論文の要旨

(Thesis Summary)

氏名（Name）Mohammad Shamim Hossain

論 文 題 目 (Thesis Title) **Characterizing the factors that are required for the maintenance of circular chromosomes using fission yeast**

(分裂酵母を用いた環状染色体の維持に必要な因子の理解)

A telomere is a region of repetitive nucleotide sequences at each end of a eukaryotic chromosome. Telomere usually protects cells from genomic instability and cellular senescence. DNA double strand break generates sticky end which fuses with end to end and creates circular chromosome. In addition, loss of telomere also creates chromosome circularization. Circular chromosomes are found in many eukaryotes and circular chromosomes in human have been linked to some genetic diseases and cancer. My research emphasizes to develop therapies to kill cancer cells that most of the cells carrying circular chromosomes. But genes that are involved in the maintenance of circular chromosome are not known in human. In additions, there has no advanced therapy yet discovered that can selectively kill cancer cells. I tried to find out the genes that are required for the maintenance of circular chromosome. I used *Schizosaccharomyces pombe*, which is genetically tractable and most of the genes related to chromosome maintenance are conserved in mammalian counterparts. I used *pot1*$^{+}$ deletion strain which has circular chromosomes. To identify genes that are required for the maintenance of circular chromosome in *S. pombe*, I tried to find out a gene that becomes synthetically lethal with *pot1* deletion strain.

The cell cycle checkpoint signaling pathways are activated during DNA damage. These activations are important for maintaining the cellular integrity. Likewise, the cell cycle checkpoint protein Rad9-Hus1-Rad1 (9-1-1) complex plays an important role to maintain cellular integrity. However, the role of 9-1-1 complex on the maintenance of circular chromosome is not known. I constructed *pot1*Δ and the 9-1-1 complex double mutant cells which were exhibited the feature of circular chromosome. I analyzed the sensitivity of this double mutant using 5-fluorodeoxyuridine (Fudr) which is an anticancer drug. I found that checkpoint-defective 9-1-1 complex (*rad9*Δ*, rad1*Δ*, hus1*Δ*)* single mutant is sensitive to Fudr even fission yeast does not express endogenous thymidine kinase (*tk*). I also found that *pot1*Δ and the 9-1-1 complex double mutant strain (*pot1*Δ *hus1*Δ*, pot1*Δ *rad1*Δ*, pot1*Δ *rad9*Δ) shows greater sensitivity to Fudr than every single mutant. I found that Fudr causes cell cycle arrest in S phase. Thus, 9-1-1 complex is required for the maintenance of circular chromosome when DNA replication is arrested.

The *S. pombe* phosphatidylinositol 4-kinase, Pik1p homologous to human PI 4-kinase IIIβ is found to associate with Golgi. Pik1 is involved in sorting proteins in Golgi. However, the role of $pik1^{+}$ in the maintenance of Golgi structures, nucleus and circular chromosome is not known. To obtain the strain that will be synthetically lethal with *pot1Δ*, I used a mutagenic alkylating agent, EMS to mutagenize the *pot1Δ* cell carrying pot1 expressing plasmid. I obtained a candidate, which had an unknown mutation in *pot1Δ* carrying pot1 expressing plasmid. I found that *pot1Δ* is synthetically lethal with unknown EMS-induced mutation. Later on, I used a browser-accessible tool, Mutation discovery and identified that this unknown mutation is *pik1* mutation. I called this mutation is *pik1-1*. There may have two possible reasons behind the lethality between *pot1Δ* and *pik1-1* double mutant. Pik1 is either required for the formation of chromosome circularization or maintenance of chromosome circularization. I found that *pik1* is not required in the formation of circular chromosome. Therefore, it could be possible that *pik1* is required for the maintenance of circular chromosome.

Cure of cancer is one of the most important tasks in biomedical research field because cancer is one of the main reasons for death. It is important to find a new drug that specifically can kill cancer cells because many drugs used for cancer therapy have strong side effects. Chromosome in human is linear. The cells from atypical lipomatous and dermatofibrosarcoma protuberans tumors contain about 85% and 70% circular chromosomes, respectively. If it is possible to find out a drug that can selectively kill cancer cell that has circular chromosome, this drug obviously is expected to be no side effect to normal cells. Yet now, no modern advanced therapy has been discovered that can selectively kill cancer cells without causing any side effect to normal cells. *pot1*Δ and 9-1-1 complex and *pot1*Δ *pik1-1* double mutant cells which exhibit the feature of circular chromosome. *pot1*Δ and 9-1-1 complex and *pot1*Δ *pik1-1* double mutant are found to be synthetically lethal in presence and absence of DNA replication inhibitor, respectively. I also found that 9-1-1 complex when replication is arrested and Pik1 are required for the maintenance of circular chromosome. In *S. pombe* most of the genes that are related to chromosome maintenance are conserved in mammalian counterparts. Therefore, it is expected that the identifying genes in *S. pombe* that are required for the maintenance of circular chromosome will also be required for the maintenance of circular chromosome in human. The 9-1-1 complex checkpoint proteins Rad1, Rad9 and Hus1 are similar in both human and *S. pombe*. The *S. pombe* Pik1 is homologous to human PI(4) KIIIβ. Therefore, inhibition of 9-1-1 complex protein when replication is arrested or PI(4)K IIIβ protein in cancer cells that have circular chromosomes are expected to kill those cancer cells.