Biological functions of G-quadruplexes in regulation of DNA replication

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G-quadruplex structures (G4) are ubiquitously present on various genomes. More than 370,000 potential G4-forming sequences are present on the human genome. Ample evidence indicates that their unusual structures pose barriers to replication fork progression and are sources for elevated genome instability. To prevent this from happening, cells are equipped with various "anti-G4" machinery including helicases that can specifically resolve G4 structures. On the other hand, G4 has been implicated in regulation of various chromosome functions, including transcription, recombination, epigenetic regulation, gene rearrangement, and retrotransposition. We have been studying mechanisms of DNA replication and have discovered potential roles of G4 in both positive and negative regulations of replication.

(1) Negative regulation of DNA replication by G4

A conserved factor, Rif1, regulates replication timing by suppressing the origin firing. Rif1 recognizes and binds to G4 present in its binding sites (Rif1BS), and this binding generates chromatin architecture inhibitory for initiation. Mammalian Rif1 also binds to G4 and regulate genome-wide replication timing. Human Rif1 also binds to phospholipids and this binding is stimulated by the presence of G4. G4 and phospholipid bindings of Rif1 are mediated by the C-terminal 123 aa of Rif1 which forms tetramers. We also show that G4 binding to Rif1 can induce disruption of the oligomeric structure of the C-terminal segment, which leads to increased phospholipid binding.

(2) Positive regulation of DNA replication by G4

2-1 Initiation of replication from RNA-DNA hybrid/G4

G4 plays a positive role for initiation of DNA replication through RNA-DNA hybrids that are formed in a manner dependent on G4-forming sequences. G4-forming sequences play an important role for origin firing at dispersed locations on the *E. coli* genome, and the initiation is inhibited by G4-specific ligands. We further showed that mere transcription of G-rich sequences on a single locus on the genome can promote DNA replication of the entire *E.coli* genome. We would like to discuss biological and evolutional implication of this finding.

2-2 G4-driven formation of nucleosome-free region

In the eukaryotic genome, G4 sequences have been shown to be frequently associated with replication origins. One potential mechanism of this association could be the ability of G4 to generate nucleosome-free region. We have discovered that nucleosome assembly is precluded at Rif1BS in fission yeast, while point mutations disrupting G4 structure of Rif1BS restored the nucleosome assembly. This result indicates that G4 formation disrupts nucleosome formation, which could facilitate replication initiation.

(3) Detection of cellular G4

It is hard to predict the presence of G4 in the cells simply from the sequences, and therefore, probes or methodology for detection of cellular G4 need to be developed.

Taking advantage of a long loop of Rif1BS G4 in which a restriction enzyme site is inserted, we show that DNA is resistant to cleavage only when it adopts G4. We are trying to apply this method for more global analyses of G4 on cellular chromosomes.

I would like to discuss general roles of G4 in regulation of various biological processes which are robust and sometimes highly plastic and adaptive, and even stochastic.

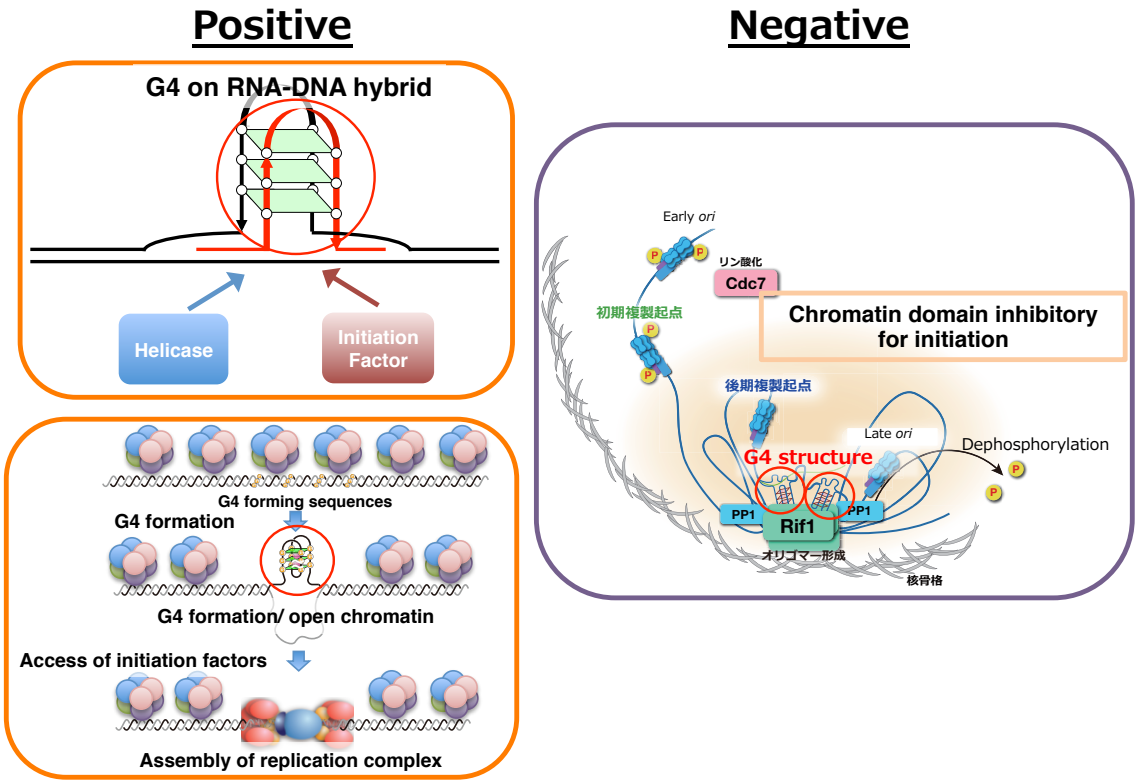

Figure Positive and negative regulation of DNA replication by G4
Left, positive regulation. Upper, Transcription of a G-rich sequence generates RNA-DNA hybrid with G4 structure and can induce DNA replication. Lower, G4 formation can induce open chromatin (free of nucleosome) and facilitates assembly of replication complex. **Right**, negative regulation. Rif1 binds to G4 and nuclear membrane and generates higher-order chromatin structures near nuclear periphery, which are inhibitory for initiation of DNA replication.

References

1. 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.
2. Yamazaki, S., Ishii, A., Kanoh, Y., Oda, M., Nishito, Y. and Masai, H. (2012) "Rif1 protein is a key regulator of the genome-wide DNA replication timing in human cells." ***EMBO J.*** 31, 3167-3177.
3. Kanoh, Y., Matsumoto, S., Fukatsu, R., Kakusho, N., Kono, N., Renard-Guillet, C., Masuda, K., Iida, K., Nagasawa, K, Shirahige, K., and Masai, H. (2015) "Rif1 binds to G-quadruplexes and suppresses replication over long distances." ***Nature Struct. Mol. Biol.*** 22, 889-897.
4. Moriyama, K., Yoshizawa-Sugata, N., and Masai, H. (2018) "Oligomer formation

and G-quadruplex binding by purified murine Rif1 protein, a key organizer of higher-order chromatin architecture***. J. Biol. Chem.*** 293, 3607-3624.
5. Kobayashi, S., Fukatsu, R., Kanoh, Y., Kakusho, N., Matsumoto, S., Chaen, S. and Masai, H. (2019) "Both a unique motif at the C terminus and N-terminal HEAT repeat contribute to G4 binding and origin regulation by Rif1 protein." ***Mol Cell. Biol.*** 39(4). pii: e00364-18.
6. Masai, H, Kanoh, Y, Kakusho, N, Fukatsu, R (2020) "Detection of cellular G-quadruplex by using a loop structure as a structural determinant." ***Biochemical and Biophysical Research Communications***, 531(1): 75-83.
7. Masai, H. and Tanaka, T. (2020) "G-quadruplex DNA and RNA: Their roles in regulation of DNA replication and other biological functions." ***Biochemical and Biophysical Research Communications***, 531(1):25-38.
8. Yoshizawa-Sugata, Y., Yamazaki, S., Mita-Yoshida, K., Ono, T., Nishito, Y., and Masai, H. (2021) "Loss of full-length Rif1 protein in 2-cell embryos is associated with zygotic transcriptional activation." ***J. Biol. Chem.***, 297(6):101367.
9. Yutaka Kanoh, Seiji Matsumoto, Masaru Ueno, Motoshi Hayano, Satomi Kudo, Hisao Masai (2023) "Aberrant association of chromatin with nuclear periphery induced by Rif1 leads to mitotic defect and cell death." ***Life Science Alliance*** 6(4):e202201603.