DEPARTEMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF GENE INFORMATION ANALYSIS

1) Interactions among proteins involved in translesion DNA synthesis: E. OHASHI, K. KAMEI, T. HANAFUSA, and H. OHMORI

Cellular DNAs in living organisms are constantly exposed to various genotoxic agents of endogenous and exogenous origins. To cope with different DNA damages generated by various genotoxins, cells are endowed with multiple mechanisms for repair and tolerance of DNA damages. One of DNA damage tolerance mechanisms is translesion DNA synthesis (TLS) by specialized DNA polymerases (mainly Y-family polymerases), which is carried out in co-operations with various partner proteins. First, all Y-family polymerases (Polη, Polι, Polκ and REV1) were reported to interact with PCNA (Proliferating Cell Nuclear Antigen), a protein that is known as the sliding clamp to increase the processivity of many DNA polymerases. Second, all Y-family DNA polymerase has one or two copies of ubiquitin-binding motif and indeed, some of the proteins (e.g., Polι) were shown to bind to ubiquitin. Furthermore, Polη was reported to interact with Rad18, an ubiquitin ligase (E3 enzyme) that forms a complex with Rad6 (ubiquitin conjugation enzyme, E2). In DNA-damaged cells, PCNA is ubiquitinated by the Rad6-Rad18 complex, resulting in increasing affinity to Y-family polymerases. Finally, Polη, Polι, and Polκ bind to the C-terminal region of REV1 (as described below in more details). Thus, these TLS proteins have various binding partners and the differences in the affinity to such partners may determine which enzyme is recruited to a site of DNA damage.

In order to compare the binding affinities of each Y-family enzyme to the partner protein based on yeast two-hybrid assay, we made quantitative measurement of β-galactosidase activity. Among Y-family DNA polymerases, Polι was found to interact most strongly with either PCNA or ubiquitin. REV1, Polη and Polι interacted with Rad18 at similar strength. Polκ interacted most weakly with either one of PCNA, ubiquitin and Rad18. As for binding with REV1, Polη interacted most strongly and Polι did second most.

Thus, Y family DNA polymerases show different affinities to distinct binding partners. However, it is possible that DNA damage signaling may change the biding

affinities, for example, by phosphorylation and regulates the accessibility of the TLS polymerases.

2) The mechanism of the interactions between hREV1 and three Y-family DNA polymerases: E. OHASHI, T. HANAFUSA, K. KAMEI, and H. OHMORI

As described above, Polη, Polι, and Polκ interact with PCNA and have a sequence similar to the consensus sequence of so-called PIP (PCNA interacting protein) box, which is frequently represented by Q-x-x-(I,L,M)-x-x-F-F (x is any amino acid residue). We and other groups have shown that Polη, Polι, and Polκ interact with a C-terminal region of REV1, another member of the Y-family DNA polymerases. The same C-terminal region of REV1 was also shown to interact with REV7, a non-catalytic subunit of Polζ, which is another TLS DNA polymerase belonging to the B-family. This has suggested us that REV1 plays a crucial role in cellular TLS events. Unlike for the PCNA binding, Polη, Polι, and Polκ did not seem to have an apparent consensus sequence for the REV1-interaction. However, our further experiments revealed that short sequences less than 10 residues are sufficient for the REV1-interaction in all of the three Pols. In addition, in all cases, two consecutive phenylalanine (FF) residues were found to be critical for the REV1-interactions. In contrast to the PCNA-binding sequence, no conserved amino acid residue is required for the N-terminal side of the FF motif, but the presence of several residues at its C-terminal side is essential while the sequence is not conserved. Using surface plasmon resonance method, we showed that short synthetic peptides of the sequence derived from the REV1-binding site directly bind the C-terminal region of REV1. Furthermore, such peptides did inhibit the binding between purified REV1 and Polκ proteins.

To examine the biological relevance of the interaction between REV1 and Polκ, we employed complementation assay using Polκ-deficient MEF (mouse embryonic fibroblast) cells, which exhibit increased sensitivity to BPDE (benzo[a[pyrene dihydroxydiol epoxide, the ultimate carcinogen of benzo[a[pyrene] and UV-irradiation. We observed that the sensitivities to BPDE or UV were recovered by transient expression of the wild-type of Polκ (in a fusion with GFP), but not by that of the FF567-578AA mutant deficient for the REV1-binding.

Thus, the interaction with REV1 is essential for *in vivo* functioning of Polκ. The biological meaning of the REV-interactions of the three Pols needs to be elucidated by further investigations.

3) Interaction of hREV1 with hREV7 and Polκ: T. HANAFUSA, E. OHASHI, H. HASHIMOTO[1], and H. OHMORI ([1]Yokohama City University)

Polζ is one of multiple DNA polymerases involved in translesion DNA synthesis (TLS) and composed of two subunits; the REV3 catalytic subunit and the REV7 accessory subunit. The REV3 subunit show a significant similarity in the amino acid sequence to the catalytic subunit of Polδ, the replicative DNA polymerase, so that Polζ is classified into the B-family, while many of other TLS DNA polymerases are classified inot the Y-family. The *Saccharomyces cerevisiae* (sc) REV7 subunit was shown to increase the polymerase activity of the scREV3 catalytic subunit by 20- to 30-fold, but the exact function of the REV7 is not yet known. The human REV7 protein (hereafter hREV7) has a significant similarity to the human MAD2 protein (hereafter designated hMAD2A), a protein involved in spindle checkpoint. For the reason, hREV7 is also called hMAD2B (hMAD2L2 or hMAD2β). In fact, *Xenopus laevis* REV7 was shown to interact with Cdh1-APC (Anaphase Promoting Complex), paralleling the effect of MAD2A on Cdc20-APC. The hMAD2B has been reported to interact with some other proteins such as MDC9 (a metalloprotease disintegrin) and PRCC (papillary renal cell carcinoma). Moreover, a recent study reported that hREV7 interacts with Elk-1, which is a transcription regulator and targets on *egr-1* or *c-fos* following exposure of cells to DNA damaging agents, and acts to promote Elk-1 phosphorylation by the JNK MAP kinases. Thus, hREV7 may play a critical role in linking TLS to cell cycle regulation or gene expression changes.

Previously, hREV7 was shown to interact with a C-terminal domain (CTD) of hREV1 (approximately 100 amino-acid in the total 1251 residues). Recently, we and other groups have reported that the same CTD of hREV1 interacts also with Polκ□ Polι and Polη. To investigate how REV1 interacts with such many other DNA polymerases, we examined the structural requirements of hREV1 for the binding to hREV7. Our recent experiments revealed that short sequences less than 10 residues of the three Pols are sufficient for the REV1-interaction, all containing the FF motif. However, hREV7

does not contain FF in the entire 211 amino acid sequence and furthermore, a previous study showed that an almost entire region of hREV7 is required for its interaction with hREV1. Therefore, it seems likely that hREV1 CTD interacts with hREV7 and the three Pols in different manners. Our results also suggested that a tertiary structure of the hREV1 CTD is required for the interaction with hREV7. However, in a sharp contrast to it, we have very recently found that a sequence of REV3 as short as 14 amino acids is sufficient for the interaction with hREV7. Thus, hREV7 seems to have multiple phases to interact with different proteins. We are now trying to purify a complex of hREV7 and hREV1 CTD for structural analysis.

4) Possible Mechanisms for the Polκ 3'-mRNA processing: Y. S. CHAN, H. TANAKA[1], H. OHMORI ([1]Osaka University)

Polκ is one of the Y-family DNA polymerases that are involved in translesion DNA synthesis (TLS). Polκ is able to accurately and efficiently bypass some DNA lesions such as dG-N^2-BPDE, a bulky adduct generated by benzo[a]pyrene that is a potent environmental carcinogen.

Both the human and mouse genes coding for Polκ□□□*POLK* for the human gene and *Polk* for the mouse gene) are ubiquitously expressed with the highest expression in testis. Besides *Polk*, many genes involved in DNA repair and recombination functions are also observed to show similar expression patterns. However, one unique feature of the *Polk* genes is that the major transcripts found in the testes are shorter than those found in other organs; mRNAs abundantly expressed in the testes are about 2.8 kb in length whereas those ubiquitously expressed in many other organs (including testis at lower level) are 4.2 kb. The major and shorter transcripts found in testes have a polyA tail immediately downstream of the translation stop codon, whereas the longer transcripts have long 3'-UTR containing multiple copies of AUUUA sequence (an essential and minimal unit of AREs). AREs are known to render mRNAs unstable when present in 3'-UTR. Most of the tissues may express these labile mRNAs to keep the amount of *Polk* mRNAs at low levels to avoid unfavorable mutations caused by the accumulation of this error-prone enzyme.

Some mouse genes are also known to generate shorter transcripts in testes than in other organs. One of such genes is *Cdcc89*, whose expression is dramatically

up-regulated during spermiogenesis. The *Cdcc89* transcripts found in the testis are about 1.5 kb in length while the transcripts found in embryonic brain are 2.3 kb. *Cdcc89* is an intron-less gene, and the two kinds of transcript have the same coding region, differing in the length of 3'-UTR. It seems plausible that a same mechanism operates to generate shorter transcripts of the *Polk* and *Ccdc89* genes, making shorter 3'-UTRs.

CstF-64 is a protein essential for polyadenylation of mRNAs, involved at the cleavage process of nascent transcripts in the downstream of the canonical polyA signal sequence AAUAAA. Recently, it has been found that a new form of CstF-64 (named τCstF-64) is most highly expressed in male germ cells, which may cause the differences polyadenylation site selections between somatic cells and male germ cells. We are planning to test whether τCstF-64 may be involved in making shorter *Polk* and *Ccdc89* transcripts in testes.

LIST OF PUBLICATIONS

Department of Genetics and Molecular Biology
Laboratory of Genetic Information Analysis

M. Kawanishi, K. Matsukawa, E. Ohashi, T. Takamura, Y. Totsuka, M. Watanabe, T. Sugimura, K. Wakabayashi, F. Hanaoka, H. Ohmori, T. Yagi.: Translesion DNA synthesis across mono ADP-ribosylated dG by Y-family DNA polymerases, *New Developments in Mutation Research*, in press

X. Bi, L.R. Barkley, D.M. Slater, S. Tateishi, M. Yamaizumi, H. Ohmori, C. Vaziri.: Rad18 regulates DNA polymerase κ and is required for recovery from S-phase checkpoint-mediated arrest. *Mol. Cell. Biol.* **26**, 3527-3540, 2006

E. Ohashi, T. Hanafusa, K. Kamei, H. Ohmori.: Interactions between hREV1 and three Y-family DNA polymerases. 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress. 京都、６月１８～２３日、２００６

A. Hishiki, H. Hashimoto, K. Kamei, E. Ohashi, T. Shimizu, H. Ohmori, M. Sato.: Structural insight of interaction between proliferating cell nuclear antigen and human TLS polymerases. Fifth East Asian Biophysics Symposium & Forty-Fourth Annual Meeting of the

Biophysical Society of Japan, 沖縄, １１月１２ー１６日, ２００６

赤木 純一、菅 貴嗣、片岡 裕貴、大橋 英治、大森 治夫、益谷 央豪、花岡 文雄：REV1 との相互作用を消失した polη のヒト細胞内での解析、日本分子生物学会 2006 フォーラム「分子生物学の未来」、名古屋、１２月６ー８日, ２００６

遺伝子動態調節研究部門　　Department of Genetic and Molecular Biology
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当グループは発足以来ずっとスタッフは大森一人だけであったが、学位取得後も学術振興会特別研究員 PD そして助手並み待遇の研究員として在籍していた大橋が８月１日から短期助手として採用された（任期は２００７年２月末まで)。理学研究科生物科学専攻の修士課程を終えた亀井はつくば市の特許事務所に就職した。代わりに、大阪大学理学部生物学科出身のマレーシアからの留学生 Chan Yuet Sim が理学研究科生物科学専攻の修士課程 M1 として我々のグループに加わり、また技術補佐員として風間が加わるなど、3，４月は構成メンバーに大きな変動があった。

我々がここ数年中心課題として扱ってきた損傷バイパス酵素 Polκをコードする遺伝子（マウスの遺伝子は *Polk*、ヒトの遺伝子は *POLK* と表記される）の発現は精巣で最も高いことがこの遺伝子を同定した当初から分かっていた。DNA 合成、組み換え、修復などに関わる遺伝子の多くは精巣での発現が高いことが知られているが、ヒト *POLK* やマウス *Polk* の両遺伝子の発現についてのユニークな特長は、精巣での主要な mRNA は約 2.8 kb の長さであるのに対して他の臓器で発現されている mRNA の長さは約 4.8 kb と、精巣での mRNA の長さが他の臓器に比べて顕著に短いことである。我々と同時期に *POLK*, *Polk* 遺伝子を同定した米国の E. Friedberg のグループは 4.8 kb と長い転写物の 3’-UTR には mRNA を不安定にする ARE (AU-rich element)が多コピー存在することを報告した。我々のグループはヒト *POLK* やマウス *Polk* の両遺伝子の転写開始点を正確に塩基レベルで決定して、１）マウス *Polk* 遺伝子の転写は二つの異なる位置□□□□、及び P1b)から開始され、上流に位置する P1a からの転写開始は精巣を含む多くの臓器で起こるが、それから約200ヌクレオチド下流に存在する□1□の転写開始はほぼ精巣特異的であること、２）マヒト *POLK* 遺伝子の転写開始付近の DNA 塩基配列は上記のマウスの配列と類似しているが、その転写開始はマウスの P1a の 6 ヌクレオチドの一箇所からのみ起こることを示した。一方、マウス *Polk*、及びヒト *POLK* の精巣における mRNA は翻訳終始コドンのすぐ下流で polyA 配列に繋がるのに対して、他の臓器からの mRNA は長い 3’-UTR を持っている。すなわち、精巣，及び他の臓器で作られる mRNA は coding region は全く同じであるが、3’-UTR において大きく異なる。

精巣は細胞分裂が盛んに起こっているばかりでなく、一部の細胞は精子として成熟するために著しい形態変化を伴う分化を起こすというユニークな組織であり、そのために固有の遺伝子を数多く発現している。大阪大学の田中らのグループはマウス精巣で半数体精子細胞の出現に伴って特異的に発現される遺伝子群 (*TISP*, transcript induced in spermiogenesis)を同定して、そのうちのあるグループ(Type 3、*TISP201~232*)では精子形成の成熟化に伴って mRNA のサイズが小さくなることを明らかにした。実際、*TISP201~232* のそれぞれの全長の cDNA と EST (expression sequence tag) library 登録されている精巣由来の cDNA のサイズ、取り分け 3'-UTR のサイズに注目して比較をすると、*TISP204* では精巣で発現される mRNA (正式遺伝子名は *Ccdc89*, coiled coil domain containing 89) は他の臓器（この場合は胎児の脳）の mRNA よりも短い 3'-UTR を持つらしいことが分かった。そこで田中らと共同して、精巣及び胎児で発現されている *Ccdc89* の cDNA を単離して塩基配列を解析したところ、胎児由来の *Ccdc89*-cDNA は 1 kb ほどの 3'-UTR を持ち、その 3'-end の polyA 配列の 20 ヌクレオチド上流に AGTAAA という配列が存在したが、精巣由来の *Ccdc89*-cDNA の 3'-UTR は約 120 ヌクレオチドと短く、その polyA 配列の 20 ヌクレオチド上流には AGTACA という配列が存在した。この *Ccdc89* 遺伝子には intro は存在せず、3'-UTR の違いは alternative splicing によるものではなく、polyA site の選択の違いによると考えられる。

真核生物における mRNA の 3'-end は一旦転写された後に AAUAAA という配列の約２０ヌクレオチド下流で切断されて、polyA が付加されることが知られている。そのような切断反応に関わる因子の一つである CstF-64 は X 染色体上に存在するので、精子形成の途中の段階でその発現が停止し、その代わりに 10 番染色体上に存在するτCstF-64 が精巣特異的に発現される。このτCstF-64 が働くと AAUAAA 以外の配列を polyA シグナル配列として利用する可能性が考えられる。今後は *Polk*、*Ccdc89* という二つの遺伝子の精巣における mRNA の 3'-end processing についての研究を損傷バイパスのメカニズムの研究と並行して進めていく予定である。