

Elucidating the biological significance of the TRF2-ORC interaction at telomeres using the specific TRF2 mutants

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<https://hdl.handle.net/2324/4060103>

出版情報 : Kyushu University, 2019, 博士 (創薬科学) , 課程博士
バージョン :

権利関係 : Public access to the fulltext file is restricted for unavoidable reason (3)



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論文題名 : **Elucidating the biological significance of the TRF2-ORC interaction at telomeres using the specific TRF2 mutants**
(特異的 TRF2 変異体を利用した TRF2-ORC 相互作用のテロメアにおける生物学的重要性の解析)

区 分 : 甲

論 文 内 容 の 要 旨

Telomeres protect the ends of linear chromosomes and are therefore essential for genome stability; however, telomeres are “difficult-to-replicate” regions. Despite the identification of several factors involved in the complete duplication of telomeres, the underlying molecular mechanisms remain elusive. Origin recognition complex subunit 1 (ORC1) binds to telomeric repeat binding factor 2 (TRF2), which facilitates loading of the replicative helicase MCM complex (i.e., replication licensing) onto telomeres. However, the role of the TRF2-ORC interaction in the telomere maintenance remains unclear because studies based on specific inhibition of the interaction are lacking.

Here, I evaluated the biological significance of the TRF2-ORC interaction using a separation-of-function TRF2 mutant defective only in binding to ORC. To develop the specific TRF2 mutant, I first investigated the detailed molecular mechanism underlying TRF2-ORC binding. Analysis of a series of TRF2 mutants using the lacO-LacI assay showed that the TRF2 homology (TRFH) domain of TRF2 and its dimerization are required for the recruitment of ORC. A major part of the data described in the “RESULTS PART I” section of this thesis were published in Higa, M., et al., *Biochim. Biophys. Acta - Mol. Cell Res.* 1864, 191-201 (2017). Based on these results and the crystal structure of the TRFH domain of TRF2, a series of alanine-substituted TRF2 mutant proteins were generated as candidate separation-of-function TRF2 mutants. Biochemical analyses revealed that TRF2 E111A and E112A mutations compromised the recruitment of ORC by TRF2 with minimal effects on the dimerization and the binding to other TRF2-binding proteins. To specifically inhibit the TRF2-ORC interaction in vivo, HeLa clones harboring E111A/E112A mutations in the TRF2 gene were established. The CHIP-qPCR assay showed that telomere-bound ORC is decreased in the TRF2 E111A/E112A clones. Exposure of cells to DNA replication stress induced substantial telomeric DNA damage and telomere instability in the two TRF2 E111A/E112A clones. The detailed molecular mechanism(s) underlying the telomere instability in the E111A/E112A clones are currently under investigation.

In this work, I showed that (1) the TRFH domain of TRF2 and its dimerization are required for the interaction with ORC and its telomeric recruitment; (2) the TRF2-ORC interaction contributes to the recruitment of ORC to the telomere; and (3) TRF2-mediated recruitment of ORC contributes to the maintenance of telomere stability under DNA replication stress conditions.