Internship Proposal

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Research project: Regulation of DNA replication

DNA replication is central to cell proliferation. It occurs only once during S phase, and once it is initiated, the entire genome needs to be precisely copied in a coordinated manner, while any obstacles that impede the DNA chain elongation need to be properly taken care of. Mechanisms behind these regulations have been intensively studied for the last few decades. Replication is permitted at early G1, when pre-RCs (pre-replicative complexes) are generated at selective loci along the chromosomes. This process, originally termed "replication licensing", is strictly prohibited until next cell cycle, once replication starts. Once in S phase, replication of the entire genome proceeds under a defined temporal and spatial program, which is dictated by the chromatin structures and organization and is also under checkpoint regulation. However, how this replication program is regulated has not been known.

We attempt to resolve how DNA replication is regulated during cell cycle and how the entire genome is coordinately replicated during S phase. Toward this goal, we use *E.coli*, fission yeast and mammalian cells. We take various approaches, some of which are described below.

1 Biochemical purification of the factors involved in each step of DNA replication using recently developed highly efficient mammalian expression system, and reconstitution of the machinery.

2 Genetic screening of new factors involved in regulation of replication program in fission yeast

3 ChIP-Chip/ ChIP-Seq approach to determine replication timing and binding sites of its regulators on a genome-wide basis

4 Analyses of the functions of the factors involved in various aspects of DNA replication using knockout cells/ mice as well as knockdown cells

5 Live imaging of the cell cycle and replication machinery using fluorescence-tagged proteins

A student may have an opportunity to be engaged in some of these experiments during his/her stay.

Recent Selected Publications

1. Yamazaki, Hayano, M. and **Masai, H.** (2013) "Replication timing regulation of eukaryotic replicons: Rif1 as a global regulator of replication timing." ***Trends in Genetics.*** 29, 449-460.

2. Yamazaki, S., Ishii, A., Kanoh, Y., Oda, M., Nishito, Y. and **Masai, H.** (2012) "Rif1 regulates the replication timing domains on the human genome." ***EMBO J.*** 31, 3667-3677.

3. Hayano, M., Kanoh, Y., Matsumoto, S., Shrahige, K. and **Masai, H.** (2012) "Rif1 is a global regulator of timing of replication origin firing in fission yeast." ***Genes and Development***, 26,137-150.

4. Matsumoto, S., Hayano, M., Kanoh. Y. and **Masai, H**. (2011) "Multiple pathways can bypass the essential role of fission yeast Hsk1 kinase in DNA replication initiation." ***J. Cell Biol.*** 195, 387-401.

5. Hayano, M., Kanoh, Y., Matsumoto, S., Kakusho, N. and **Masai, H**. (2011) "Pre-firing binding of Mrc1 defines the early-firing origins which are selectively hyper-activated upon loss of fork stabilizing factors in fission yeast." ***Mol. Cell. Biol.*** 31, 2380-2389.

6. Masai, H., Matsumoto, S., You, Z., Yoshizawa-Sugata, N. and Oda, M. (2010) Eukaryotic DNA replication; where, when and how*?* ***Annual Rev. Biochem.*** 79, 89-130.

7. Sakaue-Sawano, A., Kurokawa, H., Morimura, T., Hanyu, A., Hama, H., Kashiwagi, S., Fukami, K., Imamura, T., Ogawa, M., **Masai, H**., and Miyawaki, A. (2008) "Spatio-temporal dynamics of multicellular cell cycle progression." ***Cell*** 132, 487-498.