Regulation of replication timing and DNA repair by nuclear membrane tethering of Rif1

Hisao Masai[1], Tomohiro Iguchi[1], Sayuri Ito[1], Mikhiko Shibata[2], Naoko Kakusho[1], Rino Fukatsu[1], Asami Oji[3], Ichiro Hiratani[3], Hiroyuki Sasanuma[1]

[1]Department of Basic Medical Sciences,Tokyo Metropolitan Institute of Medical Science, Tokyo, JAPAN; [2]Kanazawa University, NanoLSI, Kanazawa, Japan; [3]RIKEN BDR, Laboratory for Developmental Epigenetics, Kobe, Japan

E-mail: masai-hs@igakuken.or.jp

Rif1, an evolutionally conserved nuclear factor, regulates genome-wide replication timing by its ability to recruit phosphatase and by its potential to generate higher-order chromatin structures near nuclear periphery. It also regulates DSB repair by facilitating NHEJ during G1 phase in mammalian cells. Rif1 binds to subsets of G-quadruplex (G4) on the genome both in vivo and in vitro and inhibits initiation of DNA replication over ~100 kb segments in fission yeast. Mammalian Rif1 also binds to G4. A portion of mammalian Rif1 is localized near nuclear periphery and is biochemically fractionated into detergent-resistant membrane fractions. We show that Rif1 associates with endomembrane and this association requires small stretches of amino acids near the C-terminus of Rif1. Point mutations in the C-terminal segment of Rif1 lead to its dissociation from the nuclear periphery, loss of mid-S replication foci pattern, perturbation of multimer formation and dramatically altered replication timing throughout the genome with subsets of replication timing domains being converted from late to early. They also affect repair of IR-induced DSB, but not cell proliferation or cellular responses to replication stress. We will discuss mechanisms of nuclear membrane association of Rif1 and its cell cycle regulation, and how it may contribute to chromatin structures and spatio-temporal regulation of DNA replication. We will also show the molecular dynamics of the full-length Rif1 protein analyzed by high-speed AFM, and will present a model on how Rif1 interacts with DNA and nuclear membrane through its C-terminal domain.