G-quadruplex sites at TAD boundaries may contribute to CTCF and cohesin recruitment

P-01.4-17

A. Varizhuk[I], E. Isaakova[I], P. Tikhonova[I], V. Tsvetkov[I], A. Bogomazova[I], V. Severov[I], I. Pavlova[I], T. Vedekhina[I], M. Lagarkova[I], G. Pozmogova[I]

[I]Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia

G-quadruplexes (G4s) are often found within or nearby CTCF-bound sites that mark TAD boundaries [1]. We used bioinformatics analysis and in vitro binding assays to verify two possible pathways of G4 contribution to CTCF recruitment and TAD demarcation. The first pathway is related to G4s formed upon replication. They reportedly bind with DNA methyltransferase DNMT1, sequester it from the CpG sites, and thus protect hypomethylated CpGs. CTCF is recruited mainly to the hypomethylated sites. The converged CTCF-bound sites are then recognized by cohesin, and the TAD boundaries are established. To verify this pathway, we estimated CTCF frequency in CpG-rich G4-harbouring sites that overlap with DNMT1 occupancy sites. We observed substantial colocalization and, for a subset of sites, confirmed proximity to chromatin loop boundaries. The second pathway is related to G4s formed upon transcription. They may recruit modulators of chromatin remodeling that create favorable conditions for CTCF interactions with linker DNA. Previously, we have identified several such chromatin modulators, including the polycomb complex subunit ASXL1 and high mobility group proteins HMGN3 and HMGB2, among top G4 binders. HMGN3 promotes chromatin decondensation, which renders linker DNA accessible for CTCF. ASXL1 attracts cohesin. HMGB2 acts as a CTCF insulator (prevents its aggregation). To verify this pathway, we analyzed G4 frequencies in HMGN3/HMGB2/ASXL1 occupancy sites. The frequencies were significantly higher than those predicted by chance. For a set of representative G4s, we also verified interactions with the recombinant proteins by physicochemical methods. In line with previous hypotheses [2], our results indicate that G4s might contribute to chromatin organization at multiple levels, including TAD demarcation. This work was supported by RSF [19-15-00128].

[1] Hou Y et al. (2019) Epigenetics 14, 894-911
[2] Varizhuk A et al. (2019) BioEssays 41, e1900091