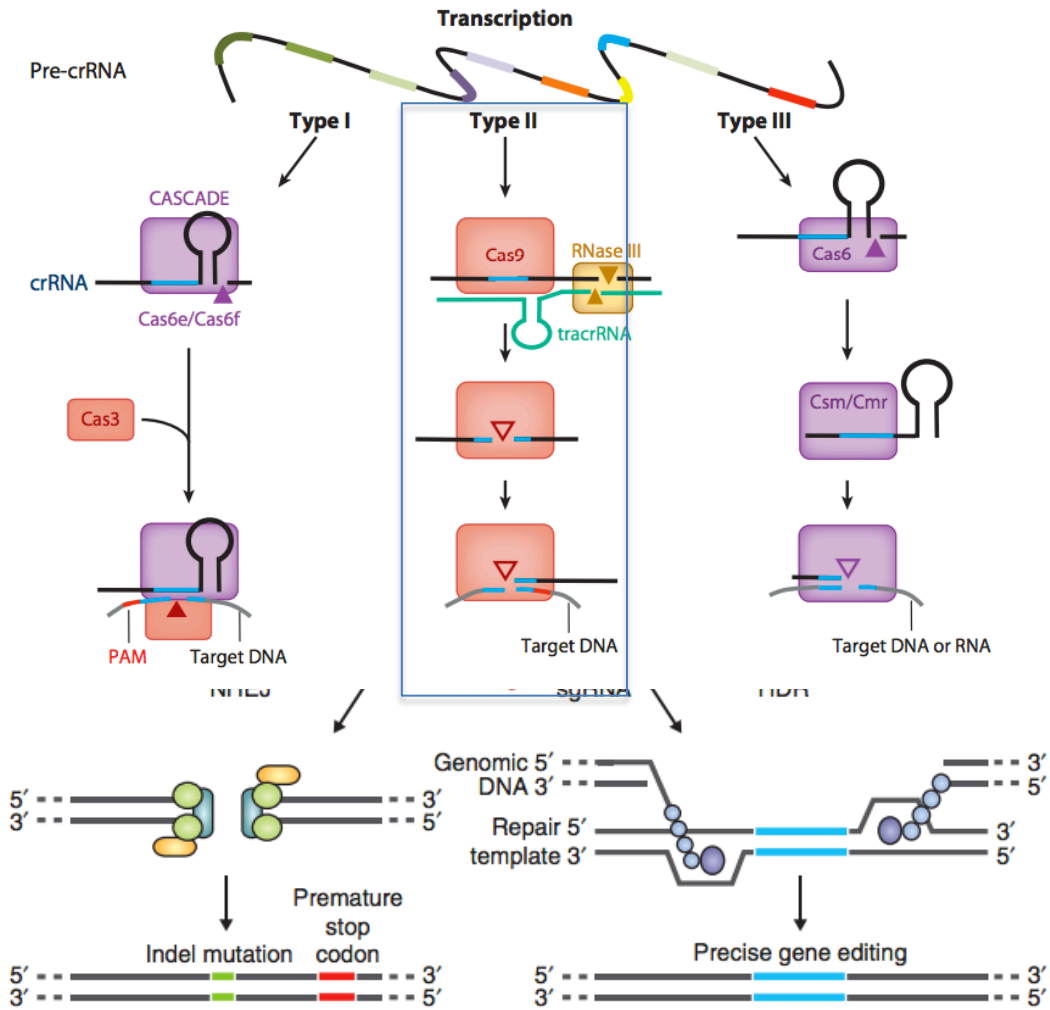
CRISPR-Cas adaptive immune system





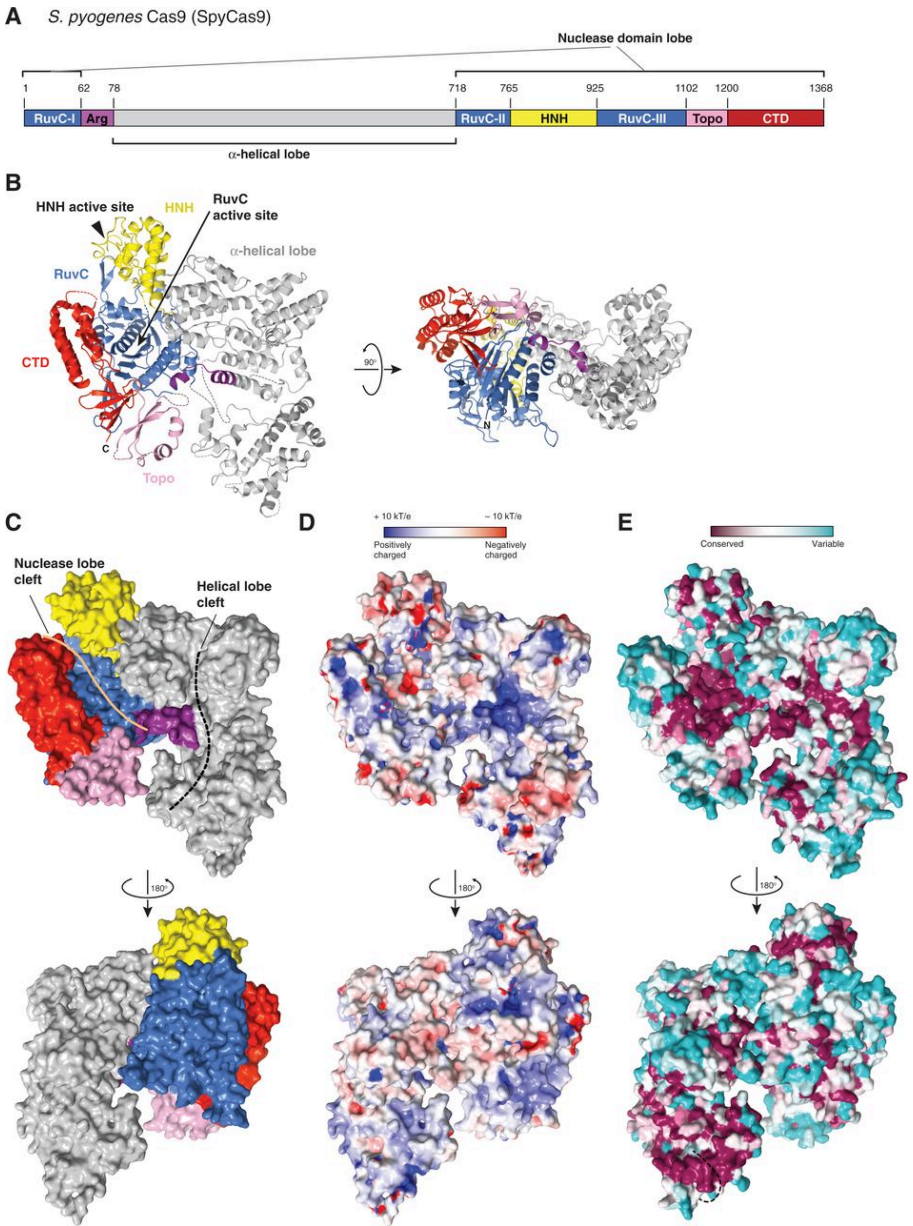
- Protospacers of invasive DNA with adjacent PAM (protospacer-associated motif) are acquired at CRISPR array of host DNA;
- Pre-CRISPR RNA (Pre-CrRNA) is transbribed and cleaved into smaller crRNAs with a single spacer and hairpin;
- The spacer of crRNA matches foreign DNA, which initiates cleavage event;

CRISPR as tool for gene editing



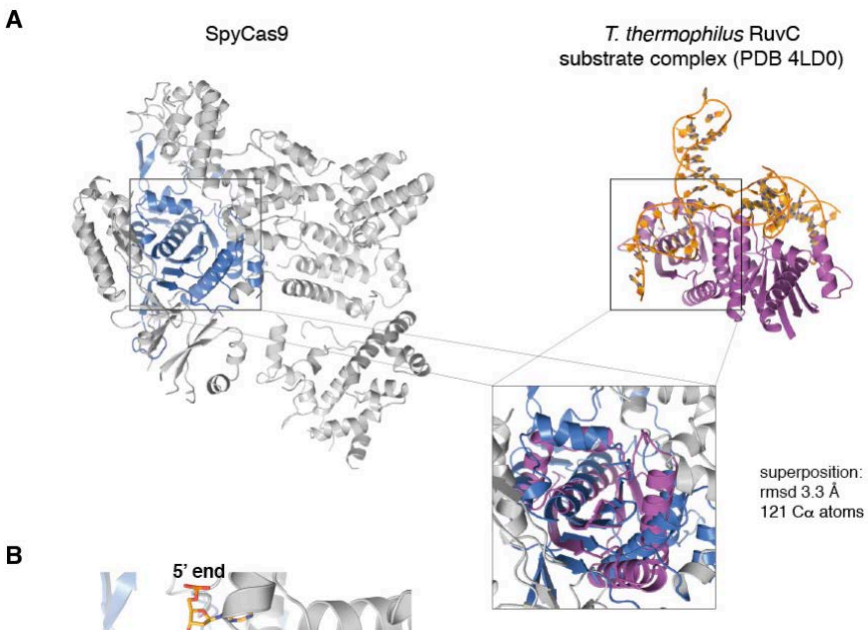
- In Type II system, single endonuclease, Cas9 is used;
- Both trans-activating crRNA (tracrRNA) and crRNA are required for target DNA recognition and cleavage;
- The cleavage is strictly dependent on the presence of PAM in targent DNA;
- By cleavage, DSBs are generated and further repaired by NHEJ or HDR.

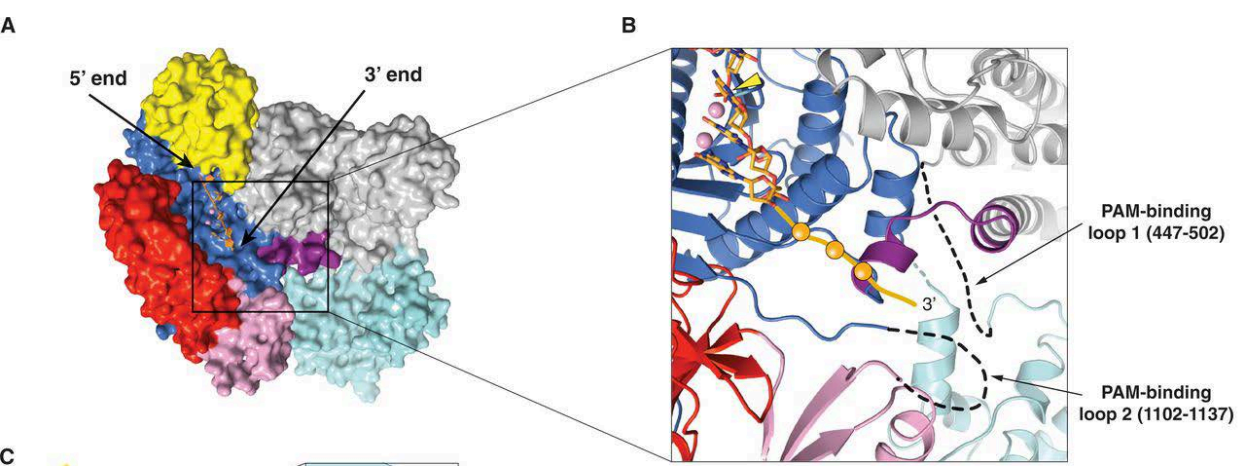
structure of SpyCas9



- Biolobed architecture: nuclease domain lobe and α-helical lobe;
- Two clefts on one face of the nuclease are conserved (E);
- Helical cleft is highly positively charged and is wide enough to accommodate duplex (RNA/RNA or RNA/DNA);
- RuvC active site (cleaving the displaced nontarget strand)locates at the bottom of nuclease lobe;

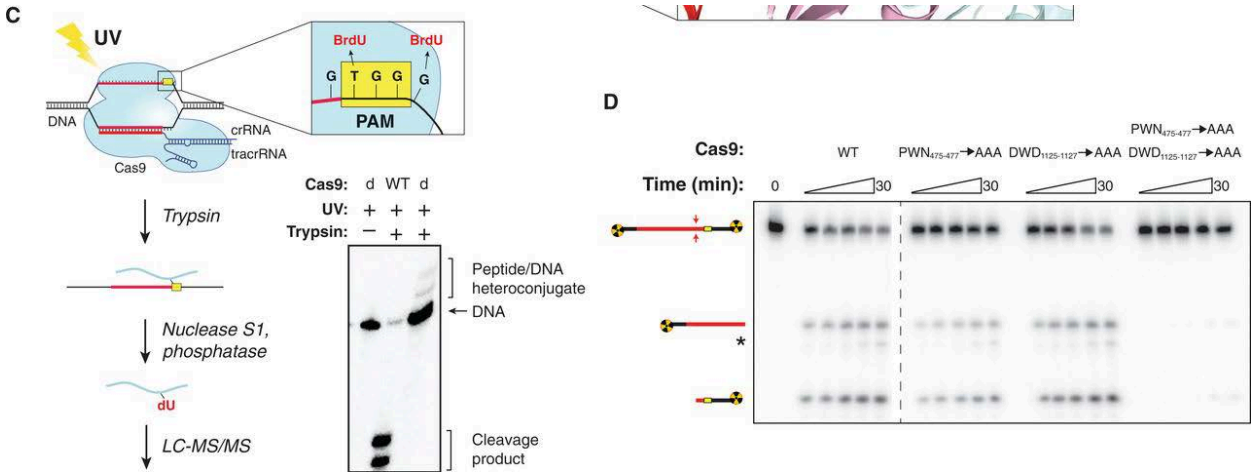
Recongition of PAM by SpyCas9





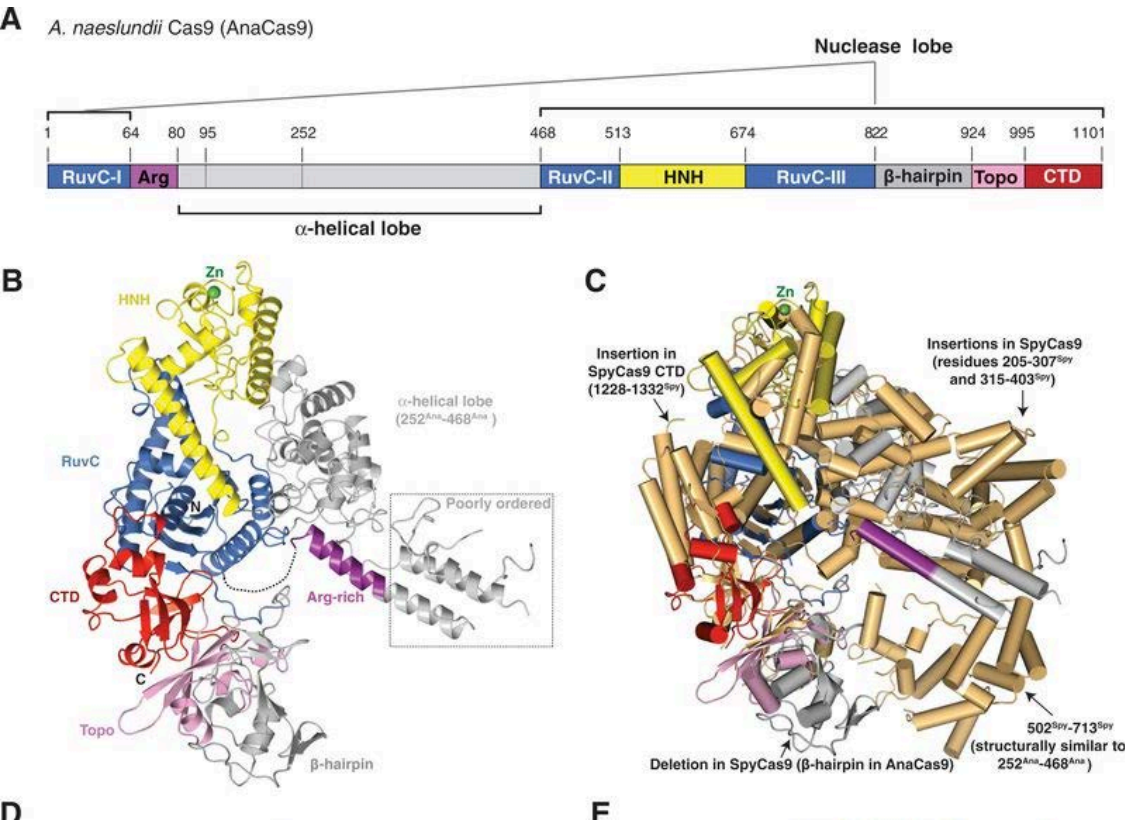
- SpyCas9 recognizes a 5'NGG-3' PAM sequence which is 3bp away from the cleavage site;
- Alignment of RuvC domain with RuvC Holliday junction resolvase-substrate complex hits PAM may be positioned at the junction of two lobes;

Recongition of PAM by SpyCas9



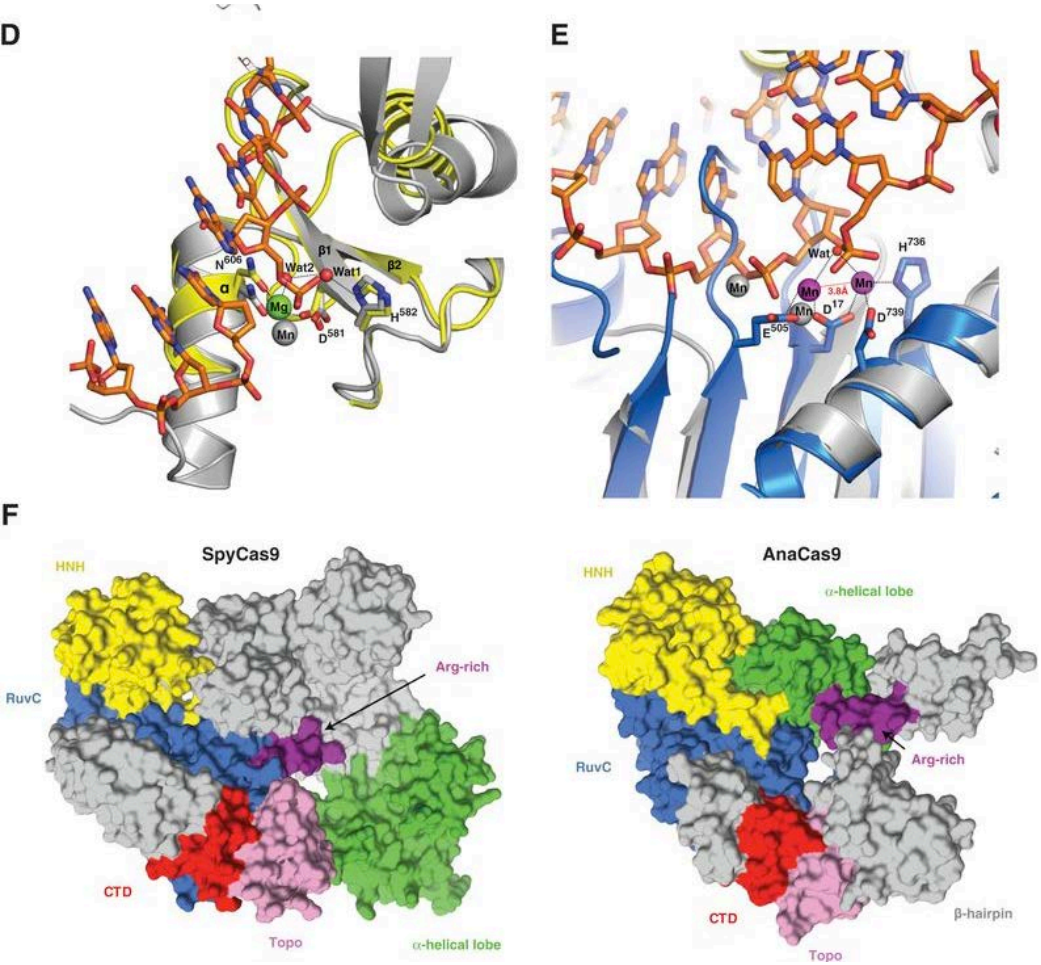
- Inactive SpyCas9(D10A/H840A) with crRNA:tracrRNA and DNA with photoactivatable BrdU on either end of the GG PAM motif on nontarget strand;
- After UV and trypsin treatment, peptide-DNA crosslink were detected;
- LS-MS identified residue 5’ to GG linked to W476 and residue 3’ to GG linked to W1126;
- Triple alanine mutation of both 475-477 and 1125-1127 almost abolished the activity;

structure of AnaCas9



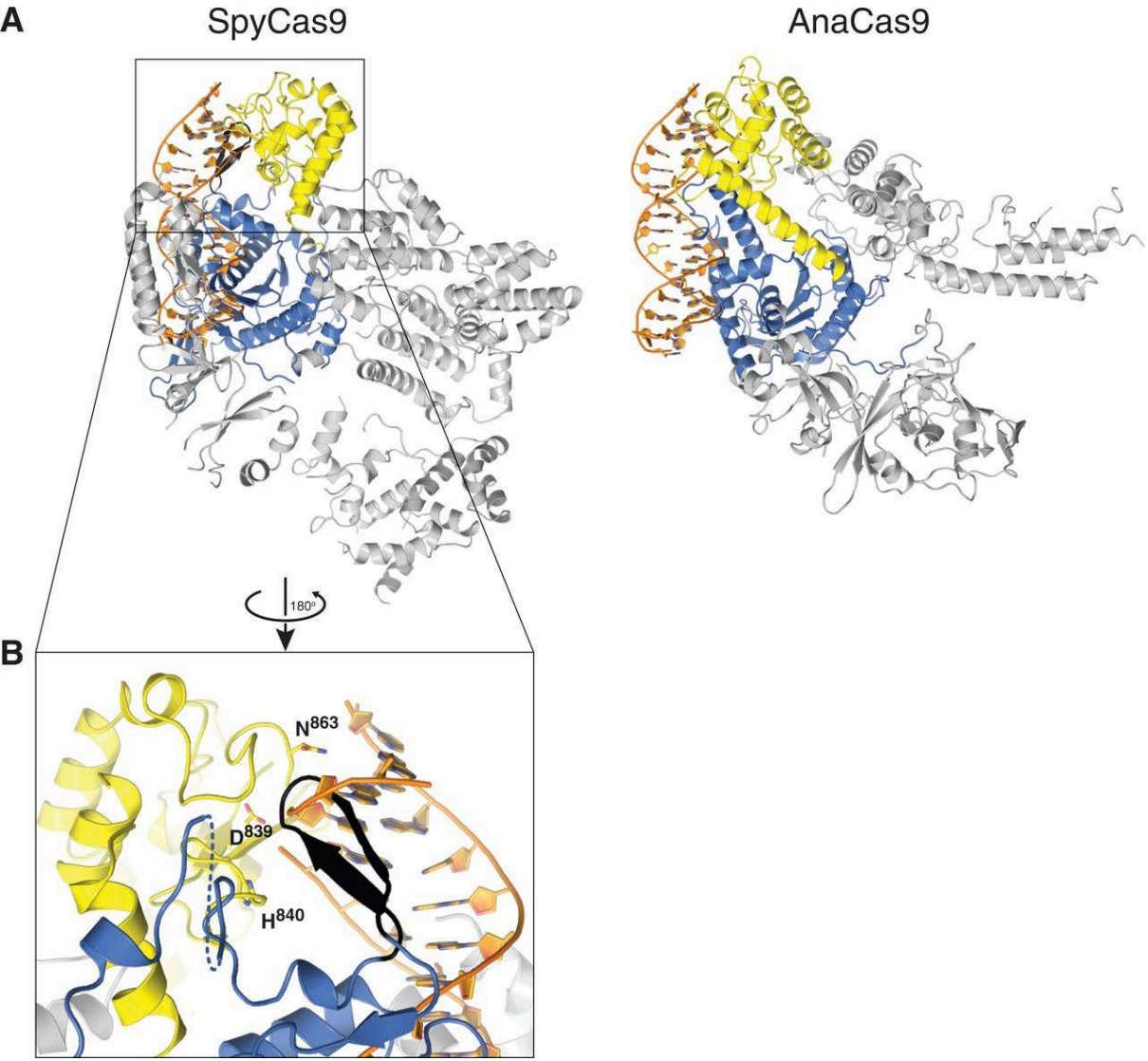
- Two lobe architecture;
- α-helical lobe is much smaller with different orientation relative to nuclease lobe;

structure of AnaCas9



- Activite site geometry proposed by structure alignment;
- HNH domain superimposed with I-HmuI-DNA complex: Mg++ for binding the scissile phosphate, H582 as the general base;
- RuvC domain superimposed with Rnase H: two metal (Mn++) mechanism;
- No biochemical evidence for the two points above;
- Conserved functional core: RuvC, HNH, Arg-rich region;

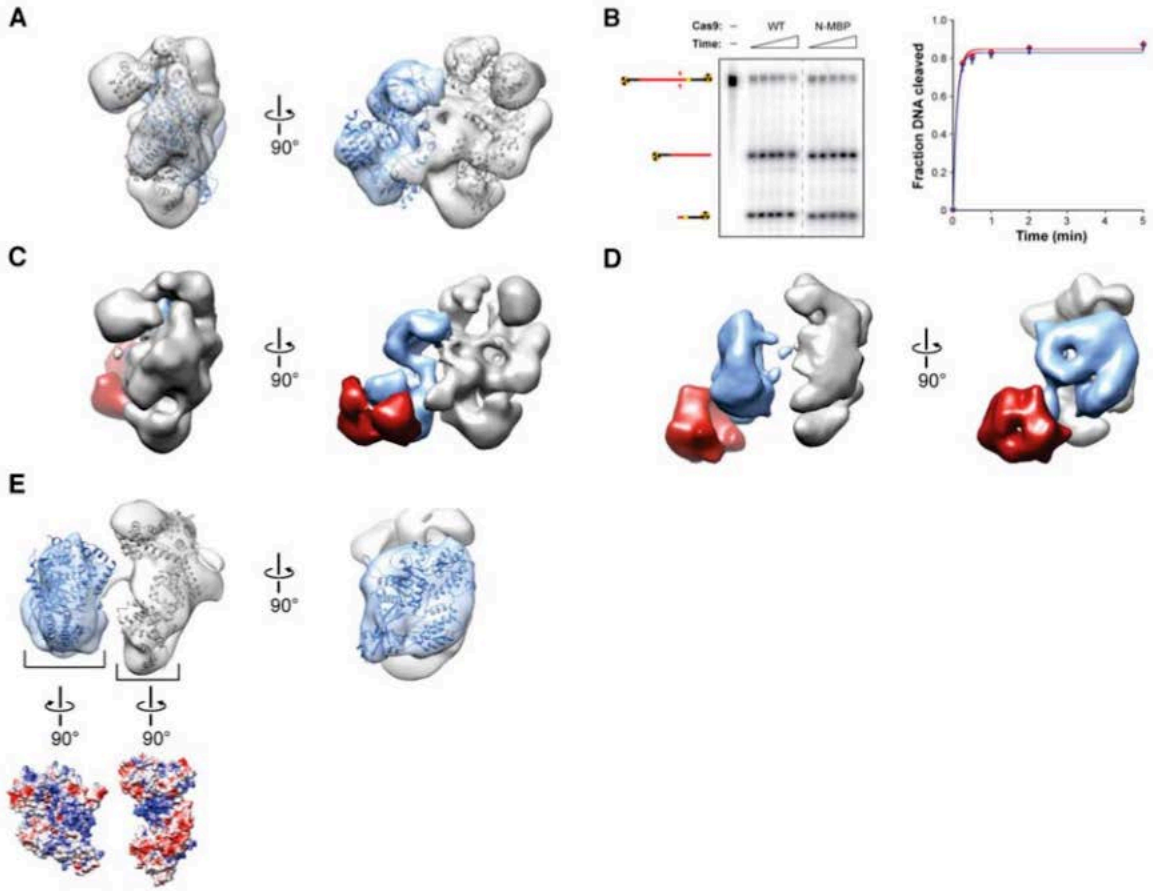
Apo-structure is inactive



- Alignment with I-HmuI/DNA complex imply the apo state is possibly not suitable for DNA binding (sterical clash);
- In SpyCas9, the HNH active site is blocked for DNA access by a beta-hairpin;
- No further evidence

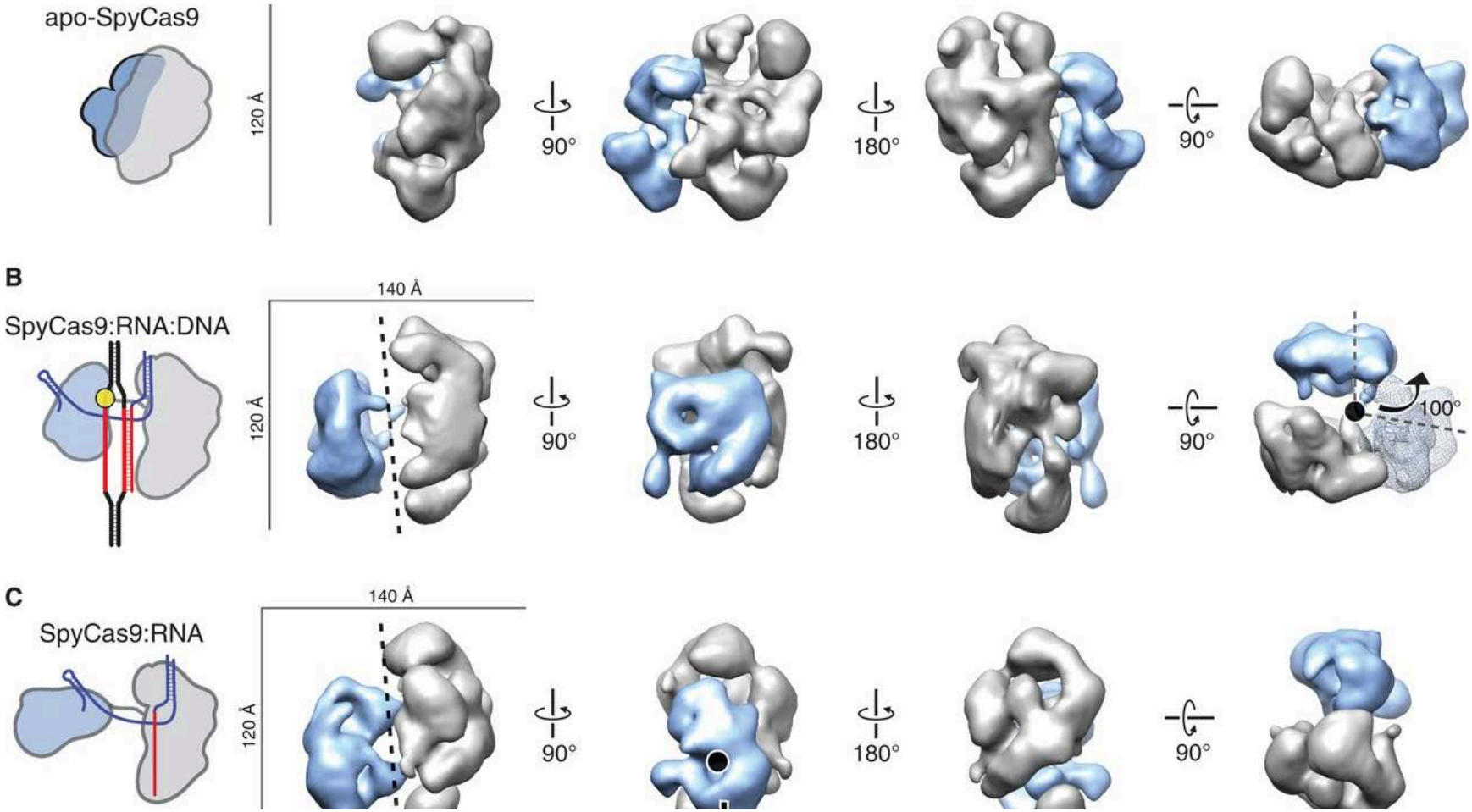
- If the apo state is inactive, how it is activated?
- Conformational change is probably involved;
- Negative-stain EM study was utilized to see these dynamic process: apo-SpyCas9, inactive SpyCas9 with crRNA:tracrRNA (SpyCas9:RNA), inactive SpyCas9:RNA:DNA, though the resolution is as low as 19A;

EM structure and X-ray crystal structure

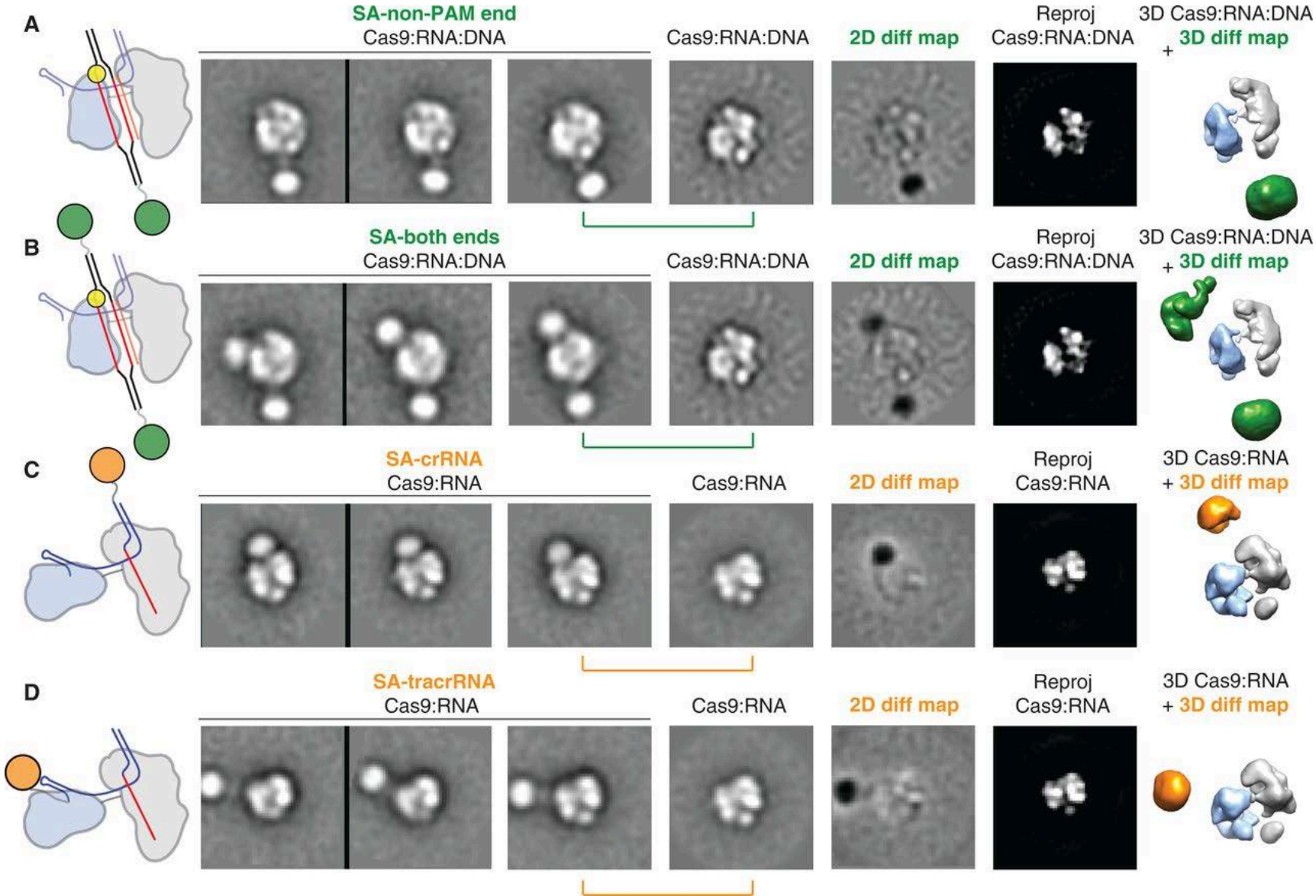


- The alpha-helical and nuclease lobe in the crystal structure can be docked as separate rigid bodies into the EM density;
- N-terminal MBP upstream of RuvC-I retains the activity and conforms to the EM docking results;

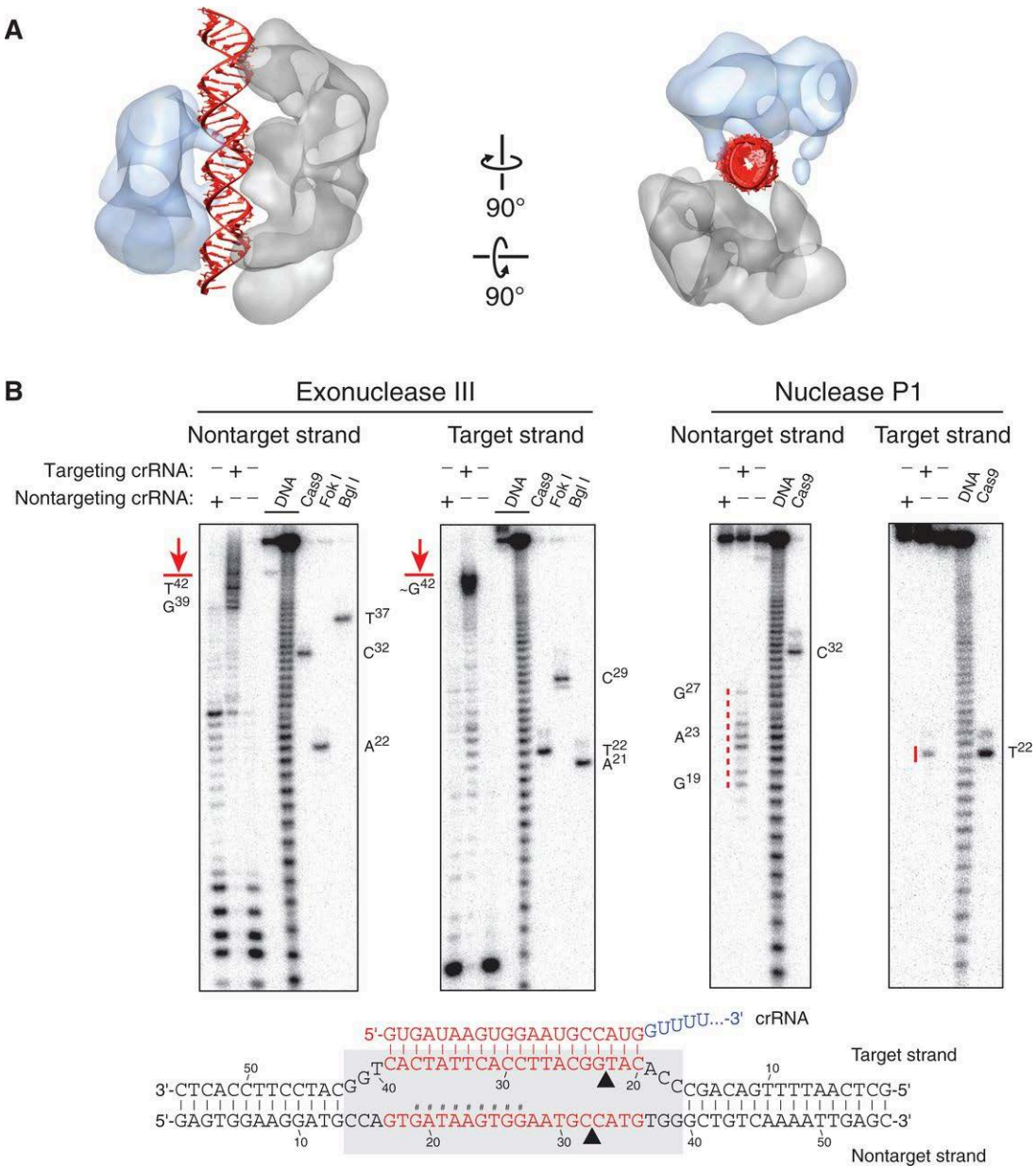
Central channel is formed by RNA loading



DNA and RNA's orientation along the channel



Central channel can accommodate DNA



conclusions

- The architecture of Cas9 is determined;
- Apo Cas9 may adopt a catalytically inactive conformation, so activation is needed;
- Guide RNA can induce conformation change in Cas9;

Take-Home Message

- Some points may need further evidence: such as the reaction mechanism in the RuvC and HNH active site, PAM binding region;
- High resolution of complex structure with ligand?

4UN3	**Crystal structure of Cas9 bound to PAM-containing DNA target** **Deposition Date** 2014-05-25 **Release Date** n/a **Status** HPUB **Authors** Anders, C., Niewoehner, O., Duerst, A., Jinek, M. **Sequence Available** NO
4UN4	**Crystal structure of Cas9 bound to PAM-containing DNA target with mismatches at positions 1-2** **Deposition Date** 2014-05-25 **Release Date** n/a **Status** HPUB **Authors** Anders, C., Niewoehner, O., Duerst, A., Jinek, M. **Sequence Available** NO
4UN5	**Crystal structure of Cas9 bound to PAM-containing DNA target containing mismatches at positions 1-3** **Deposition Date** 2014-05-25 **Release Date** n/a **Status** HPUB **Authors** Anders, C., Niewoehner, O., Duerst, A., Jinek, M. **Sequence Available** NO

Thanks!