MEETING REPORT

The 4th International Symposium on 3R; DNA Replication, Recombination and Repair

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INTRODUCTION

The 4th 3R Symposium was held from 9th to 13th of November, 2003 at a beautiful venue in Awaji Island in Inland Sea of Japan. The 4th Symposium was organized by Prof. Akio Sugino of Osaka University, and successful in the quality of presented papers and the management of the Meeting. The Symposium has been held every two years to bring the people in the field of DNA replication, recombination, Repair and various aspects of DNA damage responses, which are inseparable and merging into one fused field, into a common table. The purposes of the meeting, to be interdisciplinary and international were more fulfilled in the 4th Symposium than previous ones. There were several young foreign participants who applied for the meeting and paid for the travel expenses from their grants in addition to ~30 invited speakers from abroad, most of whom were established researchers. This is a welcome trend and should be encouraged in the 5th Symposium, which will be organized by Prof. Kiyoji Tanaka of Osaka University. This Symposium is unique in providing a meeting place for the 3R people and being regularly held outside North America and Europe with high academic standard. As reported below, most of the presented papers are related to more than one field of 3R and the participants benefited from exchanges of ideas with experts of different fields.

I. Regulation of DNA Replication

The opening talk of the Symposium was delivered by N. R. Cozzarelli (UC, Berkeley). He focused his talk on topological changes of chromosome structure during DNA replication and segregation. He mainly talked about his work on MukBEF protein complex (MukB is an SMC-like protein), which plays a role in correct partitioning of sister chromosomes after DNA replication in Escherichae coli. Using single molecule imaging technology, he showed ATP-dependent condensation of DNA by MukBEF. He proposed that the E. coli chromosome is arranged as topologically closed domains (supercoiled loops) about 10 kb in average length, based on the EM observation of DNA released from gently lysed cells, and that MukBEF plays a role in the formation of the domain structure. He also presented an ingenious method to measure the force necessary to extend DNA and applied the method to the measurement of torque and twist generation by MukBEF.

T. Katayama (Kyushu Univ.) talked about the mechanism of RIDA (regulatory inactivation of DnaA) in the E. coli chromosomal replication system. Hda protein, an essential component of RIDA, specifically interacts with Pol III β-subunit (β-clamp) to stimulate the hydrolysis of DnaA-bound ATP. He succeeded in overproduction of active Hda protein in E. coli cells by coproduction with β-clamp, and analyzed the mechanism of formation of RIDA-related protein complex. Furthermore, he addressed the relationship between replication initiation and the mode of transcription of mio gene, which is located next to oriC on the chromosome. From his genetic analyses, he proposed that the cell cycle-dependent fluctuation of transcription reading through the oriC region has some crucial meaning for the determination of timely ini-
tiation of DNA replication in *E. coli*.

The next four speakers talked about their analyses of pre-replicative complex (pre-RC) processing in eukaryotic cells. First of all, H. Araki (Natl. Inst. Genet.) presented work on formation of initiation complex (IC) from pre-RC and the functions of each component protein in *Saccharomyces cerevisiae*. He showed that three complexes, Sld2-Dpb11, Sld3-Cdc45, and GINS (Sld5-Psf1-Psf2-Psf3), are assembled on origins for replication initiation in a manner dependent on mutual interactions, and that CDK-dependent phosphorylation of Sld2 is important for the assembly of these complexes on pre-RC. To clarify the mode of interaction in the Sld2-Dpb11 complex, he delimited in each protein the regions required for the complex formation. His group’s analyses showed that two C-terminal BRCT domains of Dpb11 bind to Sld2. He also identified the region that binds to Dpb11 in the Sld2 protein. This binding region overlaps the cluster of CDK-phosphorylation sites and the binding is dependent on the phosphorylation. This phosphorylation-dependent complex formation of Sld2-Dpb11 is important for the association of DNA polymerases with replication origins. H. Masukata (Osaka Univ.) has been studying IC assembly in *Schizosaccharomyces pombe*. He previously identified a replication origin, *ars2004*, which is about 1 kb long including two long stretches of adenine/thymine and confirmed that ORC and MCM form a complex at the ori region dependent on the A stretches. To dissect the process of pre-IC formation, he performed further genetic analyses using conditional replication mutant strains, and showed that the N-terminal region of Mcm5 is involved in regulation of Cdc45 loading, but Sld3 loading is not dependent on its Cdc45 association. He suggested that DDK-dependent origin-loading of Sld3 occurs prior to Cdc45 loading as a distinct step. He also presented evidence suggesting translocation of Sld3-associated MCM within the origin. J. C. Walter (Harvard Univ.) and H. Takisawa (Osaka Univ.) have been using the DNA replication system of *Xenopus* egg extracts. J. C. Walter focused on the replicative helicase activity. Using his in vitro reconstitution system with nuclease-free *Xenopus* egg extracts, he demonstrated that the Mcm2-7 complex plays a direct role in chromosome unwinding. Furthermore, he presented data supporting the idea that MCM2-7 is required for chromosome unwinding even after the replication complex moves considerable distance from the origin. These results support for the idea that MCM2-7 is the replicative DNA helicase. In addition, he showed that Cdc45 is directly required for chromosome unwinding as a helicase cofactor. The behavior of Cdt1 in the process of pre-RC was also mentioned in his talk. H. Takisawa talked about processing of preRC to the initiation complex (IC) through pre-IC in his in vitro reconstitution system using nuclease-free *Xenopus* egg extracts. As shown in *S. cerevisiae* by H. Araki, GINS, a complex of Sld5, Psf1, 2, and 3, is required for the formation of IC, in addition to Cut5(Dpb11), Sld2, Sld3, and Cdc45, in the *Xenopus* system. He tried further dissection of IC formation by chromatin transfer experiments, and showed that CDK-dependent Cut5 function is required for the loading of Cdc45 and GINS onto pre-RC to form preIC. The replicative DNA polymerases can be loaded after pre-IC formation. He proposed that the precisely coordinated assembly of the replication proteins onto origins is important for the initiation of DNA replication.

T. Mizuno (RIKEN) focused on the geminin-Cdt1 system, which is involved in the mechanism of regulation of DNA replication initiation in metazoan cells. He investigated the interactions among Cdt1, Mcm6, and geminin using *C. elegans* and mouse proteins, and reported that Cdt1 associates with Mcm6 through the C-terminal region. On the other hand, geminin binds to the central region of Cdt1, and thus Cdt1-Mcm6 interaction is inhibited in the presence of geminin, resulting in the inhibition of licensing activity. He also showed that Cdt1 has affinity to DNA through an AT-hook-like motif, which is located in the N-terminal region. This DNA binding activity of Cdt1 is also inhibited by geminin. He suggested that the Cdt1-geminin system has evolved to regulate essential Cdt1 function in the DNA replication system in metazoan cells.

H. Masai (Tokyo Metropolitan Inst.) talked about three topics of his current research. First of all, he described the structure and function of *E. coli* PriA protein, which is a DEXH-type helicase that plays crucial roles in switching recombination intermediates to replication machinery. He showed that PriA specifically recognizes the 3’-terminus of arrested nascent DNA chains in the stalled replication forks in vitro. The binding region in PriA, called the “3’-terminus DNA binding pocket” is located in the N-terminal segment. Next, he briefly mentioned the sequence specificity of the unwinding activity of mouse Mcm4/6/7 helicase and proposed that selective activation of Mcm helicase on the DNA by stretches of thymine sequences may be critical for selection of the initiation site. Finally, he reported the genetic analyses of the cdc7 allele in mice. From their cdc7-knockdown mouse analysis, he suggested that a critical level of Cdc7 kinase is required for proper development and meiotic processes in mammals.

The last speaker of the first part (regulation of DNA replication initiation) of the DNA replication session was A. Sugino (Osaka Univ.), the chair of this symposium, from Osaka Univ. His interest is how Cdc7/Dbf4 kinase triggers the initiation of DNA replication in eukaryotic cells, and in this meeting, he talked about his research on the function of Mcm2 phosphorylation in the regulation of DNA replication initiation in *S. cerevisiae*. He made a mutant *mcm2* gene, in which 12 possible phosphorylat-
able serine residues were changed to alanine. It was very interesting that the mutant mcm2 gene fully complemented the lethality of the mcm2Δ mutant. He also demonstrated the specific binding of Mcm2 and Cdc7-Dbf4 kinase both in vivo and in vitro. With his genetic data showing the synthetic lethality of mcm2 and dbf4 mutations, he proposed that the binding of the kinase complex to Mcm2p, but not phosphorylation of Mcm2, is crucial for the initiation of DNA replication.

In the second part of the DNA replication session, the oral presentations were mainly related to the process of strand elongation. **Y. Ishimi** (Mitsubishi Kagaku Institute of Life Science) talked about MCM phosphorylation and its roles in the regulation of DNA replication. Especially, he talked about phosphorylation of MCM4 protein. MCM4 is extensively phosphorylated in HeLa cells when they are incubated with hydroxyurea (HU) or exposed to UV light. The facts that CDK is directly involved in the MCM4 phosphorylation and that the phosphorylation leads to inhibition of MCM4/6/7 helicase activity suggest that MCM4 is one of the critical targets in the checkpoint control for the DNA replication process.

**Y. Ishino** (Kyushu Univ.) described the structure and functions of a novel endonuclease, which is probably involved in stalled replication fork repair in Archaea. His group discovered a protein, named Hef, that has both helicase and endonuclease activities in a euryarchaeon, *Pyrococcus furiosus*. He reported that both of the Hef activities are highly specific for fork-structured DNA, and discussed the role of the protein in the repair system that works to restore the replication fork when it stalls for some reason. He also mentioned the different characteristics of a Hef homolog from *Aeropyrum pernix*, belonging to the other subdomain of Archaea (Crenarchaeota) than *P. furiosus*. He finally summarized his analyses of the proteins RadA, RadB, Hjc, Hjm, and PCNA, which may be involved in the stalled replication fork repair in Archaea, and discussed the replication-coupled repair system.

**A. Johnson** (Rockefeller Univ.) described the mutational analyses of RFC (replication factor C) from *S. cerevisiae* aimed at elucidating the molecular mechanism of the loading of the PCNA clamp onto the DNA strand. The clamp loader RFC is the heterotetrameric protein complex and all subunits have common sequence motifs found in AAA+ family proteins. His group introduced a mutation into one of the motifs called the “arginine finger” in individual RFC subunits to investigate the effects of ATP binding and hydrolysis on the clamp loading activity. All the RFC complexes containing the individual mutant subunits allowed PCNA loading onto DNA, but not unloading, and as a result, processive DNA synthesis was prevented. The mutant RFC in which all the subunits were mutated could not open the PCNA ring, although it retained binding ability. He discussed the clamp loading mechanism considering these results.

**T. Tsurimoto** (Kyushu Univ.) described RFC-family protein complexes and their interactions with other proteins involved in DNA transactions in human cells. In addition to the normal RFC (RFCs1-5), complex, Rad17-RFCs2-5, and Chl12-RFCs2-5 made similar clamp loader type complexes. The Rad17 complex and Chl12 complex specifically interact with the Rad1-Hus1-Rad9 hetero trimeric ring and normal PCNA ring, respectively, in the same manner as the RFC-PCNA interaction. He also mentioned WRNIP1 (Mgs1), Werner helicase interacting protein 1, which is involved in maintenance of genome stability. WRNIP1 also has RFC-like sequences; however, it forms a homo oligomeric complex and specifically interacts with DNA polymerase d. He suggested from these data that the functional networks of the replication machineries and the protein factors related to important cellular functions are crucial for accurate cell cycle processing.

**Y-S. Seo** (Korea Advanced Inst. Sci & Tech) reported the current progress of his molecular analyses of Okazaki fragment processing. The removal of RNA primer in the lagging strand maturation requires the combination of Fen1, RNase H1 and DNA ligase I. In addition to these enzymes, his group demonstrated the importance of Dna2 nuclease in this process and proposed a concrete molecular mechanism previously. In this meeting, he mentioned more details about the functions of Dna2 protein. Dna2 can be divided into three domains, and its helicase and endonuclease activities are encoded in the central and the C-terminal domains, respectively. He presented findings about the role of the helicase activity in the removal of primers containing a secondary structure and showed that the N-terminal domain is critical for targeting the Dna2 to the flap position. Based on these results, he proposed a general mechanism for the instability of trinucleotide repeats (TNR), which is directly related to many hereditary neurological disorders in humans.

**V. A. Zakian** (Princeton Univ.) talked about the relationship between Rrm3p and DNA replication in *Saccharomyces cerevisiae*. Rrm3p is conserved from yeast to human and has a 5′-3′ DNA helicase activity. In the *RRM3*-deleted cells, replication forks slow down at many places in the chromosome. Furthermore, chromosome breakage and locus-specific increases in recombination occurred via deletion of the *RRM3* gene. These replication defects were associated with activation of the intra-S phase checkpoint, because activation of the checkpoint was critical for the viability of ΔRRM3 cells. Interestingly, stable, non-nucleosomal protein-DNA complexes were observed at all Rrm3p-dependent replication sites. From these data, she concluded that Rrm3p helicase helps replication forks traverse protein-DNA complexes, naturally occurring impediments that are encountered in each S phase.

**E. Schwob** (Univ. Montpellier) investigated the mecha-
nism of the S-phase checkpoint in *S. cerevisiae*. He is very interested in the mechanism of regulation of the cell cycle, in which cells make sure of the completion of chromosome replication before entering mitosis. Using various checkpoint mutant strains, he tested whether mitosis can be delayed by DNA replication without interfering with the progression of the replication fork and got results suggesting that ongoing DNA replication indeed triggers a response that delays mitosis until replication is finished. Considering his data, he proposed that the checkpoint monitoring ongoing DNA replication depends on the number of active forks, and furthermore, that equal spacing between the origins and firing chromosomal domains prevents inappropriate entry into mitosis during DNA replication.

D. A. Gordenin (NIH) talked about factors essential for stable DNA duplication, including hypermutable At-Risk Motifs (ARMs), which are known to be poor substrates for DNA replication or repair. In addition to his previous finding that the 3'-5' exonuclease activity of DNA polymerase δ serves as a backup for Okazaki fragment processing, he showed that a common environmental carcinogen is a cause of microsatellite instability. He presented data showing that chronic exposure of yeast to environmentally relevant concentrations of cadmium, a known human carcinogen, can result in extreme hypermutability. Based on the strong similarity between cadmium mutagenesis and the mutator effects of mismatch repair (MMR)-null alleles, he concluded that cadmium is a new kind of mutagen that acts by inhibiting the MMR system rather than through DNA damage.

In addition to these invited talks, many interesting posters were presented. For example, P. McInerney (Rockefeller Univ.) showed the fate of replisome components in response to polymerization blocks on either the leading or lagging strand template using an in vitro reconstitution system with *E. coli* proteins. It is very interesting that the replisome structures are very different from each other when leading strand synthesis versus lagging strand synthesis is blocked. K. Okumura (Mie Univ.) directly visualized replication fork movement on DNA fibers extended from cultured cell nuclei and showed that the rate of the fork movement is not constant during the S-phase progression. Chromatin modification also affects the fork movement. His conclusion is that the timing of origin firing and the fork movement are regulated by some epigenetic mechanisms. (Reported by Y. Ishino)

II. DNA repair

The DNA repair session began with a talk from A. Yasui (Tohoku University) in which he presented *in vivo* evidence indicating that assembly of repair proteins at oxidative DNA damage is significantly different from that at single strand breaks (SSBs), in mammalian cells. In order to introduce SSBs, human cells expressing a foreign endonuclease involved in repairing UV damage were irradiated with UV through small pores of membrane filters. By using antibodies and fluorescence microscopy, temporal and special cellular responses to SSB at restricted areas of the nucleus were visualized. Poly(ADP-ribose) synthesis occurs at the damaged area immediately after irradiation, and simultaneously XRCC1, DNA polymerase β, and ligase III are translocated from nucleoli as well as nucleoplasmic foci to the SSBs, where poly(ADP-ribose)ylation occurs, along with accumulation of proliferating cell nuclear antigen (PCNA) and p150 subunit of chromatin assembly factor 1 (CAF-1). In contrast, DNA glycosylases, OGG1, NTH1, NEIL1, NEIL2, and NEIL3, which excise oxidized bases, rapidly assemble with DNA polymerase d/e, FEN1, and CAF-1 after introduction of laser-induced local DNA damage in the nucleus, thus suggesting that there is a significant difference between the sequential assembly of repair proteins for SSBs and for oxidative base damage.

The next talk was given by T. Lindahl (Cancer Research UK), who described several topics in base excision repair (BER), proofreading, and a novel oxidative demethylation of DNA lesions. In mammalian cells, OGG1 excises 8-oxoguanine opposite cytosine in DNA, while MUTYH excises adenine misincorporated opposite 8-oxoguanine, and thus both enzymes initiate BER to avoid mutagenesis induced by 8-oxoguanine. OGG1-deficient mice exhibit increased accumulation of 8-oxoG in cellular DNA and increased mutation frequency; however, only one group found increased lung carcinogenesis among three groups who generated OGG1-deficient mice. In this meeting, Lindahl briefly mentioned that OGG1/MUTYH double-deficient mice exhibited increased occurrence of spontaneous lung and ovary tumors, indicating that spontaneous carcinogenesis is cooperatively prevented by OGG1 and MUTYH. He next described that mice deficient in uracil DNA glycosylase (UNG) exhibit an increased occurrence of B-cell lymphoma in addition to alterations in somatic hypermutation and immunoglobulin isotype switching. TREX1 is an *E. coli* DnaQ-like proofreading exonuclease, and Lindahl showed that mice deficient in TREX1 died young with inflammatory myocarditis; however, increased mutagenesis was not observed in mouse embryonic fibroblast (MEF) cells lacking TREX1 in comparison to wild-type cells. Finally, Lindahl presented a novel strategy for repair of alkylated DNA, that is, oxidative demethylation of 1-methyladenine in DNA by a 1-methyladenine-DNA dioxygenase, which is encoded by the *E. coli* alkB gene and the two homolog (*ABH2* and *ABH3*) genes in human cells.

Y. Nakabeppu (Kyushu Univ.) described that mice deficient in any one of MTH1, which hydrolyzes oxidized purine nucleoside triphosphates, such as 8-oxo-dGTP and
2-hydroxy-dATP, or OGG1, or MUTYH exhibited an increased occurrence of spontaneous carcinogenesis in liver, lung or small intestine, respectively. Next, he described the biochemical and biological characterization of a mutant MUTYH protein carrying a missense mutation, G82D, found in human colorectal cancer patients. Finally, he showed that mice deficient in both MTH1 and OGG1 exhibit no malignancy in the lung, and he further presented data indicating that either OGG1- or MTH1-deficient MEFs exhibit increased sensitivity to oxidative stress with increased accumulation of 8-oxoG in both nuclear and mitochondrial genomes, thus suggesting that cells deficient in both MTH1 and OGG1 exhibit greatly increased sensitivity to oxidative stress, which can initiate oncogenic alterations of certain genes, and may die before achieving malignant transformation. Nakabeppu also mentioned that expression of MTH1, OGG1, and MUTYH is altered in patients with neurodegenerative disorders with increased accumulation of 8-oxoG in such degenerative tissues, thus suggesting that MTH1, OGG1, and MUTYH protect neurons from oxidative stress.

K. Sugasawa (RIKEN) described a distinct binding directionality of XPC protein complex towards a helix-distorted site. The XPC complex is a structure-specific DNA-binding factor, which prefers a junction of double-stranded DNA with a single-stranded tail branching to the 3' direction, thus exhibiting specific binding affinity not only for lesions but also for artificial bubble and loop structures that do not contain any altered bases. It was also shown that the XPC complex is specifically targeted to the 5' side of a looped sequence in a loop substrate in which only one DNA strand contains unpaired bases. Using the electrophoretic mobility shift assay and atomic force microscopy, he obtained data indicating that one lesion site may accommodate only one XPC complex. Interestingly, even in the presence of a lesion such as N-acetoxy-2-acetylaminofluorene (AAF) in the loop substrate, the XPC complex only protects the 5' side of the looped sequence regardless of the position of the AAF adduct. Furthermore, Sugasawa showed that cell-free nucleotide excision repair (NER) incision was strongly stimulated by the presence of a loop opposite the AAF lesion, and was rather inhibited by the presence of the lesion within a looped sequence, thus suggesting that the correct orientation of the initial XPC binding is crucial for initiating a productive NER reaction.

L.H.F. Mullenders (Leiden Univ.) discussed the in vivo assembly of the NER complex involved in global genome repair (GGR) or transcription-coupled repair (TCR) in UV irradiated normal and repair-deficient human cells with special emphasis on the proteins involved in DNA lesion recognition. First he described the assembly of the NER complex during GGR in normal and repair-deficient (xeroderma pigmentosum) human cells, employing local UV irradiation combined with fluorescent antibody labeling. The XPC complex recognizing the damage site appears to be essential for the recruitment of all subsequent NER factors to the precission complex, including transcription repair factor TFIHH with XPB. XPA, which is required for anchoring of ERCC1-XPF, associates relatively late along with RPA. On the damaged sites, DNA polymerase ε, δ, XPC, and Ligase I are colocalized. These findings support a concept of sequential assembly of repair proteins at the site of the damage rather than a preassembled repairosome. He also described that the cellular content of a DNA damage-binding protein (DDB), which consists of DDB1 (p127) and the XPE gene product (DDB2, p48) and binds to pyrimidine 6–4 pyrimidone photoproducts (6-4PP) with a higher affinity than to cyclobutane pyrimidine dimers (CPD), is one of rate-limiting factors for repair of 6-4PP.

G. Almouzni (CNRS & Institut Curie) described recent findings on the histone chaperon or the chromatin assembly factor CAF1 regarding formation and maintenance of heterochromatin. Using the recently developed method for the localized UV irradiation of cells, he demonstrated that, within a single cell, the recruitment of CAF-1 to UV-induced DNA damage is a strictly local phenomenon, restricted to damaged sites. Concomitantly, PCNA locates to the same sites, and thus histones are deposited to the damaged sites. This localized recruitment suggests that CAF-1 participates directly in chromatin structural
rearrangements that occur in the vicinity of the damage. Use of NER-deficient cells shows that the NER pathway—specifically dual incision—is required for recruitment of CAF-1 and PCNA. This in vivo demonstration of the local role of CAF-1, depending directly on NER, supports the hypothesis that CAF-1 ensures the maintenance of epigenetic information by acting locally at repair sites. Furthermore, he presented data concerning the quality and stability of transcriptionally silent constitutive pericentric heterochromatin organization in interphase nuclei. He showed that both H3-K9 acetylation and methylation can occur on independent sets of H3 molecules in pericentric heterochromatin, and that an RNA- and histone modification–dependent structure that brings methylated H3-K9 tails together in a specific configuration is required for the accumulation of HP1 proteins, which play important roles to form heterochromatin, in the stably repressed regions and heterochromatin subdomains.

In the poster session, there were some interesting presentations regarding the biological significance of BER. The biological significance of NER has been established based on the existence of hereditary diseases such as xeroderma pigmentosum and Cockayne syndrome; however, there had not been such a link of BER-deficiency with human diseases. In the poster session, Y. Nakatsu (Kyushu Univ.) presented data indicating that there is increased occurrence of spontaneous intestinal tumors in MUTYH-deficient mice. This is the most interesting finding regarding BER-deficient mice, because it has been reported that germ-line mutations in MUTYH are considered to be causative mutations for some familial colorectal cancer cases without germ-line mutation in the APC gene. The phenotype of MUTYH-deficient mice is most likely to represent the human disease, and this is the first BER gene shown to be responsible for human diseases. Moreover, D. Tsuchimoto (Kyushu Univ.) presented data showing that deficiency in the 2nd AP endonuclease, APEX2, causes growth retardation and dyshematopoiesis accompanied by G2/M arrest. Tsuchimoto proposed that APEX2 and UNG both act dependently on PCNA to conduct the replication-associated BER, and thus in both mice similar cell types, such as cells in the immune system, especially B cells, are affected. (Reported by Y. Nakabeppu)

III. Translesion Synthesis and Mutagenesis

This session included nine presentations dealing with a variety of aspects of mutagenesis, mutagenic nucleotides, replication errors, replication fork blockage, and various translesion DNA polymerases.

The first speaker, M. Sekiguchi (Biomolecular Engineering Research Institute), summarized the current status of knowledge about a potent mutagenic-nucleotide, 8-oxodGTP, and mechanisms preventing mutagenesis by oxidized nucleotides. MTH1, a mammalian enzyme that hydrolyzes 8-oxodGTP to 8-oxodGMP, was shown by analysis of MTH1−/− mice to prevent spontaneous carcinogenesis. However, in contrast to the phenotype of an E. coli mutT mutant, the mutator effect of the MTH1 knockout mouse was rather weak. This may be due to the presence of other MutT homologues in mammalian cells. Indeed, NUDT5 was found to be one such protein. Interestingly, this MutT-related protein hydrolyzes 8-oxodGDP very efficiently and 8-oxodGTP at a very low rate. Since the NUDT5 protein readily substitutes for MutT when it is expressed in mutT cells, NUDT5 may have a much greater role than MTH1 in preventing the occurrence of mutations in human cells.

The next talk, given by T. Kunkel (NIEHS), was about several topics in eukaryotic mismatch repair (MMR). Using a yeast model, he and his colleagues established an elegant genetic test that measures strand bias in the rate of base substitutions resulting from replication of unrepaird 8-oxo-guanine (GO) in DNA. Since the lagging strand replication was more accurate, lagging strand GO-A mismatches seemed to be more efficiently repaired by MMR. This led them to hypothesize that PCNA and 5′ ends of Okazaki fragments may be used as strand discrimination signals for MMR. They provided evidence for this hypothesis by demonstrating significant reduction of the strand bias observed with MMR-deficient strains. Using a missense-type MSH2 mutation, they showed that two roles of Msh2 protein, MMR and apoptosis signaling in response to DNA damage, could be separated. This is an important finding for understanding cancer risk in humans.

H. Maki (NAIST) and R. Fuchs (CNRS) described the fate of the replication fork when it encounters a single DNA lesion on the leading or lagging template DNA. While Maki developed an in vitro system in which the dynamics of one replication fork concurrently synthesizing leading and lagging strands can be analyzed, Fuchs established an in vivo assay that measures the kinetics of DNA synthesis on either strand carrying a single replication-blocking lesion. Both reached the same conclusion, which is that a single lesion in the lagging strand template does not affect the movement of the replication fork but one in the leading strand template severely affects the replication fork. In addition, both found a transient uncoupling of concurrent strand synthesis when the replication fork meets a single lesion in the leading strand. These findings provide useful insights into the structural aspects of replication fork blockage and a starting point for further investigations of replication fork recovery.

During the past several years a new superfamily of DNA polymerases called the Y-family has been identified. This group, so-called translesion DNA polymerases (TLS polymerases), includes evolutionarily related proteins known to be involved in mutagenesis. In
the second half of this session, five speakers talked about several Y-family DNA polymerases.

**F. Hanaoka** (Osaka Univ.) described human DNA polymerase η, which is encoded by a gene responsible for a variety of xeroderma pigmentosum (XP-V). Pol η was found to be localized at replication foci in S-phase cells, and accumulation of pol η-containing foci was observed after exposure to UV radiation. Furthermore, examination of various DNA substrate preferences demonstrates that pol η prefers template-primer DNA. PCNA and pol η physically interact, as shown by the yeast two-hybrid assay as well as by pull-down experiments. These observations fit the notion that pol η is recruited to or localized at sites of DNA replication. Yeast two-hybrid assays also revealed that pol η interacts with Rev1 protein. These findings are important for better understanding the mechanisms that control the coming and going of TLS polymerases at the site of damage.

**E. Friedberg** (Univ. Texas) discussed mammalian DNA polymerase κ. He reported that although Pol κ is ubiquitously present in most tissues, it appears to be highly expressed in the adrenal cortex and in a cell-specific manner during spermatogenesis, suggesting that it has particular functions in these specific tissues. He also presented data from biochemical studies using purified Pol κ. It was clearly demonstrated that the Pol κ can bypass thymine glycol, a product of oxidative DNA damage, in template DNA. Mouse Pol κ was found to form a complex with Rev1 protein, a dCMP transferase that is also involved in translesion synthesis. Interestingly, Rev1 improves the efficiency of the replicative bypass of thymine glycol by Pol κ. Rev1 protein interacts with several other TLS polymerases. Based on these findings, he hypothesized that the Rev1 protein regulates the access of specialized polymerases to their respective damage sites in the genome.

**E. Ohashi** (Kyoto Univ.) also described his group’s recent studies on mammalian Pol κ. Ohashi and his colleagues demonstrated that Pol κ efficiently inserts the correct C opposite benzo[a] pyrene-adducted guanines, while Pol η inserts A or G in preference to C opposite the same lesion. Using ES cells lacking Pol κ, they provided clear evidence for anti-mutagenic action of Pol κ against exposure of cells to benzo[a] pyrene. These findings led them to hypothesize that some mechanism operates to recruit an appropriate enzyme to a particular species of DNA lesion when replicative DNA polymerase meets the lesion. To elucidate such traffic control mechanisms, they searched for proteins interacting with Pol κ. Using the yeast two-hybrid assay, genes encoding such proteins were screened using a human testis cDNA library, and the C-terminal portion of Rev1 protein was found to specifically interact with Pol κ. The Rev1-Pol κ interaction was further confirmed by immunological techniques. Ohashi also showed evidence that Pol κ and REV1 co-localize in human cells. Like Friedberg, Ohashi speculated that Rev1 might play a pivotal role during TLS in vivo.

**M. Moriya** (State Univ. New York, Stony Brook) talked about translesion DNA synthesis across endogenous exocyclic DNA adducts in human cells. He has been studying genotoxicity and mutagenicity of acrolein-derived exocyclic DNA adducts. In particular, the effects of α-OH-propano dG adduct (α adduct), a potent replication-blocking and mutagenic DNA lesion, on DNA synthesis and the consequences of the replication were extensively studied in vitro and in vivo. Moriya and his colleagues examined the capabilities of various DNA polymerases, including TLS polymerases, for in vitro DNA synthesis on the template DNA carrying a single α adduct. It appeared that Pol η is involved in inaccurate TLS on the α adduct, whereas Pol 1 and Rev1 insert the correct C opposite the lesion. This was confirmed by in vivo studies with human cells lacking Pol η. Furthermore, Moriya showed that Pol β is an efficient extender for further chain elongation from the dCMP terminus formed upon TLS by Pol 1 or Rev1. From these data, Moriya concluded that Pol 1-Pol β and Rev1-Pol β promote accurate bypass synthesis, which accounted for 90% of translesion events at the α adduct.

**R. Woodgate** (NIH) discussed structural aspects of TLS polymerases. As a model TLS polymerase belonging to the Y-family, he and his colleagues chose DNA polymerase IV (Dpo4) of the archaeon *Sulfolobus solfataricus* P2. Although Dpo4 is classified into the DinB sub-family, the *S. solfataricus* enzyme possesses biochemical properties similar to eukaryotic members of the Y-family, including a propensity to bypass certain DNA lesions like a thymine-thymine cyclobutane pyrimidine dimer (CPD). By X-ray crystallography, they analyzed Dpo4 with and without CPD-containing template-primer DNA and incoming nucleotide. Presenting the 3-D structure of Dpo4 at high resolution, Woodgate clearly demonstrated that the active site of the polymerase is sufficiently large to accommodate the covalently linked pyrimidine dimer. (Reported by H. Maki)

**IV. DNA recombination**

This session included 16 talks dealing with many aspects of DNA recombination. The first speaker was **K. Mizuuchi** (NIH), who talked about the dynamics of protein-DNA complex assembly and disassembly in phage Mu transposition. He has developed a fluorescence-based real-time assay for transpososome assembly, and used it to determine the structural features of the substrate DNA. In addition, he developed a fluorescence microscope system for studying MuB-DNA complex assembly/disassembly dynamics at the single molecule level using GFP-MuB and surface-tethered DNA. His data indicate that MuB forms clusters along DNA, which
likely represent individual functional polymers of MuB. MuA accelerates disassembly of the MuB clusters with different efficiencies depending on the particular form of MuA.

**N. Okada** (Tokyo Inst. Technology) characterized new families of retrotransposition-related LINEs (UnalL2) and SINEs (UnaSINE1) from eel Unal2 and found that these LINE/SINE partners share similar 3' tails. A retrotransposition assay in HeLa cells demonstrated that the 3' conserved tail of UnaL2 is necessary for its retrotransposition. This 3' tail is recognized in trans by the UnaL2 reverse transcriptase at a surprisingly high rate, and that of UnaSINE1 can also be recognized, thus providing experimental evidence that a SINE can be mobilized by the retrotransposition machinery of a partner LINE. Short repeats at the 3' end of UnaL2 are required for retrotransposition, suggesting that UnaL2 retrotransposes in a manner reminiscent of the reverse transcriptase activity of telomerases.

Gene targeting has been very difficult in plants. **S. Iida** (Natl Inst. Basic Biol., Japan) showed two new approaches for gene tagging in rice (Oryza sativa L.). He adopted the Hygromycin phosphotransferase gene as a marker for targeting gene disruption by homologous recombination. All of the six transgenic rice plants obtained independently were made-to-order heterozygotes. The other approach is transposon tagging, and he showed the possibility of application of a new endogenous DNA transposon identified from a mutation that was responsible for a position effect of variegation.

**T. Shibata** (RIKEN) presented findings suggesting an insight into homoplasy of mitochondrial DNA (mtDNA). Homoplasy is a basic genetic state of mitochondrial DNA in which all mitochondrial DNA copies within a cell share the same sequence. A genetic study suggested that yeast Mhr1, an ATP-independent homologous pairing protein, initiates circle replication of mtDNA. The resultant concatamers are essential intermediates in the partitioning of mtDNA into growing buds. This Mhr1-dependent system selectively transmits a number of progeny DNA copies replicated on a single template DNA copy to daughter cells, resulting in homoplasy. He proposed that a mechanism based on recombination-dependent replication similar to the Mhr1-dependent replication initiation might play a role in the maintenance of the homogeneity among units of various repeated sequences.

**T. Kobayashi** (Natl. Inst. Basic Biol.) also reported findings about the mechanisms that maintain the stability of repetitive DNA sequences. The replication fork barrier site (RFB) is an ~100 bp sequence located near the 3' end of the ribosomal RNA genes (rDNA), and is a recombination hot spot that is a potential driving-force to change the copy number of rDNA. The yeast SIR2 gene encodes a histone deacetylase and is required for gene silencing in the rDNA region as well as other silenced regions such as mating type loci and telomeres. It is also required for the stability of rDNA. His results showed that Sir2 was involved in the association of the cohesin subunit Med1 (Sec1) with rDNA but not in replication arrest or DSB formation. He suggested that for rDNA stability, Sir2 prevents unequal-chromatid recombination by forming a special cohesin structure.

**H. Iwasaki** (Yokohama City Univ.) has found a new pathway of recombination repair in *S. pombe*, which is Rad51-dependent and is independent of the Rad55/57 heterodimer, the well-known accessory protein of Rad51. The new pathway involves Swi5 and Sfr1. Sfr1 bridges the interaction between Swi5 and Rad51. Sfr1 shares significant homology with the C-terminal half of Swi2. He also showed that Swi2 bridges the interaction between Swi5 and Rad51, but this interaction is specifically involved in mating-type switching.

**A. Nicolas** (Curie Institute) proposed that Mre11 is a structural component of the Spo11-containing complex that assembles on the DNA double-strand break (DSB) sites formed in the very early phase during meiotic recombination. His chromatin immunoprecipitation and microarray analyses indicated that Mre11 transiently and quantitatively associated with the chromatin of Spo11-dependent DSB regions throughout the genome. However, Mre11 binding does not require DSB formation itself. The release of Mre11 from chromatin is blocked in all mutants that accumulate unresected DSBs. Spo11-containing complex composed of Mre11 ensures a tight coupling between DSB formation by Spo11 and the processing of DSB ends.

**R. Rothstein** (Columbia Univ.) visualized in vivo DSB repair events by using a system of GFP-fusion protein and specific DSB in yeast. He showed that the repair of multiple DSBs is organized into a single repair center. Time-lapse microscopy revealed that the localization of recombination repair proteins (Rfa1, Rad59, Rad51, Rad54, Rad55) and some checkpoint proteins (Mre11, Ddc1, Ddc2) into a focal assembly was a rapid and reversible process. Furthermore, resumption of the cell cycle does not occur until DNA repair foci have disassembled. This coupling between DNA repair and cell cycle progression is dependent on an intact DNA damage checkpoint.

**A. Shinohara** (Osaka Univ.) also showed some analyses of protein assembly and disassembly during meiotic recombination using fluorescently tagged proteins. He demonstrated that Mec3 and Ddc1, which form a checkpoint PCNA-like clamp, were colocalized with Rad51. Chromatin immunoprecipitation analysis showed that Mec3 bound to resected ends of DSB, like Rad51. These observations support the view that the checkpoint clamp functions together with Rad51 during meiotic recombination.

**P. Sung** (Yale Univ.) demonstrated that the Rad51-mediated recombination reaction was strongly inhibited
by Srs2 via disruption of the Rad51 presynaptic filament. The action of Srs2 is enhanced by RPA and requires the ATPase activity of Srs2. These in vitro properties of Srs2 provide a good explanation for the in vivo anti-recombinogenic role of Srs2.

H. Shinagawa (Osaka Univ.) characterized novel recombination repair genes, rad60 and rad62, isolated from S. pombe. He had previously developed a strategy for isolation of recombination repair mutants by taking advantage of synthetic lethality with rad2, which encodes the Fen1 nuclease involved in Okazaki fragment processing. His genetic studies suggested that Rad60 and Rad61, together with SMC5-SMC6 and Brc1 proteins, play an important role in genome integrity and recombination repair.

T. Takeda (Kyoto Univ.) analyzed the BRC2 gene in chicken DT40 cells. braca2tr2 cells, which have a truncation of the C-terminal region of the second BRC motif, exhibit a similar phenotype to that of mammalian brac2 mutant cells. This cell line also exhibits a very similar phenotype to chicken Rad51 paralogue mutants, including hypermutation with a reduced rate of immunoglobulin gene conversion in the Ig V segment. He suggested that Brca2 regulates the choice of two alternative diversification pathways, accumulation of point mutations and Ig gene conversion during chicken B cell development. He also suggested that Brca2 and Rad51 paralogues share similar roles in recombination reactions in the facilitation of Rad51 function.

K. Morikawa (BERI) presented a structural view of Holliday junction processing. Based on crystal structures of RuvA, the RuvA-Holliday junction complex, RuvB, and the RuvA-RuvB complex, and electron microscopic images of the RuvA-RuvB-junction DNA ternary complex, he modeled the three-dimensional structure of the complex, which suggests an intriguing mechanism for branch migration of the Holliday junction.

J. Tainer (Lawrence Berkeley Natl Lab, Scripps Res Inst.) also showed the structural views of protein interactions. Crystallographic studies on Mre11-Rad50 complex from hyper-thermophilic archaea, *Pyrococcus furiosus*, show that the interface between Rad50 and Mre11 defines a compact complex, implying that Rad50 controls the Mre11 exonuclease. He also showed the structural model of the complex of Rad51-BRC domain and proposed a molecular model of BRC function for disruption of Rad51-filaments.

NBS1, the product of the gene responsible for Nijmegen Breakage Syndrome, forms a multimeric complex with human MRE11-RAD50 complex. K. Komatsu (Radiation Biology Center, Kyoto Univ.) demonstrated that NBS1 binds to phosphorylated histone H2AX. The recognition of H2AX requires the forkhead associated domain (FHA) and BRC domain in NBS1.

M. Resnick (NIH) showed that although there was efficient induction of DSBs, chromosome breaks were rarely detected in wild-type yeast cells. However, chromosome breaks were common in mutants of the Rad50/Mre11/Xrs2 complex. He suggested that the structural features of Rad50 are important for preventing the transition of a DSB to a chromosome break. (Reported by H. Iwasaki)

V. Checkpoint and Chromosome Integrity

When DNA replication is inhibited, replication forks stall or pause and the proteins at the fork are thought to be maintained until the replication restarts. In this session, S.M. Gasser (Univ. Geneva) showed that when replication forks stall due to hydroxyurea treatment DNA polymerases a and ε are actually maintained at replication forks and this maintenance requires Mec1 and Sgs1 in budding yeast, a counterpart of mammalian Atr kinase and a RecQ-like DNA helicase, respectively. In the ChIP assay, Sgs1 seems to move together with replication forks even in the absence of inhibitors of DNA replication. Further, Sgs1 is co-precipitated with RP-A, a single-strand DNA-binding protein, by immunoprecipitation, consistent with the idea that Sgs1 exists at replication forks where RP-A associates with unwound single-stranded DNA. From these results, Gasser proposed a model in which Sgs1 helicase resolves aberrantly paired structures at stalled forks to maintain single-stranded DNA that allows RP-A and Mec1 to stabilize polymerase association.

On the other hand, if the replication fork is collapsed, not only DNA-repair proteins but also checkpoint proteins might be assembled. The 9-1-1 clamp complex is one such protein. A.M. Carr (Univ. Sussex) reported that in fission yeast, Rad3, a counterpart of Atr in higher eukaryotes, phosphorylates Rad9, a subunit of the 9-1-1 complex, during S phase without any replication stress and in G2 phase in the presence of damaged DNA. This phosphorylated Rad9 binds to Rad4/Cut5, which is needed for checkpoint-activation when DNA is damaged or replication forks are stalled by hydroxyurea. Consistent with the importance of this phosphorylation, a rad9 mutant lacking the phosphorylation site is defective in DNA-damage checkpoint. In Carr’s model, Rad9 and Rad4/Cut5 form a pre-checkpoint complex during S phase and this complex is recruited to the fork if it is collapsed.

A checkpoint factor might recognize stalled or paused replication forks and send a signal to the mitotic apparatus to arrest the cell cycle. W.G. Dunphy (Cal. Tech.) and K. Shirahige (RIKEN) suggested that Claspin in Xenopus extract (Dunphy) and Mrc1 and Top1 in budding yeast (Shirahige) are good candidates for factors recognizing stalled replication forks. In Xenopus egg extract, Chk1 kinase, which is essential for the checkpoint function in S phase, undergoes phosphorylation and activation in response to DNA replication inhibitors. Dunphy showed that this phosphorylation and activation of Chk1
depend on Atr kinase and the binding of phosphorylated Claspin to the Chk1 kinase domain. Claspin binds to chromatin during S phase and this chromatin-binding depends on the pre-replicative complex and Cdc45 but not on RP-A. However, Atr and Rad17, a subunit of RFC-like loader for the 9-1-1 complex, require RP-A for association with chromatin. Since Atr, Rad17 and Claspin associate with chromatin independently, they might detect different aspects of a DNA replication fork.

Using a high-density microarray of chromosome VI and ChIP assays of several replication and checkpoint proteins in budding yeast, Shirahige also addressed the function of Mrc1, a counterpart of Claspin. In those assays, not only Mrc1 but also Tof1 were found to associate with replication forks. Moreover, Mrc1 and Tof1 form a complex with replication proteins, consistent with their presence at replication forks. Furthermore, in the absence of Tof1 or Mrc1, replication proteins move farther, leaving non-replicating regions behind when DNA replication is inhibited by hydroxyurea. This over-movement of replication proteins is not observed even in the absence of Mccl and Rad53, counterparts of Atr and Chk2 in higher eukaryotes, respectively. From these results, Shirahige suggested that a stable replication-pausing complex formed by Mrc1 and Tof1 is the primary signal for the S-phase checkpoint activation.

Cells overcome intra-S DNA damage and replication block by coupling chromosome replication to sister chromatid-mediated recombination and replication-bypass processes. M. Foiani (Inst. FIRC Oncologia Molecolure, Milano) suggested that molecular junctions between replicated molecules assist sister chromatid cohesion until anaphase. In 2D gel analysis of replication intermediates in budding yeast, X-shaped molecules are found to form during the initiation of DNA replication. While this formation is independent of Rad5l- and Rad52-mediated homologous recombination and is not affected by DNA damage or replication blocks, in hydroxyurea-treated rad53 cells defective in checkpoints the X-shaped molecules in 2D gels progressively degenerate and likely contribute to the general pathological structures. Finally, Foiani proposed a model in which replication initiation generates the specialized joint molecules resembling hemicatenanes and consequently primes cohesion and assists sister chromatid-mediated recombination and replication events.

Several repair and checkpoint proteins are recruited to damaged DNA. For example, Mec1 kinase of budding yeast is recruited to damaged DNA, probably through its interaction with RP-A. K. Sugimoto (Nagoya Univ.) showed, using a double-strand break generated by HO-endonuclease, that Mec1-related Tel1 kinase, a counterpart of Atm in higher eukaryotes, is also recruited to damaged DNA, and its association with the break is enhanced in the absence of Sae2, a protein that binds to the break. In two-hybrid assays, Tel1 appears to interact with the C-terminal portion of Xrs2, which is a subunit of the Mre11-Rad50-Xrs2 complex that binds to the break. Since the C-terminus of Xrs2 is similarly involved in cell survival and Rad53 phosphorylation after DNA damage, Sugimoto concluded that Tel1 associates with the DNA region via the C-terminal portion of Xrs2 and that this association is required for the activation of the DNA damage response.

To examine the functional chromosome structures of higher eukaryotes, we need suitable tools. T. Okazaki (Fujita Health Univ.) reported the recent development of mini mammalian artificial chromosomes (MACs), which seem to be good tools for elucidating the functional structures of chromosomes. MACs exists as multimeric forms of introduced precursor human DNA 50–100 kb alphoid arrays and are megabases in size and linear and/or circular in form. MACs behave as intact chromosomes; they replicate, segregate and are transmitted accurately for many cell divisions. Moreover, heterochromatin structures are formed on alphoid arrays in MACs, as they are in intact chromosomes, and repress the gene expression proximal to alphoid sequences. As expected, when insulators are inserted into the boundaries between the heterochromatin and the genes to be expressed, the gene expression occurs successfully and is regulated like the expression of the gene located on the chromosome. Thus, Okazaki concluded that MACs are not only a good model system to study essential elements of chromosomes but would also be useful as novel vectors for mammalian cells and bodies.

One of the important structures of chromosomes is the telomere, which is essential for maintenance of chromosome integrity. However, we did not have a good in vitro system for studying the molecular mechanism of telomere maintenance. F. Ishikawa (Kyoto Univ.) talked about the recent development of such an in vitro system using Xenopus extract. Using this in vitro system, the cell-cycle regulated telomere association of a telomere-binding factor, TRF1, was revealed. TRF1 associates with mitotic chromatin and dissociates from chromatin at the M phase/S-phase transition. When nondegradable cyclin is added to the interphase extract to increase the CDK activity, TRF1 starts to associate with chromatin simultaneously with the transition to M phase. Conversely, inhibition of the CDK activity by 6-DMAP in the MII-arrested extracts leads to M-phase exit and dissociation of TRF1 from chromatin without initiation of DNA replication. Thus, the telomere structure seems to be regulated by the recruitment of TRF1 in a cell-cycle-dependent manner, which appears to facilitate telomerase-mediated telomere elongation in S phase. (Reported by H. Araki)

Special lecture was delivered by M. Yanagida (Kyoto Univ.) on the role of the fission yeast securing/cut2 in
DNA repair in interphase, in addition to its well-known role in regulating sister chromatid separation in anaphase. A temperature sensitive securing mutant, cut2-EA2 is defective in nucleotide excision, chk1 or crb1, or with rad13 defective in an excision nuclease. Since an allele of separase, cut1-zu6, is also sensitive to UV, he proposed that the securing-separase complex might aid DNA repair by removing local cohesion in interphase cells.

Another special lecture was delivered by J.-I. Tomizawa (Natl. Inst. Genet.) who emphasized the importance of microscopic analysis based on thermodynamics in describing biological equilibrium, and the importance of single molecule dynamics for such analysis.