CCC sequence in a single-strand DNA (ssDNA).

In order to obtain insight into the detailed mechanism of the deamination reaction by APOBEC3G, we adopted the real-time NMR spectroscopy (*EMBO J.*, 2009). Real-time monitoring revealed that the third cytidine of the CCC segment was deaminated faster than the second one. The first cytidine, however, was not deaminated at all. Subsequently we have chased the deamination reaction of a DNA sequence containing two CCC segments in order to examine whether the cytidine deamination activity of APOBEC3G was location-dependent. Real-time monitoring revealed that the reaction rate of the 5'-side CCC was faster than that of the 3'-side CCC. The result suggests that CCC segments in the comparatively upstream region have higher deamination rates. This location-dependent deamination rate indicates that APOBEC3G slides along DNA with 3'→5' directionality. We propose that APOBEC3G approaches to the deamination sites in HIV DNA more efficiently by sliding on DNA from 3' to 5' rather than diffusing freely in three-dimensional space.

1P123 (6-4) 光回復酵素の低温赤外分光

Low-temperature FTIR study of photoactivation and photorepair processes of (6-4) photolyase

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Photolyases (PHRs) are DNA repair proteins that specifically revert UV-induced photoproducts, cyclobutane pyrimidine dimers (CPD) or (6-4) photoproducts into normal bases to maintain genetic integrity. FAD is a common chromophore for all PHRs. Both CPD and (6-4) PHRs have been proposed to repair with an electron transfer from reduced FAD to the respective photoproducts in a light dependent manner. However, the (6-4) PHR must catalyze not only covalent bond cleavage between two pyrimidine bases, but also a hydroxyl or an amino group transfer from 5'- to 3'-side base. Thus, a more complex mechanism has been postulated for (6-4) PHRs than for CPD PHRs. Light-induced difference Fourier-transform infrared (FTIR) spectroscopy is a powerful method to study structure-function relationships in photoreceptive proteins¹. Recently, we applied FTIR spectroscopy to *Xenopus* (6-4) PHR with authentic substrate to gain insights into the activation and repair processes induced by light. By use of a concentrated solution, we successfully obtained difference FTIR spectra at 277 K that correspond to the photoactivation and repair processes of (6-4) PHR². Here we extended the study by measuring the FTIR spectra at low temperatures, where we expected to trap short-lived intermediate states in the photoactivation and repair processes. The detailed molecular mechanism will be discussed on the basis of the observed temperature dependence. [1] Kandori, in *cis-trans Isomerization in Biochemistry*; Wiley-VCH, pp 53-75 (2006). [2] Zhang et al. to be submitted.

1P124 クラスター損傷DNAと修復酵素 hOGG1 の結合に関する分子動力学シミュレーション Study of the binding between clustered damage DNA and hOGG1 by molecular dynamics simulation

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Ionizing radiation leads to clustered DNA damage sites, which are defined as two or more single lesions induced within 10-20 base pairs (bp). In general, these sites are repaired with less efficiency than single lesions. So far the detail mechanism of repair of multiple lesions is not known. A cluster damage containing a single strand break (SSB) within a few base pairs opposite to a 8-oxo-7,8-dihydroguanine (8oxoG) is one example of damage that is difficult to repair. The experimental results showed that the excision of the 8oxoG lesion by repair enzyme hOGG1 is strongly inhibited by the presence of a SSB on the opposite strand with a 1bp separation from the 8oxoG lesion and probability of repair increases with increasing distance between the SSB and the 8oxoG.

In this study, we present results of molecular dynamics (MD) simulation of the models of complex of hOGG1 and cluster damaged DNA. Each model DNA contains two damages, SSB and 8oxoG. The separation of the location of the SSB from the 8oxoG on the opposite strand were +1bp, -1bp and -3bp, respectively. According to the observed results, the structure of the damaged DNA including SSB at +1bp was reversed at SSB and the section of this damaged DNA contacted with hOGG1 closely. The structure of damaged DNA including SSB at -1bp fluctuated large around SSB and the contact between this damaged DNA and hOGG1 was weak. The effect of the SSB located at -3 was small because the residue located at -3bp did not contact directly with hOGG1.

1P125 Fixed interaction between the Escherichia coli RuvA and RuvB proteins during branch migration of Holliday junction

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Escherichia coli RuvA-RuvB protein complex promotes Holliday junction branch migration during homologous recombination and recombination repair. RuvA forms tetramer and the two tetramers sandwich planer Holliday junction. RuvB is a member of AAA+ ATPase superfamily and forms a hexameric ring, which acts as a motor protein. The two rings flank the junction by interacting RuvA octameric core and promote branch migration by pumping out DNA duplex through their central cavities. Two models are conceived to explain how the DNA double helices are pulled out through the cavities of the rings. (i) RuvB hexameric rings rotate against RuvA octameric core and the duplexes are moved by interacting with inner surfaces of the rotating RuvB rings. (ii) RuvB hexameric rings are fixed to the RuvA octameric core and the duplexes are moved by interaction with RuvB subunits which undergo sequential conformational changes. Previously, we showed that I150T-RuvB mutant was defective in interaction with RuvA. Here, we show the detailed analysis of the heterooligomer composed of wild type and the mutant I150T RuvB proteins in vitro to clarify which mechanism is employed for the RuvA-RuvB directed branch migration of Holliday junction. Heterooligomer composed of wild type and I150T RuvB proteins could promote branch migration of Holliday junction DNA efficiently. This result supports the fixed interaction model for the RuvA-RuvB mediated branch migration.

1P126 粗視化シミュレーションによるヌクレオソームの動的構造解析 Nucleosome unwrapping dynamic studied by coarse-grained molecular simulations

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In eukaryotes, DNA is usually wound to the histone octamer composed of two copies of four highly-conserved basic proteins (H2A, H2B, H3 and H4), which together forms a nucleosome. The nucleosome needs to be dynamically unwrapped to some extent when some DNA-binding proteins, such as transcription factors, access the DNA sequence in DNA replication, transcription or repair. How does the nucleosome unwrapping dynamics occur is still largely unknown. Here, we studied the structural dynamics of nucleosome unwrapping by coarse-grained molecular simulation method, which we have been developing. Histone proteins were modeled by the standard Go model and DNA was modeled so that each nucleotide is represented by three beads. Interactions between proteins and DNA are the combination of Go potential and the electrostatic interaction represented by Debye-Huckel theory. In particular, the nucleosome unwrapping may start from the dissociation of the dimer of H2A/H2B making a nucleosome with tetra-histones. Simulations of this tetra-histone nucleosome showed highly floppy state. The possible histone eviction process is also addressed.

1P127 長いゲノム DNA はどのようにして染色体の中に折り畳まれて 1D1310 いるのか? How is a long strand of genomic DNA organized into a chromosome?

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Mitotic chromosomes are essential structures for the faithful transmission of duplicated genomic DNA into two daughter cells during cell division. A long strand of DNA is wrapped around the core histone and forms a nucleosome. The nucleosome has long been assumed to be folded into 30-nm chromatin fibers. However, it remains unclear how the nucleosome or 30-nm chromatin fiber is organized into mitotic chromosomes, although it is well known that condensins and topoisomerase II α are implicated in this process. When we observed frozen hydrated (vitrified) human mitotic cells using cryo-electron microscopy, which enables direct high-resolution imaging of the cellular structures in a close-to-native state, we did not find any higher order structures, or even 30-nm chromatin fibers, but just a uniform disordered texture of the chromosome (Proc. Natl. Acad. Sci. USA, 2008). To further investigate the structure of mitotic chromosome, we performed small angle x-ray scattering or SAXS, which can detect regular internal structures in non-crystalline materials in solution. Mitotic chromosomes purified from HeLa cells were exposed to the synchrotron radiation beam at SPring-8. Again, the results were striking: no structural peaks larger than 11 nm were detected. Therefore, we propose