OKAZAKI Fragment Memorial Symposium:
Celebrating the 50th anniversary of the discontinuous DNA replication model

Sakata and Hirata Hall, Nagoya University

December 17 - 18, 2018

Preface

One of the mysteries of DNA replication after the proposal of semiconservative replicaiton model and discovery of DNA polymerase was how the double-stranded DNA consisting of two antiparallel chains can be replicated by an enzyme which can extend DNA chains only in one direction from 5' to 3'. The late Dr. Reiji Okazaki and his wife Tsuneko proposed that on the lagging strand DNA is discontinuously synthesized as short DNA fragments and that the fragments are ligated to become mature, longer DNAs, on the basis of their analyses on the nascent DNA fragments produced in *E. coli* infected with T4 phage. This short lagging strand DNA fragment was named "Okazaki fragment" and this name is still used worldwide today. Subsequent studies demonstrated that the discontinuous synthesis of lagging strand through Okazaki fragments was conserved through bacteria to animal cells, and has become one of the most basic principles on DNA replication. Reiji died of leukemia at the age of 44 in 1975, due to radiation exposure in Hiroshima when he was a second year student at a junior high school. Tsuneko took over Reiji's wishes and continued to work on DNA replication at Nagoya University, clarifying further details of lagging strand DNA synthesis.

The finding of "discontinuous DNA replication" not only disclosed a universal principle of DNA replication, but also conceptually affected many fundamental problems in biology from mechanisms of biological evolution through the assymmetric mutations on the two DNA strands to determination of left-right assymmetry. It is widely regarded that Reiji would have won the Nobel prize if he had been alive. This unique innovative study was initiated and accomplished at the Nagoya University. Half a century has passed since the discovery of discontinuous DNA replication, and we would like to celebrate this occasion by having the OKAZAKI Fragment Memorial Symposium at the exact location where this one of the greatest discovery in biology was made.

We are pleased to have four distinguished scientists from abroad, and 13 domestic sepeakers along with 12 poster presentations in this symposium. We hope that this symposium will present an opportuity to reflect on the spirit and achievement of this intelectual Giant and look into the current excitement and future direction that stemmed from this historic discovery made here at the Nagoya University.

Hisao Masai
Chair
Program committee

December 17, 2018

Opening Remarks

13:00 ~ 13:10 Masahide Takahashi (Regent of Nagoya University)

Session I: **Plenary lecture**

Chair: Hisao Masai

13:10 ~ 14:00 1P1 Peter Burgers (Washington University School of Medicine, USA)
"Fifty Years of Okazaki Fragment Research"

Session II: History and implication of Okazaki Fragment/Discontinuous replication model

Chair: Hisao Masai

14:00 ~ 14:40 1P2 Robert Fuller (University of Michigan Medical School, USA)
"From oriC Replication to Studies of VPS13 and Neurodegeneration"

14:40 ~ 15:05 1P3 Mitsuru Furusawa (Chitose Laboratory)
"Okazaki fragment drives evolution"

15:05 ~ 15:25 ***Coffee Break***

Session III: From Okazaki Fragment to new fields

Chair: Takehiko Kobayashi

15:25 ~ 15:50 1P4 Hisao Masai (Tokyo Metropolitan Institute of Medical Science)
"From mechanisms of primer RNA synthesis to revelation of hidden messages of genome"

15:50 ~ 16:15 1P5 Hisao Masukata (Osaka University)
"Replication origin where the first Okazaki fragment initiates"

16:15 ~ 16:40 1P6 Hiroshi Masumoto (Kazusa DNA Res Inst)
"Developments of Human Artificial Chromosomes and Chromatin Manipulation Technologies."

16:40 ~ 17:05 1P7 Fuyuhiko Tamanoi (Kyoto University)
"From Okazaki fragments to Patient-derived Tumor Model"

17:05 ~ 17:25 1P8 Tsuneko Okazaki
"Discontinuous Mechanism of DNA replication. -How it was investigated."

17:25 ~ 17:30 **Photo Session**

17:30 ~ 18:00 **Poster Short Presentations**

18:00 ~ 19:00 **Poster Viewing with refreshments**

19:00 ~ 20:45 **Reception Party (Hananoki)**

December 18, 2018

Session IV: Okazaki fragment/ Discontinuous Replication now and beyond

Chair: Peter Burgers

9:00 ~ 9:25 2A1 Hisaji Maki (Nara Institute of Science and Technology)
"Collision with duplex DNA renders *Escherichia coli* DNA polymerase III holoenzyme susceptible to DNA polymerase IV-mediated polymerase switching on the sliding clamp."

9:25 ~ 9:50 2A2 Toshiki Tsurimoto (Kyushu University)
"Roles of PCNA and clamp loaders for leading and lagging DNA synthesis"

9:50 ~ 10:30 2A3 Philippe Pasero (Institute of Human Genetics, France)
"Nascent DNA drives inflammation under replication stress conditions"

10:30 ~ 10:50 ***Coffee Break***

10:50 ~ 11:15 2A4 Yoshizumi Ishino (Kyushu University)
"Replisome structure and its functions in Archaea"

11:15 ~ 11:55 2A5 Huilin Li (Van Andel Research Institute, USA)
"Cryo-EM suggests a coupled-sister-replisomes model at the core of the replication factory"

11:55 ~ 13:00 ***Lunch Break***

Session V: The newest trends in DNA replication and related areas after Okazaki Fragment/ Discontinuous replication model

Chair: Katsuhiko Shirahige

13:00 ~ 13:25 2P1 Tsutomu Katayama (Kyushu University)
"Mechanisms and regulations in the initiator DnaA and the initiation complex"

13:25 ~ 13:50 2P2 Hiroyuki Araki (National Institute of Genetics)
"A novel role of DNA polymerase ε at replication forks; its involvement in replication fork pausing at the barriers."

13:50 ~ 14:15 2P3 Takehiko Kobayashi (The University of Tokyo)
"Replication fork arrest induces gene amplification and cellular senescence"

14:15 ~ 14:35 ***Coffee Break***

14:35 ~ 15:00 2P4 Katsuhiko Shirahige (The University of Tokyo)
"Transcriptional Regulation by Cohesin loader"

15:00 ~ 15:25 2P5 Masato Kanemaki (National Institute of Genetics)
"Revealing a non-canonical DNA replication important for genome maintenance in human cells"

15:25 ~ **Poster Award Presentation**

Concluding remarks

15:35 ~ Hisao Masai

Oral Presentations

1P1

Fifty Years of Okazaki Fragment Research

Peter M. Burgers

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Abstract

At the 50-year anniversary of the publication of the 1968 landmark PNAS paper by Reiji and Tsuneko Okazaki, it is of interest to consider how the various models for lagging *versus* leading strand DNA synthesis have been put to the experimental test. Very soon after the paper was published, the replication field settled for the aesthetically appealing semi-discontinuity model, a fully continuous leading strand and a discontinuous, RNA-primed lagging strand. However, experimental results did not agree with this idealized model. Further investigations have led to new insights how the incorporation and subsequent repair of non-canonical nucleotides can contribute to transient nicks in the leading strand.

Here, I will describe the mechanisms that govern the maturation of Okazaki fragments in eukaryotic cells and the mechanisms that cause discontinuities in the leading strand. In particular, the relatively frequent misincorporation of ribonucleotides by replicative DNA polymerases provokes nicking by ribonuclease H2, initiating Ribonucleotide Excision Repair. RER is a specialized form of Okazaki fragment maturation, and it is the most frequent repair pathway in the cell. When RER and other repair processes have been eliminated, nascent leading strands are virtually continuous.

DNA polymerase delta (Pol δ) is responsible for the elongation and maturation of Okazaki fragments, while the flap endonuclease FEN1 is the principal 5'-nuclease responsible for the removal of initiator RNA. During the maturation process, Pol δ and FEN1 remain stably bound to the DNA by the circular clamp PCNA. This coordinated machinery removes RNA primers and produces a ligatable nick. Rapid quench-flow techniques have been used to study this process at msec resolution. Kinetic and enzyme trapping experiments support a model in which a stable PCNA-DNA-Pol δ -FEN1 complex moves processively through the iterative steps of nick translation in order to completely remove primer RNA, one or two ribonucleotides at the time. Ribonucleotides that have been misincorporated on the leading strand are also repaired by this machinery, after 5'-incision by Ribonuclease H2.

Biography

Peter Burgers is the Marvin Brennecke Professor of Biological Chemistry at Washington University in Saint Louis, Missouri. He was trained as an organic chemist at the University of Leiden, the Netherlands, and at the Max Planck Institute in Göttingen, Germany. From 1980-1982, he carried out postdoctoral studies with Arthur Kornberg at Stanford University. For more than thirty years, his laboratory has made critical contributions to the field of DNA replication and DNA repair, using a combination of biochemistry, molecular biology and genetic analysis in yeast cells.

Keywords: *DNA polymerase, FEN1 nuclease, Okazaki fragments, Ribonucleotide excision repair*

1P2

From oriC Replication to Studies of VPS13 and Neurodegeneration

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Abstract

Early progress in DNA replication owed its success to the application of traditional enzymology – reconstitution of authentic, complex enzymatic processes in extracts followed by the purification and reconstitution of functional components. I learned this approach as a student in the lab of Arthur Kornberg and, in collaboration with postdoctoral fellow John Kaguni, succeeded in establishing authentic replication of the *E. coli* origin of replication in extracts. This served as the basis for function-based purification of DnaA protein and reconstitution of *oriC* replication and discovery of the role of DnaA in opening of the origin to promote assembly of the replisome and subsequent bidirectional replication. For historical interest, I shall review this work and then discuss how this general approach has been key to dissecting the complex reactions involved in vesicular trafficking and the current work in my lab on yeast Vps13 protein. We used methods of purification and reconstitution to show that Vps13 protein is directly required for vesicular trafficking between the *trans* Golgi Network (TGN) and late endosome. Vps13 proteins are conserved in all eukaryotes and appear to function in a variety of membrane transactions, including vesicular trafficking and fusion, phagocytosis and autophagy, and to localize at organelle contact sites. Four human genes encode full-length Vps13 homologs. Each is the locus of an autosomal recessive neurodegenerative or neurodevelopmental disorder.

Biography

Bob Fuller is Professor of Biological Chemistry and Director of the interdepartmental Cellular and Molecular Biology Ph.D. Program at the University of Michigan. He received a B.S. in Molecular Biophysics and Biochemistry at Yale in 1978 and his Ph.D. with Arthur Kornberg in Biochemistry at Stanford University in 1984. He received postdoctoral training at UC Berkeley with Jeremy Thorner. He was Assistant Professor of Biochemistry at Stanford from 1987-1994, Associate Professor of Biological Chemistry at the University of Michigan from 1994-1999 and Professor of Biological Chemistry at the University of Michigan from 1999 to the present. His research has ranged from DNA replication in *E. coli* to proteolytic processing of secretory peptides and proteins and vesicular trafficking and protein localization in eukaryotic cells.

1P3

Okazaki fragment drives evolution

Mitsuru Furusawa

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Abstract

It has been found that the average mutation rate of the lagging strand is significantly higher than that of the leading strand. Evolutionary implications of this unbalanced mutagenesis were clarified using a simple model chromosome, which consisted of a linear DNA with a single terminal replication origin. The DNA pedigree evolved in a bit strange and paradoxical world in terms of evolutionary biology. 1) The mutagenic landscape is far away from normal binominal distribution. Figure 1 shows the distribution of mutations in the F1 generation in a population consisting of n-number of individuals. 2) Permanent maintenance of any once-appeared-genotype, including an original ancestor DNA (Fig. 2). 3) Unlimited mutation rate, or the increase or disappearance of the error-threshold. In short, the high fidelity of the leading strand ensures the exact heredity and the low fidelity of the lagging strand produces candidate mutants for evolution.

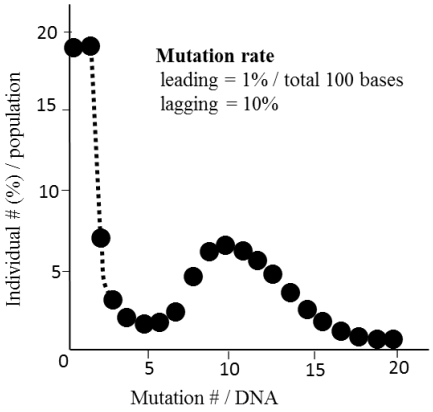

Figure 1.
Mutagenic landscape in the disparity model

Furusawa, M & Doi, H. *Genetica* 102-103, 333 (1998)
Drawn by Teraoka, M & Terasaki, T.

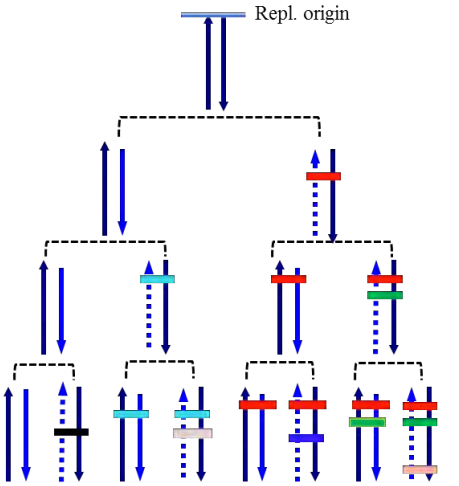

Figure 2.
DNA pedigree in the disparity model

Leading; Lagging; Mutation

Furusawa, M. & Doi, H. *J.thore.Biol.*157,127(1992)

DNA polymerase, polδ, exclusively contributes to the synthesis of the lagging strand. Therefore, mutants lacking the proofreading ability of polδ ("disparity mutator") make it possible to accelerate evolution. This technique is applicable to eukaryotic organisms. In comparison with the conventional parity mutagenesis model, the essential features of the disparity mutagenesis will be explained. Finally, experimental examples of the acceleration of evolution, using disparity mutators in *E. coli* and *Saccharomyces cerevisiae* will be shown. (Review article: Furusawa, M. Front. Oncol. 2, 144 (2012). doi: 10.3389/fonc.2012.00144)

Biography

Osaka City University (Redifferention of cancer, cell engineering. 1958-1983), Daiichi Pharmceutical Co. (Molecular Biology. Board member. 1937-2007). ERATO 'Furusawa Morphogene Project' (Evolution. Project leader. 1989-1993), Chitose Laboratory Corp. (Founder & CSA. 2002-).

Key words: disparity mutagenesis, mutagenic landscape, acceleration of evolution.

1P4

From mechanisms of primer RNA synthesis to revelation of hidden messages of genome

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Abstract

Primer RNA synthesis is essential for starting OKAZAKI fragment synthesis. The use of single-stranded DNA phage as a cloning vector permitted identification of novel signals for primer RNA synthesis. Those included the one initiated by primase alone or by RNA polymerase alone as well as those requiring more complex sets of prepriming proteins (Masai et al. ***JBC***, 1989,1990; Masai and Arai ***Cell***, 1997). The common feature for these newly isolated priming signals was the presence of unique secondary structures on the isolated single-stranded DNA.

More than 20 years after these findings, we discovered unique DNA structures as signals for regulation of DNA replication in eukaryotic cells. The sequences composed of multiple copies of guanine-tracts were found to generate G-quadruplex structure (G4), a most prevalent non-B type DNA structures on the genome. I will discuss how G4 could be regulated in cells and how it would contribute to both positive and negative regulation of DNA replication as well as other chromosome dynamics.

Biography

Hisao Masai is currently a director of Tokyo Metropolitan Institute of Medical Science. He received Ph.D from University of Tokyo in 1986. He was at DNAX Research Institute from 1981-1989 as a graduate student and as a postdoc. He became an assistant professor in 1990 and associate professor in 1995 at Institute of Medical Science, University of Tokyo, and moved to Rinshoken (the former Tokyo Metropolitan Institute of Medical Science) in 2000. He has been studying on mechanisms of DNA replication for the past 37 years using *E. coli*, yeasts and mammals as models.

Keywords: R1 plasmid, primer RNA synthesis, PriA, Cdc7, replication timing, Rif1, G-quadruplex

1P5

Replication Origin where the First Okazaki Fragment Initiates

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Abstract

Discovery of discontinuous DNA replication mechanism opened the door for study of the mysterious and exciting mechanisms of DNA replication. Okazaki fragments are generated all over the genome, and the first one is synthesized at the very specific region, DNA replication origin. It is interesting to know what is the requirement for a replication origin. Although the essential structure of eukaryotic replication origin was first identified in budding yeast, structures are diverse among organisms and the conserved rule remains elusive. We have studied structures of replication origins in fission yeast, which is diverse from budding yeast. We found that asymmetrically arrayed adenines/thymines are essential for replication origins in fission yeast. The adenines/thymines arrays provide the origin recognition complex (ORC) binding site and nucleosome-depleted region, both might be common requirements for eukaryotic replication origins. Genome-wide identification of replication origins in fission yeast demonstrated each origin is activated at specific time in S phase. Especially, the regions near the telomeres contain clusters of late origins and replicate very late. Activation of pre-RC is suppressed in early S phase at these origins by the telomere-binding protein Taz1/TRF1 dependent mechanisms. In addition, late origins in the internal chromosome regions that are associated with two-copies of telomere-sequence are also regulated by Taz1. Interestingly, the internal late origins are localized very close to the telomeres at the nuclear periphery, specifically in G1/S phase when replication timing is determined. Telomere-binding proteins play roles in specific localization and replication timing control. Our results suggest importance of the intra-nuclear chromatin localization and telomere-binding proteins for regulation of DNA replication.

Biography

I obtained a PhD degree from Osaka University in 1980 and then engaged in study of initiation of ColE1 DNA replication in Jun-ichi Tomizawa Lab at NIH. I joined to Tsuneko Okazaki Lab in 1987 and studied structures of replication origins in human cells and fission yeast until I moved to Osaka University in 1995. At Osaka University I continued the study of replication origins and regulation of replication initiation in fission yeast *Schizosaccharomyces pombe* until I retired March 2018.

Keywords: *replication origin, replication timing, telomere, intra-nuclear localization, Taz1*

1P6

Development of Human Artificial Chromosomes and Chromatin Manipulation Technologies

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Abstract

34 years ago when I started my study in Prof. Tuneko Okazaki's lab as a graduate student, the laboratory had just started to move from bacterial DNA replication to mammalian system. In eukaryotic cells, study of DNA replication is desirable to be coupled with centromere activity for maintaining an input DNA molecule stably through cell divisions. So we studied human centromere in Okazaki's lab. 13 years later, these efforts produced the first human artificial chromosome (HAC) from cloned centromeric repetitive DNA.

Since then, I have been focusing my study on the molecular mechanisms by which centromere chromatin, the chromatin landscape at centromeres on the repetitive DNA, impacts on the establishment and maintenance of proper centromere/kinetochore function for the chromosome segregation process. We are interested in the histone modifications that define centromere chromatin including a key epigenetic centromere mark CENP-A (a histone H3 variant), how these marks are dynamically regulated to influence centromere formation, maintenance or inactivation by heterochromatin formation, and what enzymes regulate them. Then, we developed a powerful system for such studies through the use of both HACs and ectopic integration sites based on engineered synthetic alpha-satellite (alphoid) repetitive DNAs with a tet operator (alphoid^{tetO}). We have shown that these systems can mimic endogenous chromosomes in kinetochore structure and mitotic behavior. We have assembled de novo centromeres on a synthetic alphoid^{tetO} array, and shown that these exhibit mitotic stability comparable to native chromosomes in cultured human cells. Moreover, the ability to target tet repressor (tetR) fusion chimeras to the alphoid^{tetO} array permits specific manipulation of chromatin within a single defined functional centromere. We have successfully used the system to manipulate the chromatin environment underlying its functional kinetochore by the specific targeting of variety of tetR fusion proteins.

Biography

Education: 1989 Ph.D in Department of Molecular Biology, Graduate School of Science, Nagoya University, 1986 Master of Science in Department of Molecular Biology, Graduate School of Science, Nagoya University**. Research and Appointments:** 2009 Head of the Laboratory, Laboratory of Chromosome Engineering, Department of Frontier Research and Development, Kazusa DNA Research Institute, 2006 Associate Professor, Division of Biological Science, Graduate School Science, Nagoya, University, 2003 Senior Research Fellow, NCI NIH, USA, 1995 Lecturer, Division of Biological Science, Graduate School of Science, Nagoya University, 1990 Assistant Professor, Department of Molecular Biology, School of Science, Nagoya University,

Keywords: Human Artificial Chromosome (HAC), CENP-A, CENP-B, Centromere, Heterochromatin

1P7

From Okazaki Fragments to Patient-derived Tumor Model

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The notion of discontinuous DNA replication is based on the discovery of Okazaki fragments. Therefore, Drs. Reiji Okazaki and Tuneko Okazaki spent a lot of time isolating and characterizing these short DNA chains obtained by pulse labeling and sucrose gradient centrifugation. I was fortunate to experience a part of this effort when I carried out my graduate study in the Okazaki laboratory in 1970's. The first experiment I carried out was to purify T4 DNA polymerase. The 3' to 5' exonuclease activity of this and other enzymes was used to determine the direction of DNA chain growth. In the first part of my talk, I would like to discuss how the study of enzymes has contributed to the investigation of Okazaki fragments. I will then describe my journey from DNA replication to the study of oncogenes, signal transduction and anticancer drugs. My scientific journey coincided with the development of Molecular Biology that has become more and more translational. I have pursued cancer therapy as a translational aspect of my research and have developed anticancer drugs targeting the Ras signal transduction. After spending 40 years in the USA, I have recently established a lab at Kyoto University. One of the topics we are pursuing concerns patient-derived chicken egg tumor model. This is a convenient and versatile animal model that shows promise in Personalized Medicine for cancer. In this method, we purchase fertilized eggs and carry out experiments inside the egg. At day-10, we make a small window on the egg shell and transplant tumor biopsy from cancer patients. Surprisingly, we observe formation of a tumor inside the egg after 3 to 4 days of incubation. We are exploiting this model to identify anticancer drug that is tailored towards an individual patient. We hope to achieve tailor-made treatment for each individual cancer patient.

Dr. Tamanoi received PhD in Molecular Biology from Nagoya University in 1977 and has carried out postdoctoral research at Harvard Medical School. He worked as a senior investigator at Cold Spring Harbor Laboratory from 1980-1985, became Assistant Professor and then Associate Professor at the University of Chicago (1985-1993), and Full Professor at UCLA) in 1997. In April of 2017, he received joint appointment at the Institute for Integrated Cell-Material Sciences, Institute for Advanced Study, Kyoto University, Japan. Dr. Tamanoi served as Director of Signal Transduction and Therapeutics program at Jonsson Comprehensive Cancer Center and as Research Director of California NanoSystems Institute.

Keywords: *Okazaki fragments, Molecular Biology, Cancer Therapy, Chicken Egg Tumor Model*

1P8

Discontinuous Mechanism of DNA replication. -How it was discovered.

Tsuneko Okazaki
Nagoya University

Abstract

My late husband, Reiji Okazaki and I were fellow of Arthur Kornberg's laboratory at Stanford University from late 1960 to early 1963 to study the enzymatic mechanisms of DNA synthesis. At that time, DNA polymerase (now called DNA polymerase 1) was highly purified from *E.coli* and extensive analysis of properties was going on. What was revealed was rather unexpected: The DNA polymerase 1 could not use intact double stranded DNA as template-primer, while good template-primers were 3'OH-ends of nicked or gapped DNA. Moreover, in accordance with the synthesis, 5'ends of the nick or gap on the template DNA were degraded so that no net increase of DNA was observed. Within cells, DNA believed to exist long double helical molecules without nick or gap, it was difficult to imagine how they can be replicated by DNA polymerase I. Moreover, DNA polymerase synthesized chains only from 5'-to-3' direction, it was questioned how the other 3'-to-5' elongating strand (lagging strand) could be synthesized simultaneously. In Stanford University, I engaged to purify DNA polymerase from *Bacillus subtilis* and it had similar property to that of *E. coli* did. When Reiji and I returned to the Chemistry Laboratory, Faculty of Science, Nagoya University, 1963 we decided to analyze *in vivo* mechanism of DNA-chain growth, in the same molecular accuracy as *in vitro* analyses with purified DNA polymerase.

Several important works on the molecular manner of *E. coli* chromosome replication were reported then: they all showed that both strands of DNA were replicated sequentially and almost simultaneously from the replication origin. How could it be possible if enzyme only replicates 5'- to 3'-direction!

To solve this question, we decided to mark the growing ends *in vivo* by very short administration of ^{3}H-thymidine **(pulse labeling)** and then analyze the ends by degrading with the direction specific exonucleases; *E.coli* exonuclease 1 (degrades 3'-to-5') and *B. subtilis* exonuclease (degrades 5'-to-3'). First, we prepared model substrates and degradation patterns by these exonucleases were analyzed. We then pulse labeled *E. coli* DNA and labeled molecules were analyzed. We found that the nascent molecules were around 1000 nucleotides in length. These nascent molecules were nicknamed **OKAZAKI fragments** since the summary talk by Rollin Hotchkiss at 1968 Cold Spring Harbor Symposium where our work was presented. Subsequent studies (1977-1983) uncovered multistep reactions in **Discontinuous DNA Replication;** short RNA primer synthesis, primer-dependent Okazaki fragment synthesis, primer removal and gap-filling between Okazaki fragments by RNase H and DNA polymerase I, and long lagging strand formation by joining with DNA ligase between Okazaki fragments. Unfortunately, Reiji Okazaki passed away 1975 without knowing either structure of primer RNA or the full process of discontinuous DNA replication.

2A1

Collision with Duplex DNA Renders *Escherichia coli* DNA Polymerase III Holoenzyme Susceptible to DNA Polymerase IV-mediated Polymerase Switching on the Sliding Clamp

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Abstract

Organisms possess multiple DNA polymerases (Pols) and use each for a di erent purpose. One of the five Pols in *Escherichia coli*, DNA polymerase IV (Pol IV), encoded by the *dinB* gene, is known to participate in lesion bypass at certain DNA adducts. To understand how cells choose Pols when the replication fork encounters an obstacle on template DNA, the process of polymerase exchange from the primary replicative enzyme DNA polymerase III (Pol III) to Pol IV was studied *in vitro*. Replicating Pol III forming a tight holoenzyme (Pol III HE) with the sliding clamp was challenged by Pol IV on a primed ssDNA template carrying a short inverted repeat. A rapid and lesion-independent switch from Pol III to Pol IV occurred when Pol III HE encountered a hairpin stem duplex, implying that the loss of Pol III-ssDNA contact induces switching to Pol IV. Supporting this idea, mutant Pol III with an increased affinity for ssDNA was more resistant to Pol IV than wild-type Pol III was. We observed that an exchange between Pol III and Pol IV also occurred when Pol III HE collided with primer/template duplex. Our data suggest that Pol III-ssDNA interaction may modulate the susceptibility of Pol III HE to Pol IV-mediated polymerase exchange.

Biography

I learned molecular genetics at Prof. Mutsuo Sekiguchi's lab at Kyushu University. After getting Ph.D there, I worked for Prof. Arthur Kornberg as a Post-Doc from 1983 to 1987. Since then, I have been working on fidelity of DNA replication and spontaneous mutagenesis with genetical and biochemical approaches. In 1999, my research group showed DNA replication errors produced *in vivo* and *in vitro* by replicative apparatus of *Escherichia coli*. Knowing that almost all of the replication errors are corrected by mismatch repair, I shifted my focus on oxidative DNA damage as a major cause of spontaneous mutations.

Keywords: *Replicative DNA polymerase, translesion DNA polymerase, hairpin structure*

2A2

Roles of PCNA and clamp loaders for leading and lagging DNA synthesis

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Abstract

Three replicative eukaryotic DNA polymerases, α, δ, and ε play their individual roles in the replication fork. PCNA clamp and its loader RFC, which have been identified as essential replication factors in eukaryotes, are involved in coordinated synthesis of the leading and lagging strands. Upon synthesis of primers by Polα, RFC binds the 3′ end, loads PCNA there through its ATP hydrolysis, and switches to Polδ through their dynamic interactions. As RFC loads PCNA to 3′ ends in a distributive manner, Polδ is recruited at every primer end and converts it to a lagging strand preferentially.

Polε has been shown to be the major leading-strand DNA polymerase. Reconstitution studies of the replisome demonstrated that Polε and CMG DNA helicase proceed together on the template strand in a 3′ to 5′ direction as the CMGE complex to synthesize the leading strand. However, several additional factors are required to achieve a fully active replisome. The second PCNA loader CTF18-RFC consists of RFC2-5 and its specific subunits CTF18, DCC1, and CTF8. This complex is involved in replication-fork progression, and directly interacts with Polε via its specific subunits. PCNA-loading activity of CTF18-RFC is intrinsically weak but it becomes active by association with Polε. Once CTF18-RFC–Polε loads PCNA, it forms a novel complex including PCNA and can synthesizes DNA processively. Thus, CTF18-RFC is a processive PCNA loader in accordance with the movement of Polε and contributes the processive DNA synthesis. These functionally distinguishable PCNA loadings by two different PCNA loaders will be crucial for proper division of labor for DNA polymerases in the eukaryotic replication fork.

Biography

Toshiki Tsurimoto is a Professor in the Faculty of Science at Kyushu University since 2003. He was born in 1954 in Himeji, a city with a famous castle in Japan, and completed his undergraduate and Ph.D. studies at Osaka University. After that, he joined B. Stillman's lab for three years as a postdoc and started researches on eukaryotic DNA replication. His interests lie in the mechanism of progression of the replication fork in higher eukaryotes using biochemical approaches. He enjoys driving in mountain areas in Kyushu island and visiting spas there.

Keywords: *Keywords [DNA polymerase, RFC, CTF18-RFC]*

2A3

Nascent DNA Drives Inflammation under Replication Stress Conditions

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Abstract

A large body of evidence indicates that nascent DNA can be extensively processed and remodeled in response to replication stress. This processing contributes to detect, signal and repair stalled replication forks. Remarkably, recent evidence also indicate that nascent DNA can also be displaced from replication sites and can accumulate in the cytosol, where it is detected as non-self by the innate immune system. Central to this process is SAMHD1, a human protein previously characterized as a dNTPase that protects cells from viral infections. Mutations in SAMHD1 are implicated in cancer development and in a severe congenital inflammatory disease called Aicardi-Goutières syndrome. The mechanism by which SAMHD1 protects against cancer and chronic inflammation is unknown. Here, we show that SAMHD1 promotes degradation of nascent DNA at stalled replication forks by stimulating the exonuclease activity of MRE11. This function activates the ATR–CHK1 checkpoint and promotes fork restart. In SAMHD1-depleted cells, single-stranded DNA fragments are released from stalled forks and accumulate in the cytosol where they activate the cGAS–STING pathway to induce expression of pro-inflammatory type I interferons. SAMHD1 is thus an important player in the replication stress response, which prevents chronic inflammation by limiting the release of ssDNA from stalled replication forks.

Biography

Philippe Pasero received his Ph.D. in Cell Biology from the University of Aix-Marseille in 1993. He was a postdoctoral fellow at ISREC (Lausanne, Switzerland) with Susan Gasser, where he studied DNA replication in budding yeast. He joined the lab of Etienne Schwob at IGMM (Montpellier, France) in 1998, where he pioneered the use of DNA combing to monitor yeast DNA replication. He started his research group at the Institute of Human Genetics (Montpellier, France) in 2003. Research in the Pasero laboratory focuses on the cellular responses to replication stress in yeast and human cells, using single molecules and NGS-based assays.

Keywords: *DNA replication, checkpoints, replication stress, inflammation, cancer*

2A4

Replisome Structure and Its Functions in Archaea

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Abstract

Since primer synthesis and elongation are repeated many times in the lagging strand synthesis, a sophisticated mechanism should work to coordinate helicase, primase and polymerase for cooperative working in the replication fork progression. In Eukarya, primase forms a stable complex with DNA polymerase α, and the Polα-pri complex binds to helicase via Polα to synthesize short RNA by primase, followed by short DNA by Polα. This primer is taken over by Polδ to extend more to synthesize the nascent DNA strand. In the hyperthermophilic archaeon, *Thermococcus kodakarensis*, MCM-GINS-GAN helicase complex, PriS-PriL primase complex, and DP1-DP2 DNA polymerase D (PolD) complex are considered to be working together. We found the strong interaction between PolD and Primase, and also PolD and GINS, respectively. In addition to a central nexus of connecting helicase and primase, the archaeal PolD may play dual roles corresponding to the functions for eukaryotic Pol α and Pol δ in the replisome, to link the primase to the replicase and extending synthesized RNA primers. Both DNA polymerase and primase functionally worked under conditions, where they can form a complex. We will show the functional complex formation of primase, PolD, and CMG-like helicase in the replisome in *T. kodakarensis*

Biography

Yoshizumi Ishino graduated from the faculty of Pharmaceutical Science, Osaka University. He studied on DNA ligase and the *lig* gene from *E. coli* at Research Institute for Microbial Diseases, Osaka University, and earned Ph. D (1986). He found a unique repeated sequence, which is now recognized as CRISPR, in *E. coli* in his first post doc research in Osaka. He stayed at Yale University as a postdoctoral fellow (1987-1989), and came back to Takara Shuzo, Japan as a senior scientist in Biotechnology Research Laboratories. In 1996, he moved to Biomolecular Engineering Research Institute, a national project funded by the Government (METI) and 18 companies, and managed the nucleic acids-related enzymes group. In 2002, he was appointed a full professor in Kyushu University.

Keywords: *Keywords*: *Replisome, PolD, primase, Archaea, hyperthermophile*

2A5

Cryo-EM Suggests a Coupled-Sister-Replisome Model at the Core of the Replication Factory

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Abstract

We have been studying by cryo-EM the yeast replisome architecture over the past several years. Our earlier work determined the Cdc45–Mcm2-7–GINS (CMG) helicase in the apo form. Recently, we installed a double block on the double strand section of a forked DNA, and captured in vitro an active CMG helicase stalled at the fork, in the presence of ATP. Cryo-EM showed that the helicase advances on the fork with its Mcm2-7 N-tier ring in front contacting the fork, and the C-tier AAA+ motor ring pushing from behind. This N-tier–ahead–of–C-tier orientation of helicase is novel, has important implication for origin activation, and predicts that the DNA polymerase α-primase rides ahead of the helicase while the leading strand polymerase ε follows from behind.

Most recently, we studied how the trimeric Ctf4 scaffolds a replisome. We found Ctf4 is able to stably bind more than one copy of the helicase. This observation has led us to suggest that the sister replisomes arising from the same origin may be physically linked together by Ctf4. I will discuss the potential implication of such replication factory model.

The work described here is a close collaboration with Dr. Michael O'Donnell at the Rockefeller University. Roxanna Georgescu at the O'Donnell Lab, and Zuanning Yuan, Ruda Santos, and Lin Bai at the Li lab contributed to the work.

Biography

After earning his PhD in China in 1994, Huilin Li moved to the US for a postdoc training in cryo-EM structural biology at Lawrence Berkeley National Lab. He was a group leader in Brookhaven National Lab Biology Department from 2002 – 2009, a full professor at Stony Brook University Biochemistry Deaptment from 2010 – 2016. He moved to Van Andel Research Institute in 2016. He is now a Professor at the Center for Cancer Biology, Program lead of the Structural Biology Program. His lab pursues structural mechanism of eukaryotic DNA replication.

Keywords: *DNA replication, Replisome architecture, structural biology, cryo-EM.*

2P1

Mechanisms and Regulations in the Initiator DnaA and the Initiation Complex

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Abstract

Replication initiation of the chromosome is regulated to occur only once per the origin during the cell cycle. In *E. coli*, the initiation complex includes ATP-DnaA oligomers, a DNA-bending protein IHF and the origin *oriC*. Recently, based on structural and functional analyses of these factors we constructed a 3D structure model of the initiation complex and suggested mechanisms in unwinding of *oriC* DNA and loading of DnaB helicases on the single-stranded DNA regions. Also, we found that after replication initiation, ATP-DnaA is inactivated by enhanced ATP hydrolysis, resulting in production of the inactive ADP-DnaA and repression of extra initiations of replication. A major system for this regulation depends on a complex of the DNA-bound clamp and Hda protein. After completion of the Okazaki fragments, the clamp subunit of DNA replicase remains bound to DNA, which supports timely activation of this system. In addition, Ogawa and Okazaki found that the chromosomal *datA* locus bearing a cluster of DnaA-binding sites (DnaA boxes) and an IHF-binding site is required for repressing extra initiations of replication. We found that the IHF-*datA* complexes also promote the ATP hydrolysis of ATP-DnaA. In contrast, the chromosomal loci termed *DARS1* and *DARS2* both bearing specific clusters of DnaA boxes were found to promote re-activation of DnaA by exchange of DnaA-bound ADP to ATP. These independent systems concordantly form the DnaA cycle which promotes timely changes of the nucleotide forms of DnaA for regulation of replication initiation.

Biography

1985, B.Sc. from Nagoya Univ.. 1990, Ph.D. from Kyoto Univ.. 1990-1994, Post-doctoral fellow at Stanford Univ. (HFSP long-term fellow) and Georgetown Univ., 1994-2002 Assistant/Associate professor at Kyushu Univ.. 2002-present, Professor at Kyushu Univ.. Kihara Prize (2017) from the Japan Society of Genetics. Major publications: Cell (1998), EMBO J (1999, 2001) by revealing the clamp-dependent DnaA regulation, Genes Dev. (2007) by revealing a novel DnaA-stimulating protein, Genes Dev. (2009) by revealing DARS, Nat Rev Microbiol (2010) by summarizing the DnaA cycle. PNAS (2013) by revealing *datA*-dependent DnaA-ATP hydrolysis, PNAS (2016) and NAR (2017) by revealing the structure and mechanism of the initiation complex.

Keywords: *oriC*, DnaA, IHF, the clamp, Hda, *datA*, *DARS*

2P2

A Novel Role of DNA Polymerase ε at Replication Forks; Its Involvement in Replication Fork Pausing at the Barriers

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Abstract

DNA replication machineries assemble at replication origins and travel on chromosomes to synthesize DNA. During DNA synthesis, they often face obstacles, such as unusual secondary and tertiary structures of DNA, damaged DNA, and proteins bound to DNA. Cells manage the overriding of these obstacles by the machineries and maintain faithful replication throughout chromosomes. It has been known that replication forks stall or pause at the obstacles and their proper pausing of replication forks is important for genome integrity although the detailed mechanism underlying the process has not been well elucidated. Here, we successfully reconstituted fork-pausing reactions from purified yeast proteins on templates that have binding sites for the LacI, LexA, and/or Fob1 proteins; the forks paused specifically at the protein-bound sites. Moreover, although the replicative helicase Cdc45-Mcm2-7-GINS (CMG) complex alone unwound the protein-bound templates, the unwinding of the LacI-bound site was impeded by the presence of a main leading-strand DNA polymerase, Polε. This result suggests that Polε modulates CMG to pause at these sites and further implies that the replication machinery senses obstacles and pauses its progression.

Biography

Dr. Hiroyuki Araki started his career with the molecular analysis of genetic recombination in bacteriophage T7 as a PhD student and then moved to molecular genetics of budding yeast. In the field of DNA replication, he first contributed to isolation and analyses of the genes encoding subunits of DNA polymerase ε (Polε). His group isolated the *DPB11* and *SLD* genes as those genetically related to Polε and later showed their functions at the initiation step of DNA replication, which is regulated by CDK activity. Dr. Araki's group has continued to study on several aspects of chromosome replication using budding yeast.

Keywords: *Polε, replication fork pausing, CMG helicase*

2P3

Replication Fork Arrest Induces Gene Amplification and Cellular Senescence

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Abstract

Over the course of evolution, the size of the cell has been increasing and cellular functions have become more complicated. These changes have meant that there is an increasing demand for ribosomes in the cell. As a result, eukaryotic cells have huge tandem clusters of ribosomal RNA genes (rDNA) in their genome. The problem arises in maintaining these genes. Because of the highly repetitive structure and busy transcription, the rDNA became one of the most unstable regions in the genome and it is losing the copies by intra-chromosomal recombination. Then the cells developed a unique gene amplification system to recover the copies. Therefore, the rDNA is repeating contraction and expansion of the cluster. This unstable feature affects cellular functions, such as senescence.

In my talk, I will show the interesting relationship between rDNA instability and replicative lifespan in yeast.

Biography

In 1992, Takehiko Kobayashi obtained a Ph.D. from the Kyushu University under the supervision of Prof. T. Horiuchi. After working three years as a postdoc with Dr. Melvine DePamphilis at Roche Institute of Molecular Biology in New Jersey and National Institute of Health in Maryland, USA, He returned to Japan and promoted to associate professor in National Institute for Basic Biology, Okazaki, Japan. In 2006, he became a full professor in National Institute of Genetics, Mishima, Japan. In 2015, he moved to Institute of Molecular and Cellular Biosciences (present, Institute for Quantitative Biosciences, IQB), The University of Tokyo. His research interest is the relationship between genome stability, cellular senescence and rejuvenation.

Keywords: *ribosomal RNA gene, genome stability, cellular senescence, gene amplification*

2P4

Transcriptional Regulation by Cohesin loader

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Abstract

Heterozygous mutations of **NIPBL** were found in 60% of the cases of Cornelia de Lange Syndrome (**CdLS**), a human developmental syndrome with a mental and physical growth disorder. The phenotype is believed to be caused by dysregulation of gene expression during development, however little is known about molecular function of Nipbl in transcriptional regulation. Nipbl is known to function as a cohesin loader together with Mau2 to load cohesin on chromosome. To understand cohesin loader's function in transcription, we adopted in vitro transcription assay using synthetic DNA template that consists of 4XGAL4 as an enhancer, adenovirus late promoter, and luciferase gene. In the HeLa cell nuclear extracts (NE), Pre-Initiation Complex (PIC) including RNA PolII, Mediators and GTFs were assembled on this synthetic template as soon as GAL4-VP16 activator protein was added. The role of cohesin and its loader in regulation of transcription will be discussed.

Biography

In 1993, Katsuhiko Shirahige obtained a Ph.D. from the Osaka University under the supervision of Prof. H. Yoshikawa. In 2004 he was promoted to associate professor in Tokyo Institute of Technology and in 2008, he became a full professor. In 2010, he moved to Institute of Molecular and Cellular Biosciences (present, Institute for Quantitative Biosciences, IQB), The University of Tokyo. His current research interest is to understand the regulation and dynamics of human holo enhanceosome.

Keywords: *Chromosome Structure, Chromosome Function, Cohesin, Transcription, Chromatin loop*

2P5

Revealing a non-canonical DNA replication important for genome maintenance in human cells

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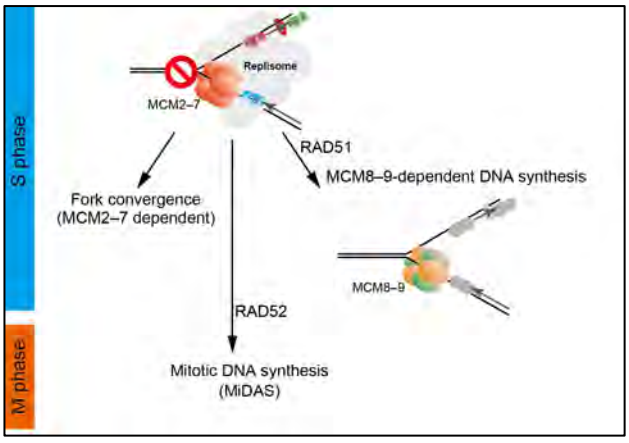
Abstract

Loss of chromosome integrity is related to genetic disorders and cancer formation. Replication fork progression can be disrupted at difficult-to-replicate loci, which has the potential to challenge chromosome integrity. This replication fork disruption can lead to the dissociation of the replisome and the formation of DNA damage. To model the events stemming from replisome dissociation during DNA replication perturbation, we utilized a degron-based system for inducible proteolysis of a subunit of the replicative MCM2-7 helicase. We show that MCM2-depleted cells generate DNA double-strand breaks and activate a DNA damage response pathway. Remarkably, these cells maintain some DNA synthesis without MCM2, and this requires the MCM8-9 complex, a paralog of the MCM2-7 helicase. We show that MCM8-9 functions in a homologous recombination (HR)-based pathway downstream of RAD51. This RAD51/MCM8-9 axis is distinct from a RAD52-dependent DNA synthesis pathway called MiDAS (mitotic DNA synthesis) that operates in mitosis at difficult-to-replicate loci. We propose that stalled replication forks can be restarted in S phase via HR utilizing MCM8-9 as an alternative replicative helicase.



Biography

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Keywords: *DNA replication, genome instability, fork stalling, auxin-inducible degron, genetic engineering*

Poster Presentations

P-1 MCMBP is Important for the Maintenance of MCM Homeostasis

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*P-2 Age-dependent Ribosomal DNA variation in mammalian cells

Eriko Watada, Yufuko Akamatsu, Takehiko Kobayashi

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*P-3 Regulation of replication fork progression in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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*P-4 Genome-wide Profiles of RNA-DNA Hybrid and G-quadruplex in *E. coli* and Its Implication in An Alternative Mode of Chromosome Replication

Tomoko Sagi, Yuimka Seki, Taku Tanaka, Hisao Masai

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P-5 Genetically Encoded Tool for Visualization of Sister Chromatid Fusion Revealed Micronuclei Formation as an Initial Event

Katsushi Kagaya[1, 2], Sanki Tashiro[3], Naoto Noma[3], Hiroyuki Irie[3], Io Yamamoto[3], Diana Zamora Romero[3], Mari Tsuboi[3], Fuyuki Ishikawa[3] and Makoto T Hayashi

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P-6 Dynamic property of the lagging strand DNA synthesis by human DNA polymerase δ and regulation of damage tolerance pathways

Yuji Masuda , Rie Kanao and Chikahide Masutani

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*P-7 Elucidation of Function of an Interaction between Origin Recognition Complex and Mitochondrial Seryl-tRNA Synthetase in Human Cells

Arakawa Miho, Shin-Ya Isobe, Koji Nagao, and Chikashi Obuse

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P-8 The Elg1-Rfc2–5 complex (Elg1-RFC) is the major PCNA-unloading complex in *Xenopus* egg extracts

Yoshitaka Kawasoe, Sakiko Shimokawa, Toshiki Tsurimoto and Tatsuro Takahashi

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P-9 *cis*-determinants of Fission Yeast DNA Replication Origins Revealed by *in silico* Modeling

Koji Masuda, Claire Renard, Katsuhiko Shirahige and Takashi Sutani

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*P-10 DNA Replication-coupled Cohesin Acetylation Revealed by Quantitative ChIP-seq

Mai Ishibashi, Masashige Bando, Takashi Sutani, Katsuhiko Shirahige

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*P-11 Roles of Cdc7 kinase complex in brain development

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*P-12 Chromatin recruitment of Lig1 is regulated in Uhrf1-dependent manner

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P-1

MCMBP is Important for the Maintenance of MCM Homeostasis

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MCMBP (MCM-binding protein) is an essential interaction partner of the MCM proteins. MCMBP is conserved in most eukaryotes, except for *S. cerevisiae* and *C. elegans*, and has a sequence similarity to MCMs. Past cross-organism studies suggested that MCMBP functions in replication-related processes, such as sister-chromatid cohesion, pre-RC formation and replication termination. However, MCMBP function in human cells remains unclear yet. In our study, we employed auxin-inducible (AID) technology to generate conditional HCT116 cells in which MCMBP can be rapidly depleted by the addition of auxin. We confirmed MCMBP localization to the nucleus and its acute degradation caused mild growth defect. Interestingly, the protein level of the MCM2-7 was significantly reduced when MCMBP was depleted for a long period of time, without any changes in the mRNA level of these genes. Furthermore, MCMBP-depleted cells showed an increased population of late-S phase, similar to the phenotype of siMCM2/5-treated cells. Finally, we found that MCMBP did not affect pre-RC formation and MCM removal from chromatin. We propose that MCMBP is important for the maintenance of MCM homeostasis at the protein level.

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*P-2

Age-dependent Ribosomal DNA variation in mammalian cells

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The ribosomal RNA gene (rDNA), which consists of tandem repetitive arrays, is one of the most unstable regions in the genome. However, it is not clear how the region changes with age in mammalian cells. Here we show that the rate of methylation of rDNA increases in bone marrow cells of 2-year-old mice to compare with 4-week-old mice in two strains, BALB/cA and C57BL/6. In addition, in the BALB/cA cells, the copy number of rDNA increases and the amount of pre-rRNA transcripts is reduced with age. While, the ratio of 28S rRNA to the other mRNA of housekeeping genes doesn't change so much in young and old cells. We discuss about the reason for the age-dependent variation of rDNA in bone marrow cells.

*P-3

Regulation of replication fork progression in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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We are interested in molecular mechanism of replication fork progression in the archaeal cells. One of the components of eukaryotic replicative helicase is Cdc45, which has sequence similarity to bacterial RecJ. Archaea has eukaryotic-like MCM and GINS. *Thermococcus kodakarensis* has two Cdc45/RecJ-like proteins. We reported that the one, named GAN, associates with GINS and formed ternary complex with MCM and GINS, although GAN does not show the promoting effect on the helicase activity of the MG complex. We show here that DNA polymerase D, present only in Archaea, stimulates helicase and ATPase activities of CMG by interacting via GINS. On the other hand, we showed that HAN interacts with Hef, the ortholog of human FANCM, and its 3′-5′ exonuclease activity is stimulated. We propose that HAN is involved in the stalled fork repair, and may be related to switch between helicase and endonuclease of Hef depending on the stalled fork structure. We will summarize the possible roles of GAN and HAN in the archaeal replication fork progression.

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*P-4

Genome-wide Profiles of RNA-DNA Hybrid and G-quadruplex in *E. coli* and Its Implication in An Alternative Mode of Chromosome Replication

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Chromosomal replication of *Escherichia coli* is usually initiated at the origin of replication (*oriC*), which is present at a unique site on the genome, in a manner dependent on the replication initiation factor, DnaA. However, a second alternative replication system (stable DNA replication: SDR) is known in *E. coli* that does not depend on the *oriC*-DnaA replication system. This mechanism constitutively operates in a mutant of RNaseH, which degrades RNA on RNA-DNA hybrids. In this replication system, it is estimated that replication starts from multiple sites on the genome, but the details have not been clear yet. In this study, we try to clarify the genome-wide profiles of RNA-DNA hybrids and G-quadruplexes that are formed on RNA-DNA hybrids, and elucidate their roles in replication control. We will discuss the roles of recombination factor (RecA) and RNA-DNA hybrid in the alternative mode of DNA replication.

P-5

Genetically Encoded Tool for Visualization of Sister Chromatid Fusion Revealed Micronuclei Formation as an Initial Event

Katsushi Kagaya[1, 2], Sanki Tashiro[3], Naoto Noma[3], Hiroyuki Irie[3], Io Yamamoto[3], Diana Zamora Romero[3], Mari Tsuboi[3], Fuyuki Ishikawa[3] and Makoto T Hayashi[2, 3]

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Chromosome fusion is highly deleterious among oncogenic chromosome rearrangements. Although artificial induction of chromosome fusions has been shown to cause multiple tumor-driving abnormalities, conventional methodologies failed to regulate the timing, the number and the type of chromosome fusion in a given cell. Here, we have developed a human cell-based sister chromatid fusion visualization (SFV) system, by which a single sister chromatid fusion can be artificially induced by CRISPR/Cas9 and traced by mCitrine expression. Live cell imaging of SFV and data analysis by generalized linear modeling revealed that a single sister chromatid fusion causes micronuclei formation.

P-6

Dynamic property of the lagging strand DNA synthesis by human DNA polymerase δ and regulation of damage tolerance pathways

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DNA-damage tolerance protects cells via at least two sub-pathways regulated by PCNA ubiquitination in eukaryotes: translesion DNA synthesis (TLS) and template switching (TS), which are stimulated by mono- and K63-linked polyubiquitination, respectively, by RAD6-$(RAD18)_2$ and with additional enzymes, such as MMS2-UBC13 and HLTF in humans. In a reconstituted system of the lagging strand DNA synthesis with pol δ, we demonstrate that switching reactions between pol δ and pol η are stimulated by replication-coupled mono-ubiquitination of PCNA on K164, depending on UBZ of pol η. We also demonstrate that HLTF has a potential to access to the stalled primer ends of the lagging strand DNA synthesis with pol δ in a HIRAN domain-dependent manner. In the HLTF-RFC-PCNA complex at the primer ends, when PCNA is mono-ubiquitinated by RAD6-$(RAD18)_2$, the resulting ubiquitin moiety is immediately polyubiquitinated by coexisting HLTF, indicating a coupling reaction between mono- and polyubiquitination. Therefore, replication-coupled recruitment of HLTF could lead TS. We also demonstrate a channeling reaction from TLS to TS, when TLS failed after mono-ubiquitination of PCNA.

*P-7

Elucidation of Function of an Interaction between Origin Recognition Complex and Mitochondrial Seryl-tRNA Synthetase in Human Cells

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ORC (Origin Recognition Complex) recognizes and binds to the replication origin on the chromosome in eukaryotic cells and have an activity for loading of MCM helicase. LRWD1 was identified as an ORC binding protein in human cell and conserved among higher eukaryote. To investigate functions in LRWD1, we performed proteomic analysis and identified SARS2 (Serine Aminoacyl tRNA Synthetase, mitochondrial), in addition to G9a complex, a histone H3K9 methyl transferase. SARS2 is involved in serine tRNA synthesis in mitochondria. SARS2 directly interacts with LRWD1 and has ability to form complex with ORC. Interestingly, depletion of SARS2 reduced the population of S phase cells, suggesting that the SARS2-LRWD1-ORC interaction is important for controlling of initiation of DNA replication. In addition to supplying cellular energy, mitochondria are involved in other tasks, such as cell death, signaling, and control of the cell cycle. The SARS2-LRWD1-ORC interaction may be implicated in relationship between mitochondrial functions and DNA replication on genome.

P-8

The Elg1-Rfc2–5 complex (Elg1-RFC) is the major PCNA-unloading complex in *Xenopus* egg extracts

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The replication clamp PCNA is a central player in various reactions coupled with DNA replication. The loading of PCNA onto DNA is regulated by three clamp-loader/unloader complexes, RFC, Ctf18-RFC, and Elg1-RFC. RFC is the major PCNA-loader complex during lagging-strand DNA synthesis and DNA repair reactions, and Ctf18-RFC is suggested to load PCNA onto the leading strand by forming a complex with DNA polymerase ε. Recent studies also revealed that Elg1-RFC functions as a PCNA unloader. However, Elg1 is not essential for cell viability, and RFC and Ctf18-RFC exhibit the PCNA-unloading activity *in vitro*, raising a question as to whether Elg1-RFC is the only PCNA unloader *in viv*o.

In this study, we examined the dynamics of PCNA unloading in *Xenopus* egg extracts and a reconstitution system. The PCNA-unloading reaction was attenuated by depletion of Elg1 but not by that of Rfc1 or Ctf18, suggesting that Elg1-RFC is the major PCNA unloader in *Xenopus* egg extracts. In contrast, Elg1-RFC did not unload PCNA in a reconstituted reaction. A possible regulatory mechanism of PCNA unloading by Elg1-RFC will be discussed.

P-9

cis-determinants of Fission Yeast DNA Replication Origins Revealed by *in silico* Modeling

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A eukaryotic chromosome possesses multiple distinct DNA elements called replication origins, from which DNA replication initiates. The replication origins form a base for assembly of the pre-replication complex (pre-RC), and faithful duplication of chromosomes in S phase relies on functional integrity of the origins. It remains, however, to be understood how the replication origins are specified in the genome in any of the eukaryotic species. Here we revealed through bioinformatic analysis that replication origins in fission yeast *Schizosaccharomyces pombe* are associated with three features; a ≥15 bp-long motif with stretches of As, an AT-rich region that is a few hundred bp long, and a transcriptionally silent region that is ~1 kb long. Machine learning-based approach demonstrated that these three features, in combination, are sufficient to predict computationally the replication origin position along chromosome arms with high accuracy. Moreover, we found that the A-rich motif is correlated with chromosomal binding of the Orc, whereas the AT-rich region associated with loading of the Mcm replicative helicase. Taken together, these data indicate that replication origins in eukaryotes can be defined by DNA sequence and transcriptional landscape, and provide an insight into how each feature contributes to pre-RC assembly.

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*P-10

DNA Replication-coupled Cohesin Acetylation Revealed by Quantitative ChIP-seq

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The cohesin complex plays a key role in modulating higher-order chromosome structure, thereby mediating sister chromatid cohesion and controlling developmental gene expression. Stable binding of cohesin to chromosomes requires acetylation on a cohesin subunit Smc3, and in vertebrates, two paralogues Esco1 and Esco2 are responsible for this reaction. Here we show that Esco2 differs from Esco1 in its acetylation timing and target regions on the human genome, by quantitative ChIP-seq analysis utilizing spike-in control. We compared ChIP-seq profiles of acetylated Smc3 (Smc3ac) between control and knock-down (KD) cells. In HeLa genome, we detected Smc3ac broadly distributed throughout the genome by optimizing binning size in ChIP-seq analysis. We also discovered that such broad acetylation was dependent on Esco2 and occurred coordinately with DNA replication, which marks a sharp contrast with constant cohesin acetylation at cohesin peaks in euchromatin by Esco1. Collectively, we conclude that Esco2 fulfills replication-coupled cohesin acetylation throughout the genome.

*P-11

Roles of Cdc7 kinase complex in brain development

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The Cdc7-Dbf4(ASK) kinase complex is evolutionally conserved and plays crucial roles in initiation of DNA replication as well as in other chromosome events including meiotic recombination and repair of DNA damages through trans-lesion DNA synthesis. We previously reported that Cdc7 knockout mice die in an early embryonic stage (E3.5-6.5), while hypomorphic Cdc7 mutant mice survive but are deficient in germ cell development (Kim *et al*. **EMBO J.** 2002, 2003). In order to more precisely examine the roles of Cdc7 during development, we have generated mice with Cdc7 of a flox version, and induced knockout of Cdc7 in various organs and tissues. Combination of Nestin-Cre with the Cdc7 flox generated mice that lose Cdc7 expression in neural stem cells. Unexpectedly, the mice were born without significant phenotype, but exhibited growth retardation and did not survive beyond 3 weeks of age. The mutant mice showed convulsion as well as impaired body movement. Brain layer formation in cortex was severely impaired, indicating crucial functions of Cdc7 in brain development. In contrast, the similar knockout of Dbf4/ASK, the activation subunit of Cdc7, in neural stem cells, did not result in neonatal death, suggesting that Cdc7 function in brain may be independent of the Dbf4/ASK subunit that has been identified so far.

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*P-12

Chromatin recruitment of Lig1 is regulated in Uhrf1-dependent manner

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Genome-wide demethylation may cause genetic instability and hence facilitate or accelerate tumor progression, but the nature of DNA damage induced by hypo-methylation has not been clearly established. To maintain the DNA methylation pattern during DNA replication, Uhrf1, a specific binding protein to hemi-methylated DNA, plays a crucial role by catalyzing multiple mono-ubiquitylation of chromatin proteins to promote the recruitment of Dnmt1. Here, we demonstrate the novel regulatory mechanism of lagging strand synthesis that requires Uhrf1 for Lig1 accumulation on replicating chromatin. In *Xenopus* egg extracts, Uhrf1 and Lig1 interacts each other and Lig1 chromatin binding is significantly compromised in the absence of Uhrf1. In contrast, Lig1 depletion did not affect Uhrf1 association with chromatin and subsequent Dnmt1 recruitment. We also found that the SRA and TTD domains of UHRF1 are indispensable for UHRF1-dependnent chromatin recruitment of Lig1. We are currently testing if this pathway is necessary for the maintenance of genome stability.