The Environment Mutagen Society of Japan

W2-3 Translesion DNA synthesis and mutagenesis of 4-aminobiphenyl-DNA adducts in human cells

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DNA bulky adducts block DNA replication, which leads to cell death and mutations. To avoid the block by the adducts, cells have the translesion DNA synthesis (TLS) mechanism. Recently, DNA polymerases to carry out TLS have been identified, however, its mechanism has not been elucidated well so far.

There are many mutagenic polycyclic aromatic hydrocarbons (PAHs) in the air. One of the PAHs, 4-aminobiphenyl (ABP), is also found in tobacco smoke and is suspected to be carcinogenic. ABP is metabolically activated and forms a major DNA adduct, *N*-(2'-deoxyguanosine-8yl)-4-aminobiphenyl (dG-ABP). Mutation hotspots in *p53* gene of human bladder carcinomas caused by ABP have been shown to coincide with ABP-adducted sites.

To investigate the mechanisms of mutagenesis and TLS of dG-ABP, we constructed a *lacZ* shuttle vector plasmid containing a single dG-ABP in *p53* mutation hotspot sequence inserted into the *lacZ* gene. We introduced the plasmids into nucleotide excision repair deficient human cells (XP2OS(SV)), and analyzed replicated plasmids. The result showed that the TLS occurred with an efficiency of 24%, and the mutation frequency of the plasmids was 10%. We previously showed that dG-ABP hardly blocked DNA replication in *E.coli*. As our result suggest that human and *E.coli* cells have different TLS mechanisms for dG-ABP, the human TLS assay is important to evaluate chemical carcinogenesis in humans.

ヒト細胞における4－アミノビフェニルDNA付加体のTLSを介した突然変異

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W2-4 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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