Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (83): Bulge-containing G-quadruplexes is a new target motif to regulate gene expression in therapeutics

([1]*Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University*, [2]*Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University*) ○Sunipa Sarkar,[1] Hisae Tateishi-Karimata,[1] Tatsuya Ohyama,[1] Naoki Sugimoto[1,2]

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The DNA sequences with the potential to form G-quadruplexes (G4s) located in cancer related genes as *c-MYC, KRAS,* and *BCL2*. We previously showed that the frequency of transcript mutations depends on the stability of the G-quadruplexes formed in the template DNAs.[1] Interestingly, the G4 formations are highly variable in response to changes in the cellular environments and regulates gene expression.[2,3] Thus, the roles of DNA G-quadruplexes during tumor progression have attracted attention. In recent years, novel bulge containing G4 structures (buG4s) have been reported (Figure 1). These buG4s may also regulate gene expression, however, the detailed mechanisms are still unknown.

In this study, we searched for G-rich sequences in cancer-related genes and analyzed the structure and function of these sequences under the molecular crowding environment. As results, we found four buG4 sequences in *c-MYC* promoter region. We analyzed the structures of these G-rich sequences using circular dichroism (CD) spectroscopy and native gel electrophoresis experiments, suggesting that these G-rich sequences form the intramolecular buG4 structures in the crowding condition. We further analyzed the thermal stability of these structures using UV melting curves. Importantly, buG4s were shown to be dramatically stabilized by the addition of cosolutes inducing molecular crowding conditions. Moreover, in vitro transcription reaction indicates that buG4s can suppress the transcriptional regulation. Our study will help to understand how the formation of such buG4 structures presents an opportunity for the potential therapeutic modulation of gene expression.

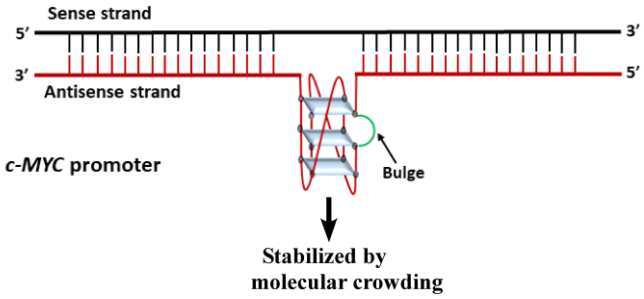

Figure 1. Schematic illustration of the gene and the formation of the bulge containing G4 (buG4) structure.

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