2022 年度　第4回　生命科学技術国際卓越講義

東京大学 THE UNIVERSITY OF TOKYO

World-leading Innovative Lectures in Life Science & Technology The University of Tokyo

WINGS LST Life Science & Technology

Seeing is believing: break-induced mitotic recombination from 1936 to 2022

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Date: Thursday, 17th, November, 2022

Time: 5：00PM ~ 5:50PM Lecture

6:00PM ~ 6:30PM Interview Session with Students and Young Researchers

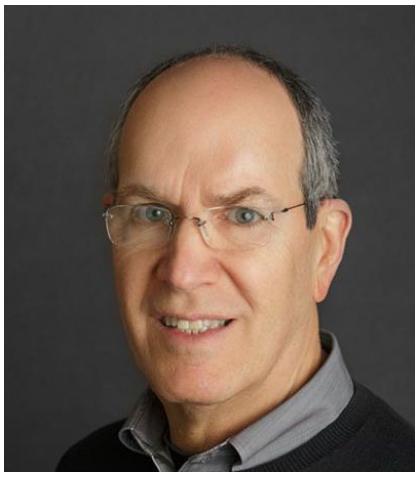
(Attending an interview session needs another registration (see your e-mail))

Venue: Zoom (meeting URL will be sent after registering)

Participants: Up to 500 participants

Please register by this QR code or clicking the following link

Registration Form



Abstract:

In 1936, Curt Stern showed that “twin spots” arose in *Drosophila* after a mitotic recombination event between the centromere and two distal markers that were linked in repulsion (y, yellow body color and sn, singed bristles; Genetics *21*: 625–730). His analysis suggested that, after the crossover, one of the two chromosome segregations that can take place during mitosis leads to Loss of Heterozygosity (LOH). The other mitotic segregation does not lead to LOH, although one daughter cell contains the two reciprocally crossed-over chromosomes. Stern hypothesized that the crossover had to occur in G2 to generate the LOH. To examine the steps in mitotic recombination that occur after induction of a site-specific double-strand break (DSB) in diploid budding yeast cells, we have created a strain that contains “Yellow” and “Red” fluorescent tags flanking each side of an I-*Sce*I cut site. The homolog, without a cut site, contains a single “Magenta” fluorescent tag at the homologous position of the Red site. Crossover events can be visualized by the change in linkage of the Yellow tag with respect to Red and Magenta. In addition to the I-*Sce*I enzyme, we have designed a CRISPR-Cas9 guide that also directs cutting very close to the I-*Sce*I site. Thus, we can compare the repair outcomes of an I-*Sce*I-generated break (3’-protruding end) to a CRISPR-Cas9 break (blunt end). In both cases, nuclease expression is induced continuously and >99% of the surviving colonies undergo gene conversion of the cut site. Cells from the colonies are examined using epifluorescence microscopy to determine whether the chromosomes have undergone crossovers associated with the induced gene conversion event. After I-*Sce*I and CRISPR-Cas9 induction, crossovers and non-crossovers each account for approximately half of the repair outcomes. For I-*Sce*I, 10 of the 26 crossovers give rise to LOH and for CRISPR-Cas9-induced breaks, 4 of the 14 crossovers are LOH. Importantly, the colonies containing non-LOH crossovers have cells with two reciprocal recombinant chromosomes, allowing us for the first time to directly visualize the mitotic segregation class that Stern predicted 86 years ago. Finally, no G1 crossovers were recovered since “pure” non-LOH colonies were not observed. Our results fit a model whereby the efficient repair of a DSB in mitotic diploid cells results in a G2 crossover half of the time.

At the same time, we have also been studying how homologous recombination after induction of a DSB is linked to changes in chromosome movement. We find that end resection, the recruitment of recombination proteins, increased chromosome mobility, the pairing of homologs and gene conversion are temporally linked in response to a DSB. In *mre11*Δ mutant cells, which exhibit a delay in the initial processing of a DSB, chromosome mobility and all subsequent recombination events are also delayed. Interestingly, overexpression of the Dna2 nuclease, but not ExoI, suppresses the *mre11*Δ delay in end resection and restores the original timing of chromosome mobility and all subsequent downstream HR events. Thus, changing the timing of chromosome mobility results in a corresponding change in essential downstream HR events, reinforcing its mechanistic role in the DNA repair process.

Organizer: World-leading Innovative Graduate Study Program for Life Science and Technology

Cooperation: Graduate Program for Leaders in Life Innovation, The University of Tokyo

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