Deoxyribonucleic acid (DNA) is a molecule that carries the genetic information in all known living organisms. The accurate replication and transmission of DNA is fundamental for cellular homeostasis and organism viability. However, cells are continually exposed to environmental and endogenous DNA damage agents that threaten genomic stability. Resulting alterations of DNA structure induced by the agents are genetically not suitable for its essential role in the replication and transmission of genetic information.

DNA double strand breaks (DSBs) are one of the most cytotoxic forms of DNA damage, because an unrepaired DSB is enough to trigger permanent growth arrest and even cell death. In addition, DSBs potentially induce chromosomal rearrangements such as deletions, translocations and amplifications. These rearrangements could result in the activation of oncogenes and/or the loss of tumor suppressor genes, which accelerate malignant transformations. Therefore DSB repair pathway is believed to be critical for the cell survival, the maintenance of genome integrity and the tumor suppression. Almost all organisms have acquired and evolved the DSB repair pathway and cell cycle checkpoint to protect genome integrity from excessive mutations. DSBs can occur accidentally during normal cell metabolism such as DNA replication, and by exposure of cells to exogenous agents such as ionizing radiation (IR) or some anticancer drugs. In addition, DSBs are the essential intermediates during programmed recombination events such as meiosis and immunoglobulin V(D)J recombination in the early stages of the immune system.

Classically, DSBs are known to be repaired through the two major pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). In NHEJ reactions, the broken ends of DSBs are firstly protected by Ku70/80 dimer. Ku dimer recruits

DNA-dependent protein kinase catalytic subunit (DNA-PKcs) within the broken ends to form DNA-PK complex. The assembled DNA-PK complex then recruits the following proteins on the end including XRCC4 and XLF to trim the broken ends. DNA ligase IV ligates the trimmed ends and finally complete NHEJ, which can result a deletion of DNA information and could lead to a shift of the codon reading-frame if DSBs are induced within introns.

Opposite to NHEJ, HR utilizes an undamaged homologous sequence as a template for DSBs repair. The lost information at the broken ends will be therefore filled and completely repaired. The reactions of HR pathway are initiated by nucleolytic degradation of the 5' end of DSBs to yield 3' single-stranded DNA (ssDNA) tail, a process referred to as 5'-3' resection. Replication protein A (RPA) complex binds to the ssDNA to protect that against a further processing, and is then replaced with RAD51 to form a nucleoprotein filament that catalyzes homologous pairing and strand invasion. After the strand invasion, DNA polymerases synthesize the lost region by using homologous sequence as a template. When the synthesis is completed, resolvases cleave the intermediate of HR called Holliday Junction and then HR repair is finally carried out.

Since an easily accessible homologous template is found on a sister chromatid, HR is believed to activate primarily during the late S and G2 phases of the cell cycle in which a sister 6chromatid exists. Opposite to HR, NHEJ dose not require a template, it is not restricted to a particular phase of the cell cycle. The initiation of resection should be a critical determination for repair pathway choice. Because resection has once initiated, the DSB end structure is not suitable for binding of Ku70/80 dimer, end protection complex and the end is committed to HR. The pathway choice mechanisms of DSB repair have been investigated in these decades. S.P.

Jackson and other groups reported that end resection is regulated during the cell cycle by protein expression and/or phosphorylation of CtIP by CDKs to ensure HR is restricted during the late S and G2 phases. Others reported protein expression of RAD51, which is a critical mediator of HR, and RAD52, which is a mediator of RAD51, are also permitted during these phases. Recently, the complexity of DSB ends have been also reported to be a factor to determine the repair pathway choice, however the molecular mechanism underlay the choice has not been elucidated.

As described above, the regulation of DSB repair to choose the correct pathway is important for cell survival because the output from HR and NHEJ are different. However, the mechanism of the repair pathway choice is disclosed only when cells are irradiated with high dose of IR. It is reported that NHEJ is a dominant pathway to repair DSBs in mammalian cells irradiated with more than 2 Gy of IR, whereas there is no reports for cells irradiated with low doses of IR. To measure the effect of natural radiations to living cells, we should seek the mechanism to repair the DNA damages induced with low dose of IR. To this end, we tried to determine the regulatory mechanism of HR without irradiation in yeast cells. Next, the dose-dependent regulation of HR and NHEJ in mammalian cells were analyzed. We firstly established the method for purifying and analyzing protein complexes with proteomic analysis. Using the established method, a regulatory mechanism of HR in fisson yeast *Schizosaccharomyces pombe*, in which HR is dominantly utilized for DSB repair was identified. We found that HR is suppressed through 26S proteasome-dependent protein degradation to avoid an excessive recombination. Furthermore, we explored a newly mechanism of the DSB repair pathway choice especially responsible for low doses of IR in mammalian cells. The

reporter assays showed that HR is activated sensitively from low dose of IR but easily saturated by increased dose of IR, whereas NHEJ is activated by high dose of IR. In these studies, we concluded that HR is suppressed in cells without DSBs and activated rapidly following DSB inductions to maintain genome integrity, however HR is gradually suppressed by the activated NHEJ with increased dose of DSBs, resulting the switch of a utilized repair pathway from HR to NHEJ.