Motif I of the Cas3 SF2 helicase domain regulates directionality of Cas3 movement during CRISPR interference and primed adaptation

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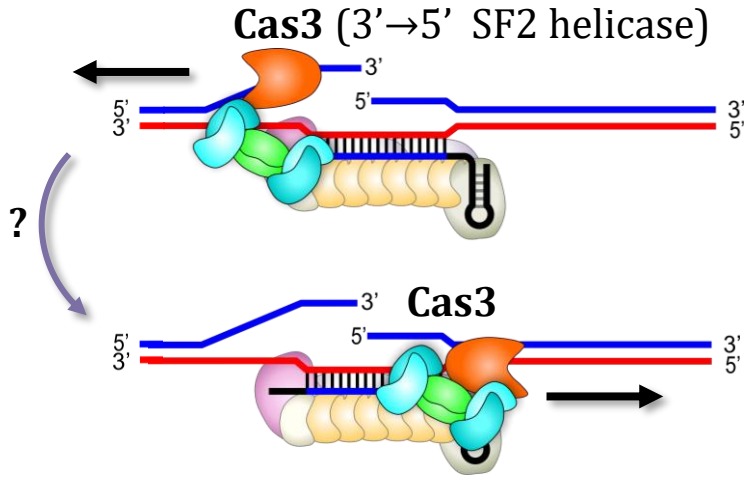
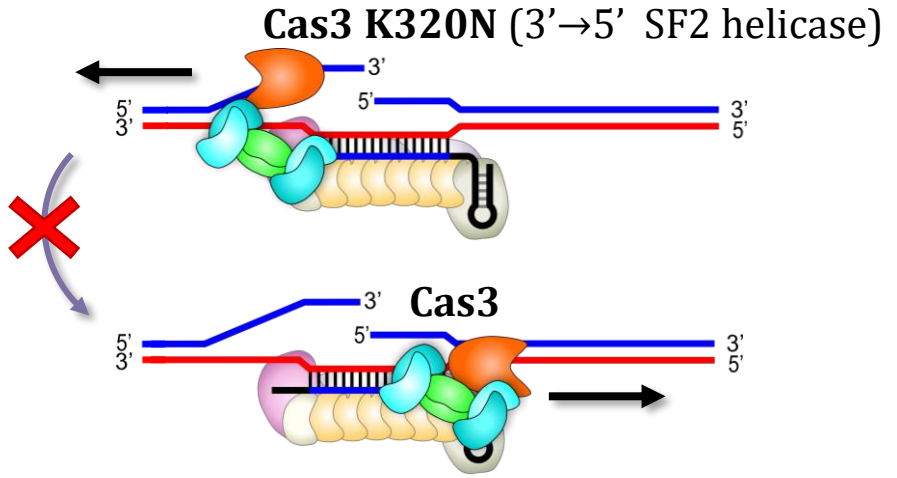
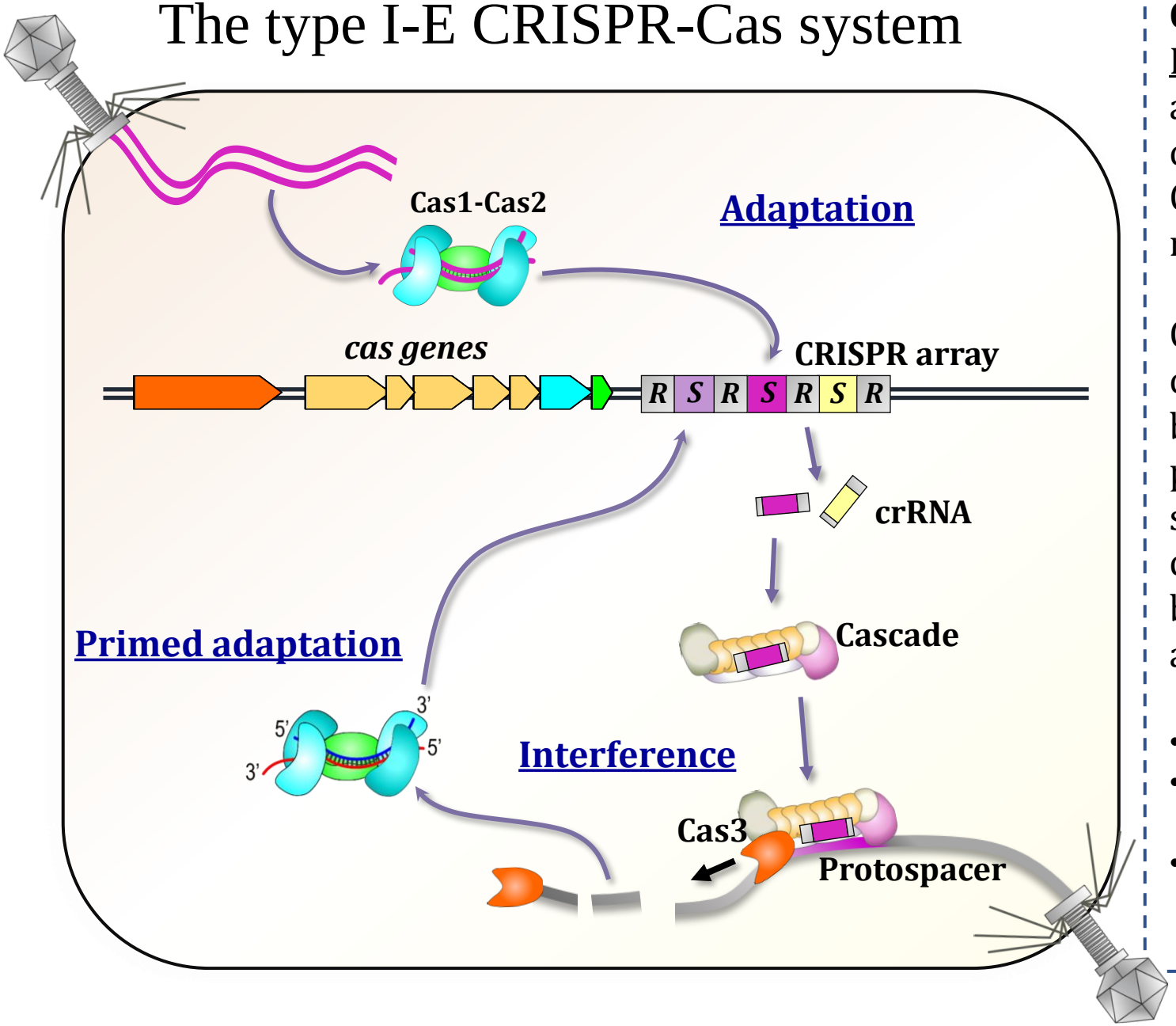




The type I-E CRISPR-Cas system



CRISPR-Cas (CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats) is an adaptive immunity system in prokaryotes. It consists of *cas* genes and CRISPR arrays.
CRISPR arrays include spacers (S) separated by repeats (R).

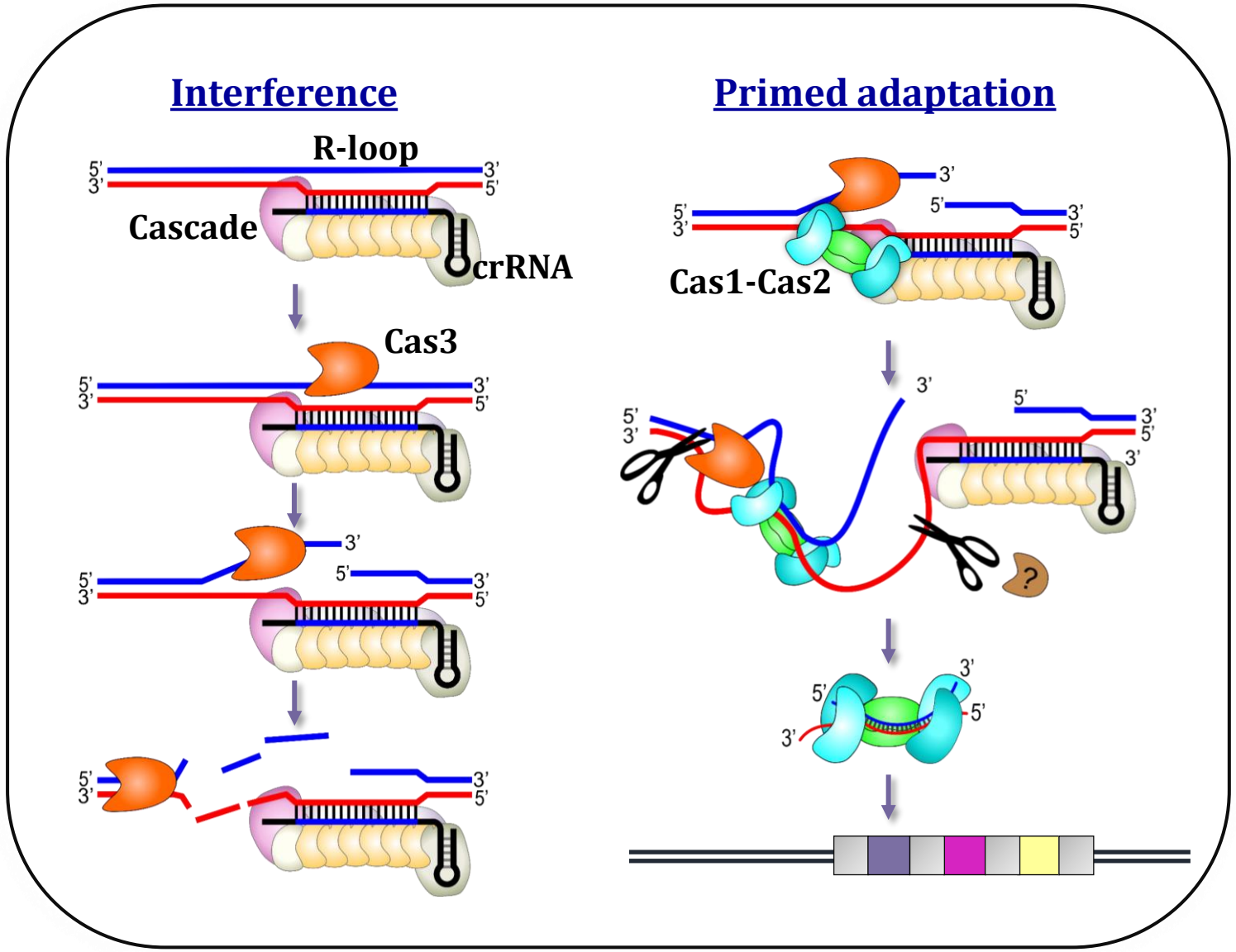
CRISPR arrays are transcribed yielding short crRNAs, each containing a single spacer. crRNAs bound by the Cascade complex search for protospacers - sequences complementary to spacers. Protospacer recognition leads to degradation of the protospacer-containing DNA by Cas3 protein. The degraded region serves as a source of new spacers.

- ***Adaptation*** – spacer acquisition
- ***Interference*** – degradation of protospacer-containing DNA by Cas proteins
- ***Primed adaptation*** - spacer acquisition from the region degraded during interference

The role of Cas3 in CRISPR interference and primed adaptation

Cas3 is a superfamily 2 helicase fused to an HD nuclease domain. Once the Cascade-crRNA complex recognizes the complementary protospacer during *interference*, an R-loop between the protospacer DNA and crRNA is formed. This leads to Cas3 recruitment. Cas3 nicks single-stranded DNA within the R-loop, loads onto the generated 3’ end, moves in 3’→5’ direction using ATP hydrolysis, and degrades the unwound DNA.

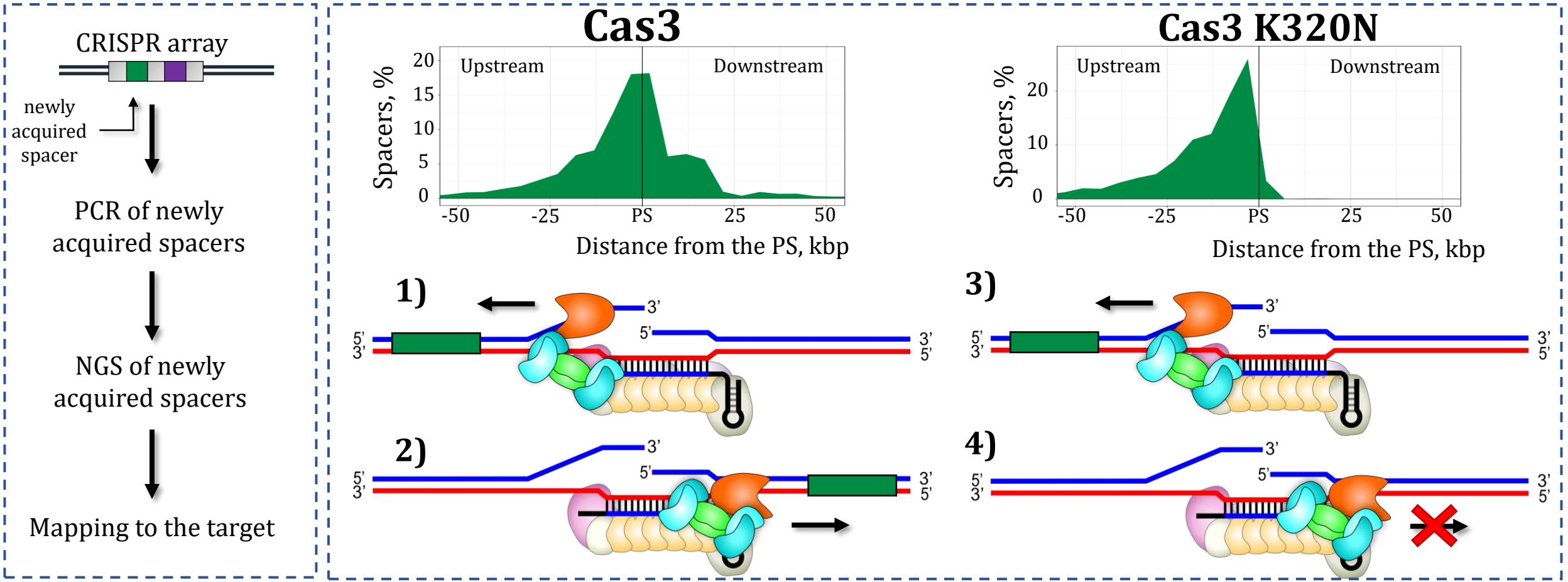
During *primed adaptation* Cas3 forms a complex with Cascade, Cas1, and Cas2. This complex moves along the target due to the Cas3 helicase activity. Eventually, short spacer precursors bound by Cas1 and Cas2 are generated and integrated into the CRISPR array.



Shiriaeva et al., 2019; Musharova et al., 2021; Dillard et al., 2018

Cas3 bidirectional movement requires lysine 320

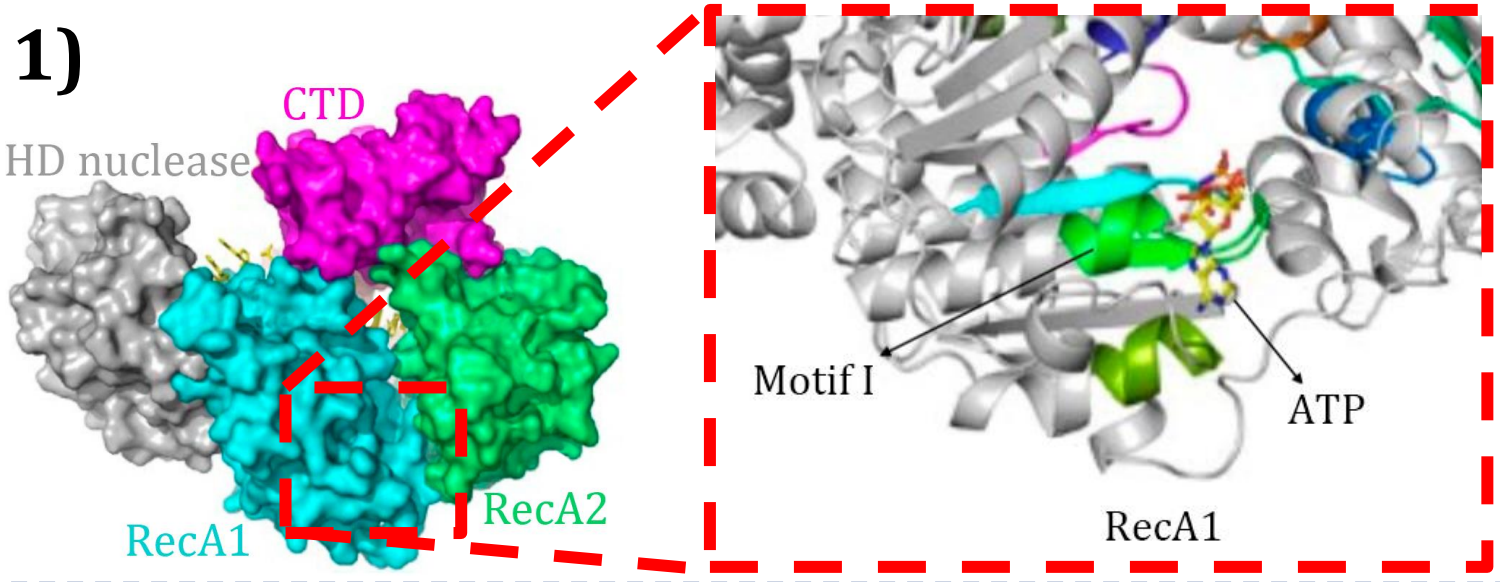
Cas3 has 3'→5' polarity (Sinkunas et al., 2011). It was shown initially that Cas3 moves unidirectionally upstream of the protospacer (PS) starting from the 3' end within the nicked R-loop (Sinkunas et al., 2013; Mulepati et al., 2013). However, when we amplified CRISPR arrays, subjected them to NGS, and mapped newly acquired spacers to the target, we found that spacers originate from both the region upstream (1) and downstream (2) of the protospacer. This suggests that Cas3 can somehow switch to the complementary DNA strand and start movement downstream of the protospacer. We also found that the K320N substitution abolishes spacer acquisition downstream of the protospacer (4).



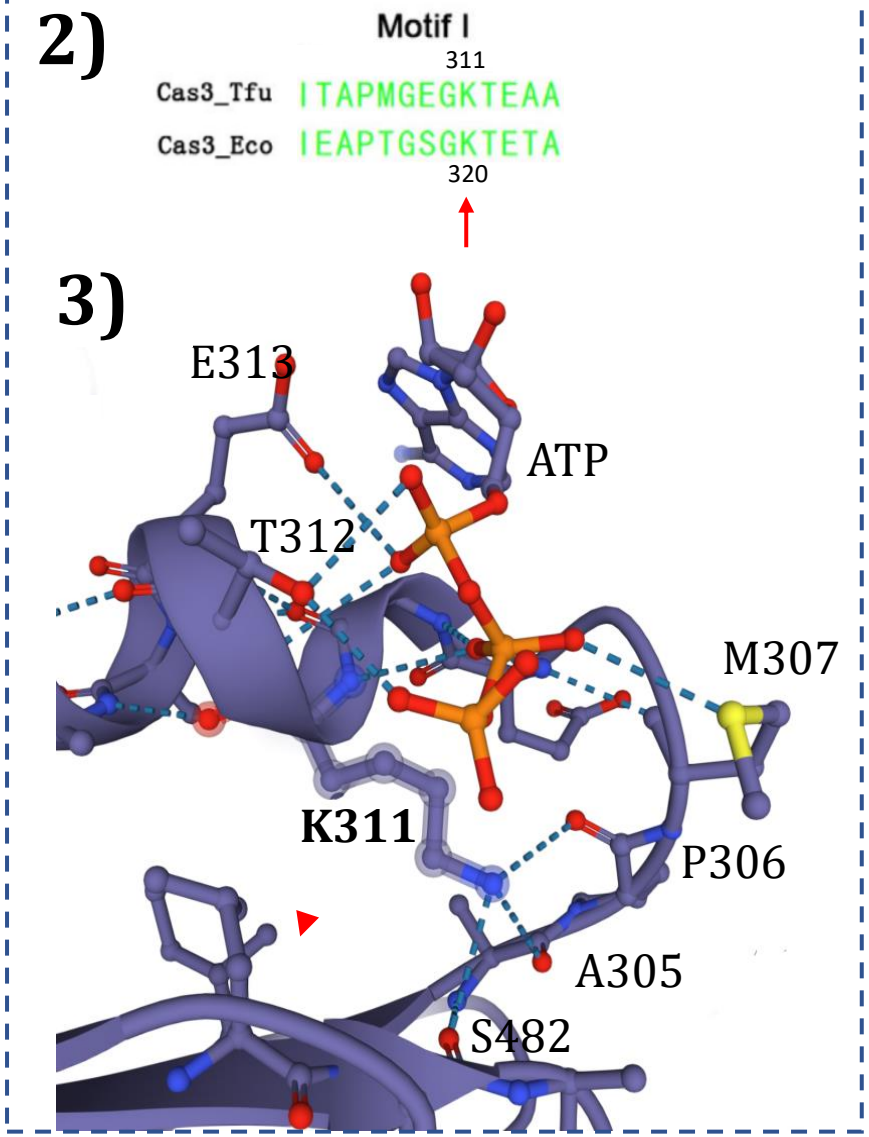
The K320 residue is located in the ATP-binding motif I of Cas3

There is a structure of a Cas3 homolog from *Thermobifida fusca* obtained in the Prof. Ke lab (*Huo et al., 2014*). The Cas3 helicase domain consists of RecA1 and RecA2 domains **(1)**. The *E.coli* Cas3 K320 corresponds to the *T. fusca* Cas3 K311 located in the motif I of the RecA1 domain **(1, 2)**. The *T. fusca* K311 is involved in ATP binding **(3)**. It's unclear why this residue affects the directionality of the Cas3 movement.

1)



2)



Contact us if you have any suggestions or questions!