Session 21: CRISPR Technologies for Targeted Genome Editing

Lectures

L21.1

Temporal control of CRISPR/Cas9 activity

Maria Hubmann[2], Ivana Bilusic-Vilagos[1], Monika Borowska[1], Ulrich Elling[2], Krzysztof Chylinski[1]

[1]Vienna Biocenter Core Facilities (VBCF), CRISPR-Lab at Protech, Vienna, Austria; [2]Institute of Molecular Biotechnology (IMBA), Vienna, Austria
Krzysztof Chylinski <krzysztof.chylinski@vbcf.ac.at>

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated) systems constitute a family of prokaryotic adaptive defense mechanisms providing immunity against foreign genetic elements including bacteriophages and plasmids. To destroy foreign nucleic acids CRISPR-Cas systems utilize short RNAs, called crRNAs or CRISPR RNAs, that are in part complementary to the invader DNA. crRNAs are complexed with one or multiple Cas proteins, one of them being an executioner Cas nuclease that cleaves target DNA upon crRNA binding. So-called class II CRISPR-Cas systems use minimal DNA targeting machineries comprising only guiding RNA molecule(s) and a large single RNA-guided DNA endonuclease, namely Cas9 or Cpf1. Thus, class II systems were chosen to be adapted to programmable genome editing tools. CRISPR/Cas9 was the first CRISPR-based genome editing tool successfully used in a plethora of cell types and model organisms for gene deletion, insertion, mutation, transcription regulation, DNA visualization and epigenetic reprogramming. Although the tool quickly became the state-of-the-art in genome editing approaches, it still requires optimization in delivery, efficiency and specificity. This work concentrates on spatial and temporal control of genome editing using CRISPR tools. To this end we optimize the use of purified CRISPR/Cas9 components, namely recombinant Cas9 protein and *in vitro* transcribed sgRNA, both *in vitro* and *in vivo*. We further developed a tight, rapid and highly efficient system allowing for time-controlled expression of CRISPR/Cas9 components based on action of inducible recombinases. This virus-based system can be used to timely activate and deactivate genome editing or to restrict its action to particular tissue or cell types.

L21.2

Evaluation of CRISPR/Cas9-mediated genome editing efficiency

Magdalena Dabrowska[1], Karol Czubak[2], Wojciech Juzwa[3], Wlodzimierz J. Krzyzosiak[4], Marta Olejniczak[1] and Piotr Kozlowski[2]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Department of Genome Engineering, Poznań, Poland; [2]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Department of Molecular Genetics, Poznań, Poland; [3]Poznan University of Life Sciences, Department of Biotechnology and Food Microbiology, Poznań, Poland; [4]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Department of Molecular Biomedicine, Poznań, Poland
Marta Olejniczak <marta.olejniczak@ibch.poznan.pl>

Genome editing technology based on engineered nucleases has been increasingly applied for targeted modification of genes in a variety of cell types and organisms. Despite great progress in sgRNA design algorithms, the efficiency of a specific double strand breaks induction within the target sequence is still difficult to predict. Additionally, unspecific targeting of other genomic regions (off-targets) is difficult to avoid and therefore remains one of the most important challenges of genome editing approaches, especially in the context of their clinical applications. Several methods have been developed to evaluate the activity of sgRNAs and frequency of INDEL mutations; however, all of them have their specific limitations, including preferential detection of some mutation types, sensitivity to polymorphisms that hamper mismatch detection, lack of multiplex capability, or sensitivity to assay conditions. Unlike other methods, qEva-CRISPR (**q**uantitative **Eva**luation of **CRISPR**/Cas9-mediated editing) detects all types of mutations, including point mutations and large deletions, and its sensitivity does not depend on the mutation type. It may become a method of choice for unbiased sgRNA screening to evaluate experimental conditions that affect genome editing or to distinguish homology-directed repair from non-homologous end joining (Dabrowska M *et al*., 2018 *Nucleic Acids Res*).