P041 Analysis of a fork regression pathway for the UV-damaged DNA by using an *in vitro* DNA replication system

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DNA damages that escaped from DNA repair systems often block DNA replication fork progression. Translesion DNA synthesis (TLS) is an important pathway for cells to tolerate these DNA damages. Recently it is becoming clear that other pathways are also important for DNA damage tolerance. A fork regression pathway that induces chickenfoot DNA intermediates is one of them. This pathway was found in bacteria and yeast, but not yet in mammals.
To investigate whether the chickenfoot DNA intermediates are induced by DNA damages (cyclobutane pyrimidine dimer (CPD) and 6-4 photoproduct (6-4PP)) caused by UV in mammalian cells or not, we employed an *in vitro* DNA replication system. This system enables us to analyze the DNA replication reactions by human cell-free extracts with templates containing single site-specific defined lesions. We combined this system with the neutral-neutral two-dimensional agarose gel electrophoresis (2D gel) method and the atomic force microscopy (AFM).
As a result, we detected abnormal replication intermediates and arrested replication forks derived from the CPD and the 6-4PP lesions by the 2D gel method. Furthermore structure analyses of DNA by AFM revealed that chickenfoot DNA replication intermediates were induced by the 6-4PP lesion. These data suggest that the fork regression pathway is involved in mammalian DNA damage tolerance mechanisms.

***In vitro* DNA 複製系を用いた紫外線損傷 DNA に対する複製フォーク後退機構の解析**

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P042 Mutations induced by 7,8-dihydro-8-oxoguanine in yeast and involvement of DNA polymerase η

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Previously we analyzed the relationship between DNA sequences and mutations induced by 7, 8-dihydro-8-oxo-guanine (8-oxoG) using the yeast oligonucleotide transformation assay. In a *rad30-deficient mutant,* which is a yeast polymerase η (ypolη) deficient strain, it was demonstrated showed that oligo-CCX(X=8oxoG) showed higher G→ T mutation frequency than oligo-CGX and assumed that the lesion bypass efficiency is sequence dependent. Recently we have examined what nucleotide was incorporated to the opposite site of 8-oxoG by the standing-start primer extension assay *in vitro*. The results showed that A was more frequently incorporated to the opposite site of 8-oxoG in oligo-CGX than in oligo-CCX in the presence of ypolη which means the ypolη could minimize the mutation potential in oligo-CCX more effectively than in oligo-CGX. Furthermore, by comparing with the *E.coli* replication polymerase Klenow fragment exo⁻(KF), the ypolη has lower mutation frequency than the KF. Both *in vivo* and *in vitro* results were corresponding to each other and the G→T mutation potential of 8-oxoG and the TLS action of ypolη against it maybehighly sequence dependent and there could be other polymerase(s) response to minimize the G→T mutation in CGX sequence. In addition, we also compared the mutation potential difference between yeast and human polymerase η(hpol η) and it showed ypol η has higher fidelity than hpol η.

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