Precision Genome Editing using Modulators of dsDNA Break Repair Pathways

Supervisory team:
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Project description:
CRISPR/Cas9 relies on introducing specific double-stranded DNA breaks (DSBs) into the target genome which are then repaired by the predominant host cell DSB repair mechanisms: Homology-Directed Repair (HDR) or Non-Homologous End-Joining (NHEJ); the activity of these pathways depends on the type and dividing state of the cell. Both DNA repair mechanisms can be utilized productively for DNA insertion, each with its own merits. However, in the absence of any selection pressure, the DNA insertion efficiency is low (<10% typically) preventing exploitation of CRISPR/Cas9 systems for scarless large DNA insertions to their full potential.

We now aim to potentiate the efficacy of DNA integration into the target genome. Chemicals have been shown to enhance DNA repair by selectively modulating components of the cellular DNA repair machinery. We postulate that nanobodies can likewise act on cellular DNA repair factors, inhibiting either HDR or NHEJ and thus boosting efficacies. Nanobodies (Camelid single-domain antibody fragments) are small (15 kDa), and fold within cells.

Starting from a naïve nanobody library we will select highly specific, high-affinity binders using Ribosome Display in vitro selection and evolution, a method established in the Berger-Schaffitzel laboratory. Many constituent proteins of the two DSB repair pathways are available in recombinant, highly purified form in Mark Dillingham's lab; these will be used as antigens for nanobody selection. We have already shown that this is possible by selecting nanobodies against KU70/80. DNA encoding for the best nanobody candidates with desirable characteristics (such as blocking protein-protein interactions, inhibiting Ku70/80 DNA binding, etc) will be incorporated into viral vectors that already contain the CRISPR/Cas9 system to boost DNA insertion efficacy. To this end, the selected nanobody binders will be characterized biochemically, biophysically and structurally (using crystallography or electron cryo-microscopy).

In collaboration with Binyam Mogessie we will establish Trim-Away for nanobodies which bind their targets but do not interfere with function. Trim-Away is a highly effective technique to acutely degrade endogenous proteins in mammalian cells with the help of antibodies. Antibody-bound targets are recognized by TRIM21 that recognizes the Fc domain of antibodies with high affinity. Thus, we will fuse the mammalian Fc domain to the selected nanobody. TRIM21 will then target the nanobody-antigen complex (e.g. a protein involved in DSB repair) to the proteasome for degradation. This approach offers exciting possibilities for acute, transient protein depletion (within minutes of application, avoiding secondary, compensatory effects) to study basic mechanisms of DSB repair and to boost DNA insertion efficiency.

References: [1] Hanes, J., Schaffitzel, C. et al. (2000) Nat. Biotechnol. 18, 1287-1292. [2] Clift, D. et al. (2017) Cell 171, 1692-1706.