Jiang et al. (2013); CRISPR-Cas9; Nature Biotech

Overview:

This paper by Jiang et al. is a detailed investigation of the basic mechanisms and practical considerations in applying the CRISPR-Cas9 technology to genome editing.

Experimental organism

The organism in which the experiments are carried out is Streptococcus pneumonia. Streptococcus is one of the bacteria that are naturally competent for the uptake of DNA. This is quite helpful for the sorts of experiments described, and makes the interpretation of the results straightforward.

[**A historical note**: Streptococcus pneumonia, also called Pneumococcus, was the first experimental organism in which DNA was demonstrated to be the genetic material. The earliest experiment was performed by Frederic Griffith, who utilized two types of Pneumococcus strains, a virulent one which killed mice when injected into them, and an avirulent one which did not cause the disease. Griffith heat-killed virulent cells, mixed these cells with the avirulent ones, and injected mice with the mixture. The mice became infected and died. The heat killed cells alone were harmless. Griffith concluded that that he had transformed the avirulent bacteria with a heat resistant 'transforming principle' from the virulent bacteria. Subsequently, experiments by Oswald Avery, Colin McLeod and Maclyn McCarty characterized the transforming principle to be DNA. It was shown to be resistant to heat, protease and RNase treatment, but lost its transforming activity upon DNase treatment.]

Significant results

The first set of experiments suggested that the introduction of CRISPR-Cas9 into cells containing a susceptible locus caused cell death. However, there were survivors in which the susceptible locus was replaced by the wild type locus present in the transforming DNA. The efficiency of this gene replacement (or gene editing) was enhanced in presence of the CRISPR-Cas9 system compared to its absence. This observation suggested the possibility of using the CRISPR-Cas9 system for editing genes in a targeted fashion.

When the amount of the 'editing DNA' in the transforming DNA was increased, the frequency of editing was also increased.

To assess the importance of the 'PAM' sequence (will be explained in class in more detail), this sequence was randomized, and the susceptibility or resistance of this randomized library to CRISR-Cas9 was assayed. The susceptible ones will not survive in this biological assay, while the resistant ones will. Their analysis showed the consensus triplet motif of PAM to be '5'NNG3'.

A similar randomization and screen was performed for the 20 nucleotide positions adjacent to the PAM sequence. At the first three to five positions immediately proximal to PAM, complementarity with the crRNA sequence is quite important. At the next five or six positions, complementarity is not critical. The overall effect on targeting varies depending on the particular base pair present in the DNA. Beyond position ten-twelve, the requirement for complementarity is considerably relaxed. However, in all cases, the complementary base almost always performs with the best or close to the best efficiency. Thus, when targeting a specific DNA sequence, the presence of a consensus PAM sequence is of prime consideration. The crRNA is then designed to be complementary to the adjacent 20 nucleotides of the DNA strand opposite to that harboring PAM.

Using the rationale described above, in a proof-of-principle experiment, successful editing of the LacZ gene of Streptococcus by introducing a point mutation, more than one point mutation or a deletion was demonstrated.

In the next set of experiments, the capability of performing sequential editing of loci and simultaneous editing of multiple loci was demonstrated.

In S. pneumonia, the CRISPR-Cas9 system provides a selection for the edited gene (resistant to killing by the CRISPR system) by eliminating cells containing the unedited gene (and therefore susceptible to killing). However there is a small but detectable fraction of cells that survive even in the absence of editing (escaper cells). In S. pneumonia, with the strong DNA uptake and integration systems, this background is not a serious problem.

There is a small enhancement of editing via homologous recombination promoted by CRISPR-induced double strand break, but this effect is rather modest.

While the CRISPR-Cas9 system works well in a bacterium with an efficient homologous recombination (integration) system, in its basic form the system is not so useful for bacteria with less robust homologous recombination systems, for example E. coli or Salmonella. The efficiency of editing (integration of the edited) gene is not sufficient, compared to the frequency of escape from CRISPR, to easily identify edited cells. Such bacteria have to be made more robust in

homologous recombination first before CRISPR-Cas9 can be implemented in them conveniently. This is achieved by reconstituting the phage lambda ‘red’ system to make them ‘recombineering’ strains. In such strains homologous recombination is sufficiently high to overcome the background produced by escaper cells. Finally, the double strand break generated by CRISPR-Cas9 stimulates red-mediated homologous recombination to further enhance the level of editing.

Summary

Among the currently available gene targeting systems, the CRISPR-Cas9 system is the most convenient as well as the most versatile and efficient one. Within a very short time of the discovery and characterization of CRISPR biology and biochemistry, its general principles have been exploited to efficiently and rapidly engineer eukaryotic genomes. The CRISPR field is a rapidly advancing one, and the progress in CRISPR technology continues to be impressive.