Paper: **Therapeutic applications of CRISPR/Cas9 system in gene therapy**

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1. **What is the significance of this research topic?**

The CRISPR/Ca9 technology is one of the most significant breakthroughs in the last 10 years in genome modification. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas system is part of the immune system found in some bacteria and archaea. When a virus invades these bacteria, their DNA is “captured” and incorporated in the host DNA as spacers within the CRISPR array, each spacer is separated from the other by repeat units. The next step is transcription of CRISPR repeats and spacers into CRISPR RBA (crRNA). The last step is the activation of the Cas9 protein, which upon conformational change, finds the target DNA which then is followed by the attachment using base pair complementarity of the crRNA to viral RNA, which leads to the cleavage of the foreign RNA complex and its elimination. Cas9 nuclease activity can be easily replaced by a sequence of interest making Cas9 compared to other DNA-binding platforms (meganucleases, zinc fingers (ZF), transcription activator-like effectors (TALEs) is very efficient. Another important advantage of the CRISPR-Cas9 system, is its ability to cleave multiple distinct targets sequences in parallel, inducing multiple mutations in different genes which extend the possibilities for modeling complex diseases without lengthy breeding strategies with less animal testing when using mice from single gene knockouts.

**Who is working in this area?**

1. **What methods are used to study the concepts described in the paper?**

Among the different proteins involved in the CRISPR complex (Cas-1 to Cas10), Cas9 is the only enzyme within the Cas gene cluster that plays a role in locating and DNA cleavage. The Cas9 protein has 6 domains:

Rec 1 and Rec 2 domain binds the complementary region of the guide RNA. Rec1 role is essential compared to Rec 2as for the binding of repeat/anti-repeat target DNA.

Bridge helix (NH) arginine-based structure which modulates target DNA cleavage and mismatch tolerance.

Photospacer Adjacent Motif (PAM)-Interacting (PI) domain confers PAM specificity, and is responsible for initiating binding to target DNA.

HNH and RuvC domains are nuclease domains that cut single stranded DNA.

A single guide RNA (sgRNA) can be engineered by fusing a crRNA containing the target DNA sequence to a noncoding trans-activating crRNA (tracrRNA) to activate the Cas9 protein. The guide RNA forms a T-shape comprised of one tetraloop and 2 or 3 stem loops, and is constructed to have a 5’ end complimentary to the target DNA sequence.

1. **Does the review article lead to new questions or hypotheses in this technical area (by the authors, by other researchers)?**
2. **What are some practical applications of the research discussed in the article?**

**6. How does this topic relate to other areas of cell biology, bioengineering, or medicine?**

<https://www.jax.org/news-and-insights/jax-blog/2014/march/pros-and-cons-of-znfs-talens-and-crispr-cas>

<https://sites.tufts.edu/crispr/crispr-mechanism/rna-binding/>