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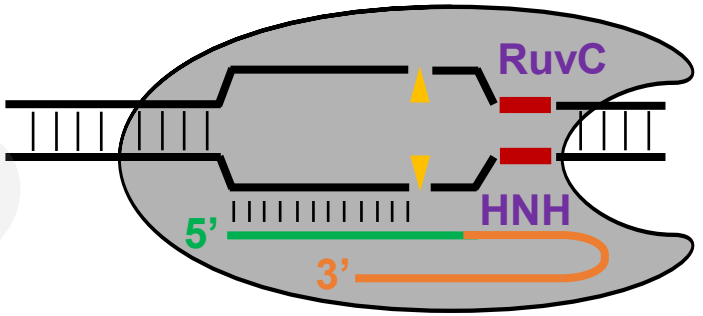
# Target binding and residence: a new determinant of DNA double strand break repair pathway choice in CRISPR/Cas9 genome editing

**Key words: CRISPR/Cas9 genome editing, DSB repair pathway choice, Target binding affinity, Target residence**

# *Backgroud:*

**Cas9-sgRNA binds DNA tightly and remains bound to its target for a period of time even after DNA cleavage, one key issue often ignored in CRISPR/Cas9 development is the possible effects of target binding and residence time on the efficiency and specificity of CRISPR/Cas9 genome editing.**




Tight binding and long residence time

# *Summary*

**This review is mainly focused on the key determinants of target binding and residence of Cas9-sgRNA and the effect of Cas9-sgRNA target binding and residence on CRISPR/Cas9 genome editing, in particular on DNA double strand break repair pathway choice**

# *Innovation points*

- **Unique DSB induction and repair in CRISPR/Cas9 genome editing**

- **Determinants of target binding and target residence of Cas9-sgRNA**

1. **Interaction between the PI domain and the PAM**
2. **Base pairing between the spacer of sgRNA and the target strand**
3. **Non-specific interactions between Cas9 and target DNA**
4. **Local DNA and chromatin metabolism**

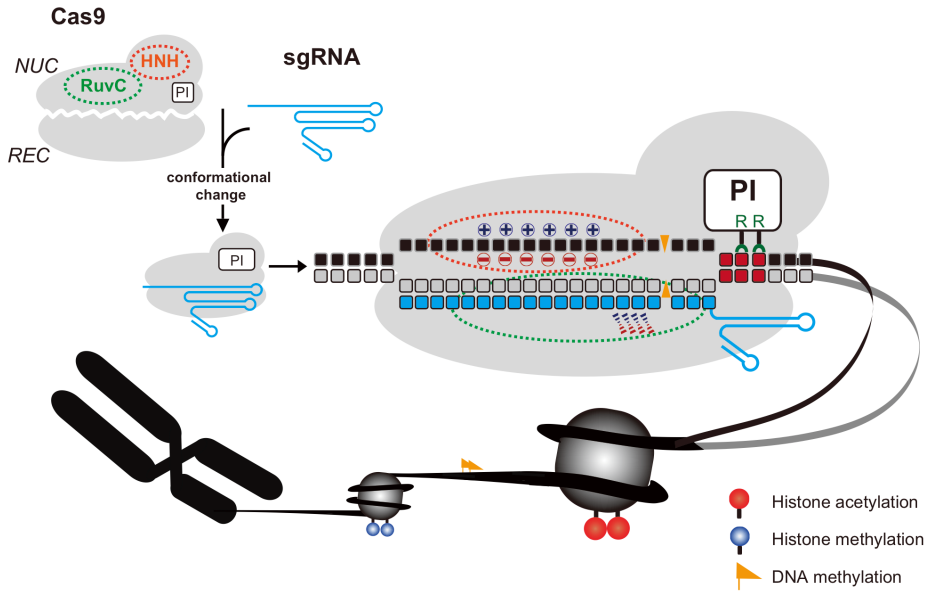



Figure 1

# • Effect of CRISPR/Cas9 target binding and residence on genome editing

1. dCas9-based applications: Cas9-sgRNA residence allows effective dCas9-based applications
2. Choice of DSB repair pathway: Cas9-sgRNA residence at the cleaved DNA influences the choice of repair pathway
3. Off-target effects: distinct choice of repair pathway at on- and off-target sites

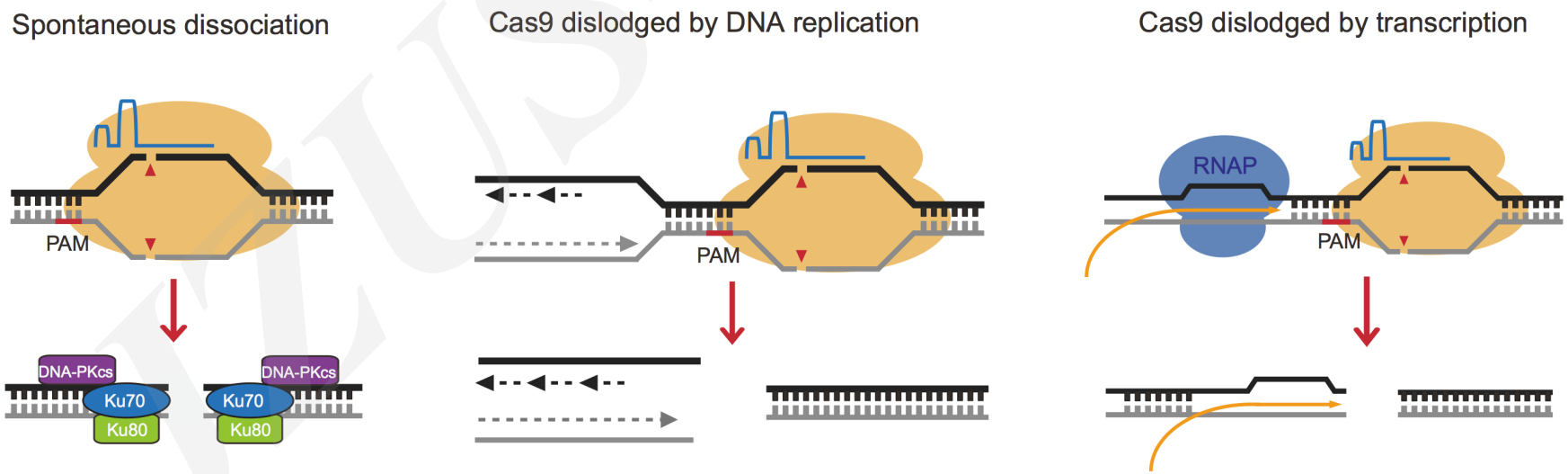



Figure 2

# *Opinions*

**Binding affinity and residence of Cas9-sgRNA at its target should be taken into account in using and improving CRISPR/Cas9 genome editing. This will provide an opportunity to Improve the efficiency of CRISPR/Cas9 genome editing and minimize the off-target activity of Cas9-sgRNA.**