The Institute of Medical Science
The University of Tokyo

UTokyo

IMSUT Genome Editing Symposium 2024

Genome editing technologies now extend to develop safer, precise, and versatile tools for manipulating biological functions. This symposium spotlights cutting-edge "post-Cas9" advances and their innovative applications especially in animal models to explore frontiers across genome editing technology and applications. This symposium is co-sponsored by the JSPS International Leading Research 23K20043.

Date : Friday, June 21th, 2024
Place : IMSUT Auditorium

13:00-13:05 Introduction
Tomoji Mashimo（The University of Tokyo, Japan）

13:05-13:55 Non-human primate genome editing, disease modeling, and beyond
Tomomi Aida（Massachusetts Institute of Technology, US）（via Zoom）

13:55-14:25 Development and applications of genome editing technologies using type I CRISPR-Cas3
Kazuto Yoshimi（The University of Tokyo, Japan）

14:25-14:35 Breaks

14:35-15:25 Discovery and development of CRISPR-associated transposases for RNA-guided DNA integration
Samuel H. Sternberg（Columbia University, US）

15:25-15:55 Gene-manipulated animals and their applications in the study of reproduction
Masahito Ikawa（Osaka University, Japan）

15:55-16:00 Closing remarks

Contact Information: Tomoji Mashimo, Division of Animal Genetics, IMSUT
mashimo@ims.u-tokyo.ac.jp, Ext. 72228

NHP genome editing, disease modeling, and beyond

Tomomi Aida 1,2,3,4,5
1McGovern Institute for Brain Research, Massachusetts Institute of Technology, 2Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, 3Emugen Therapeutics, 4MIT-JPN (MIT Japanese Postdoc, PhD student, Professional Network), Massachusetts Institute of Technology, 5JBroadies (Japanese Broadies), Broad Institute of MIT and Harvard

Genetically modified animal models have revolutionized our understanding of in vivo gene function and disease modeling. However, many failures in clinical translation underscore the limitations of rodent models, necessitating the exploration of non-human primates (NHPs). With the advent of advanced genome editing technologies, the creation of genetically modified NHP models holds promise for faithfully recapitulating human diseases and accelerating therapeutic development. In this meeting, I will discuss our decade-long efforts in generating genetically modified NHPs and their application in disease modeling and in vivo interventions for functional genomics and gene therapy. Our work focuses on achieving precise and controllable genome editing in NHPs, with prime editing emerging as the leading approach. We have made significant strides in enhancing prime editing efficiency while it unexpectedly induced frequent undesired indels. Through large-scale prime editing and cross-species prime editing, we identified molecular and cellular mechanisms underlying undesired outcomes, that may further improve prime editing. We have successfully generated genetic NHP models for neurodevelopmental, psychiatric, and neurodegenerative diseases, showcasing unique phenotypes that cannot be recapitulated in mouse models. These results validate the utility of genetic NHPs models. Finally, we have developed in vivo genome editing strategies to investigate the genetic basis of brain function at cellular and neural circuit levels. These approaches provide powerful alternatives for functional genomics in wildtype animals, accelerating scientific discoveries and facilitating gene therapy development. In summary, our research demonstrates the feasibility of generating and utilizing genetic NHP models for disease modeling and in vivo genetic interventions. These findings lay the groundwork for advancements in functional genomics and further innovative therapeutics.

Development and applications of genome editing technologies using type I CRISPR-Cas3

Kazuto Yoshimi
Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science (IMSUT), The University of Tokyo

Advances in genome editing technology have facilitated the genetic modification of various species and cells. Previously, we demonstrated the potential of I-E CRISPR-Cas3, which is derived from E. coli and classified as class 1, for genome editing in human cells. The recognition sequence of CRISPR-Cas3 consists of 27 nucleotides, which is longer than the 20 nucleotides of CRISPR-Cas9, therefore it is expected to have a low off-target effect. The system can induce large deleted mutations at the target site, ensuring reliable gene disruption. This makes it a promising and safe genome editing tool for gene therapy and industrial applications in vitro and in vivo. In the CRISPR-Cas3 system, a cascade complex containing crRNA recognizes and binds to the target DNA sequence, followed by Cas3 cleaving the DNA. By purifying and analyzing highly active CRISPR-Cas3 proteins in vitro, we found that CRISPR-Cas3 has a collateral cleavage activity against the surrounding single-stranded DNA after the target recognition. This ssDNA cleavage activity is PAM-dependent and essential for introducing double-stranded DNA breaks at the target site. In addition, we have used this DNA cleavage activity to develop the CONAN (Cas3 Operated Nucleic Acid detection) method, which enables rapid diagnosis of COVID-19. Recently, he has successfully applied the CRISPR-Cas3 system to mouse and rat embryos, which has realized simple, rapid and precise gene modification as well as Cas9. In this talk, I would like to introduce the features of CRISPR-Cas3 and the various applications.

Discovery and development of CRISPR-associated transposases for RNA-guided DNA integration

Samuel H. Sternberg
Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

In recent years, genome editing technologies have advanced from nuclease-based methods that generate DNA double-strand breaks (DSBs), which can cause undesired byproducts, to next-generation CRISPR approaches that perform controlled chemistry using DSB-independent strategies. Base editing and prime editing are ideally suited for small-scale modifications, but methods to achieve large-payload gene insertion have been lacking. Towards this goal, I will present work from my lab describing a new family of CRISPR-associated transposases (CASTs) that perform highly accurate, targeted integration of DNA payloads via RNA-guided DNA transposition. Unlike conventional CRISPR systems that combine RNA-guided targeting with DNA cleavage, CAST systems exploit nuclease-deficient CRISPR systems for RNA-guided targeting, leading to the site-specific recruitment of transposase enzymes for DNA insertion. We have resolved molecular details of this pathway using a combination of high-throughput sequencing, biochemistry, genetics, and cryo-electron microscopy, revealing a hierarchical assembly pathway and a structural roadmap to guide engineering efforts. Beyond applying CAST systems as a powerful new tool for bacterial and microbiome engineering, we recently reported our efforts to reconstitute kilobase-scale gene knock-ins in mammalian cells. Our long-term goal is to harness CASTs as a versatile platform technology for integrating therapeutically relevant DNA payloads into the genome to treat human disease.

Gene-manipulated animals and their applications in the study of reproduction

Masahito Ikawa 1, 2
1 Research Institute for Microbial Diseases, Osaka University, 2 The Institute of Medical Science, The University of Tokyo

The CRISPR/Cas9 system has ushered in a new era for reverse genetics. Using the CRISPR/Cas9-mediated approaches, our laboratory has knocked out 421 male reproductive organ-specific genes and analyzed their phenotypes in vivo. Surprisingly, even with the highly testis-specific genes (TPM>10 and 10 times higher expression than any other non-reproductive tissues), only a third is individually essential for male fertility (111/351=31.6%). Nevertheless, this KO screening approach has allowed us to decipher molecular mechanisms critical for male reproductive function. Among them, we recently elucidated the long-sought lumicrine system. Testicular NELL2/NICOL goes through the efferent duct's lumen and stimulates the ROS1 signaling pathway in the epididymal epithelial cells. Thus, differentiated epididymal epithelial cells secrete proteases such as OVCH2 that mediate sperm ADAM3 trimming and sperm maturation. I will also introduce recent findings on the molecular mechanism of mammalian fertilization.