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Meeting report

**Abstract**

The first US-Japan meeting on "error-prone DNA synthesis" convened at the Maui Prince Hotel, from December 20 through December 21, 2004. In keeping with the traditions of US-Japan conferences, the meeting was small and intimate, and comprised of 22 participants: 11 from Japan, 10 from the United States, and one from Israel. The program featured platform presentations from each of the participants, with considerable time for discussion of topics related to each of the themes of the meeting. The following pages present an introduction to the unanswered questions on error-prone DNA synthesis. These questions were the dominant themes that permeated the extensive discussions. Edited abstracts of each of the presentations, organized into primary topic themes, are presented.

## The first US-Japan meeting on error-prone DNA synthesis, Maui, Hawaii, December 20–21, 2004

Until recently, six DNA polymerases, Pols α, β, δ, ε, γ and ζ, belonging to the A, B or X families, were known to be present in eukaryotic cells. However, within the last few years, additional DNA polymerases, including an entire new family, the Y-family, have been discovered and characterized. These polymerases are evolutionarily conserved and are distinguished by their ability to carry out translesion DNA synthesis. Although they share the ability to copy past potential blocking lesions in DNA, they have low processivity and they exhibit low fidelity, particularly when copying unaltered DNA templates. Mutations and/or overexpression of these error-prone DNA polymerases in both prokaryotes and eukaryotes can result in enhanced mutation rates. One of the subgroups of xeroderma pigmentosum (XPV) encodes a mutation in the Y-family DNA polymerase, Pol η, and exhibits an elevated mutation frequency and an exceptionally high incidence of skin cancers. The association of mutations and/or overexpression of error-prone DNA polymerases with cancer in humans and mice are beginning to be evaluated. These error prone DNA polymerases were considered against a background of the replicative DNA polymerases that have been extensively studied in mammalian cells. Mutations in replicative DNA polymerases that enhance infidelity are mutators in prokaryotes and eukaryotes. Allelic substitution with low fidelity DNA polymerases results in an elevated incidence of cancer in mice.

The following questions were addressed: What is the association between error-prone DNA polymerases and different human cancers? How are error-prone DNA polymerases recruited to DNA lesions? What is the function of these polymerases in normal cells? Do these polymerases copy DNA containing endogenous lesions or alternative DNA structures? Are there cellular factors that prevent mutagenesis by error-prone DNA polymerases? Does mutagenesis result from bypass of lesions by error-prone DNA polymerase or do other factors enhance the bypass effectiveness of replicative DNA polymerases? Would inhibition of these error-prone DNA polymerases prevent cancers or curtail tumor progression? Although the workshop was predominately focused on Y and B family DNA polymerases, the consequences of mutator replicative and repair polymerases were also discussed. Together, the studies presented indicated that alterations in DNA polymerases are important in the initiation and progression of cancers.

We have rearranged the abstracts into the following categories: (1) Replicative and repair DNA polymerases, (2) Y-family DNA polymerases, (3) B-family DNA polymerases, (4) Other DNA polymerases and (5) Induction of lesions. However, this classification does not adequately reflect the overlap between the presentations or the extensive discussion that characterized this meeting.

### 1. Replicative and repair DNA polymerases

#### *1.1. DNA polymerase α*

##### *1.1.1. DNA polymerase α and genomic stability*

**Motoshi Suzuki**

Suzuki has isolated active mutants in *Saccharomyces cerevisiae* DNA polymerase α (Pol α) that are associated

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with a defect in error discrimination. Among them, purified yeast L868F DNA Pol α has a spontaneous error frequency of 3 in 100 nucleotides and 570-fold lower replication fidelity than the wild-type enzyme. In vivo, L868F DNA Pol α confers a mutator phenotype and is synergistic with *msh2* or *msh6*, suggesting that DNA polymerase α-dependent replication errors are recognized and repaired by mismatch repair. In vitro, L868F DNA Pol α catalyzes efficient bypass of a *cis*-syn cyclobutane pyrimidine dimer, extending the 3′-T 26,000-fold more efficiently than wild-type. In the translesion DNA polymerase, Pol η, Phe34 is equivalent to Leu868; the F34L mutant of *S. cerevisiae* DNA Pol η has reduced translesion DNA synthesis activity in vitro. Suzuki's data suggest that high fidelity DNA synthesis by DNA Pol α is required for genomic stability in yeast. The data also suggest that the phenylalanine and leucine residues in translesion and replicative DNA polymerases, respectively, may have played a role in the functional evolution of these enzyme classes.

### *1.2. DNA polymerase* β

#### *1.2.1. Cancer-associated mutants of DNA polymerase* β

Tieming Lang, Mausumi Maitra, Shibani Dalal, Daniela Starcevic, Ka-Wai Sun, Daniel DiMaio, and **Joann B. Sweasy**

Several small-scale studies suggest that at least thirty percent of the 149 human tumors examined to date express variants of the DNA polymerase β (Pol β) gene. None of these mutations are common polymorphisms found in non-tumor tissue. To determine if the presence of Pol β variant proteins is linked to the etiology of human cancer, the Sweasy laboratory expressed these proteins in immortalized but nontransformed mouse C127 cells. Expression of the I260M prostate, K289M colon, and D160N gastric cancer-associated Pol β variants in these cells resulted in focus formation, a hallmark of cellular transformation, whereas expression of wild-type Pol β did not induce focus formation. Studies were conducted to determine if DNA synthesis by the cancer-associated variants was less accurate. Combined results from mutation spectra and presteady-state kinetic assays demonstrated that the variants synthesize DNA inaccurately within certain sequence contexts. Based on their findings, Sweasy proposed that Pol β variant proteins synthesize DNA inaccurately during the gap-filling step of base excision repair. This could result in mutations in key growth control genes with sequences that are at risk for mutation, leading to tumorigenesis or its progression.

### *1.3. DNA polymerases* δ *and* ε

#### *1.3.1. Function of DNA polymerases* α,δ, *and* ε *at the replication forks in Saccharomyces cerevisiae*

**Akio Sugino**, Takashi Seki, Aki Hayashi-Hagihara, and Yasuo Kawasaki

The role of different DNA polymerases in eukaryotic DNA replication is controversial. Early in the eukaryotic cell cycle, a pre-replication complex is assembled at each replication origin with ORC, Cdc6, Cdt1 and Mcm2-7 proteins. At the onset of S-phase, RPA is loaded at the origin followed by Cdc45-dependent loading of Pols α, δ, and ε. Sugino et al. examined the cell cycle-dependent localization of Pols α, δ and ε in *Saccharomyces cerevisiae* using chromatin immunoprecipitation and DNA microarray analysis. They showed that Pols α, δ, and ε co-localize to early firing autonomously replicating sequences on yeast chromosome III and VI at the beginning of the S-phase, suggesting that all three DNA polymerases function together at multiple replication forks. They reported that Pol ε is recruited to the replication origin region by a concerted action of Cdc45-Sld3, Dpb11-Sld2, and the GINS complex during initiation of chromosomal DNA replication before the recruitment of Pol δ to the replication fork by RFC and PCNA. The dependence of translesion synthesis (TLS) DNA polymerases on PCNA for their function led Sugino to suggest that TLS DNA polymerases specifically promote lesion bypass during DNA synthesis on the leading DNA strand. He further speculated that other lesion bypass mechanisms, which depend on a copy-choice type of DNA synthesis or on recombination, may then be used during Pol α and Pol δ-catalyzed lagging-strand synthesis.

#### *1.3.2. Discrete cancer phenotypes in mice deficient for DNA polymerase-*δ *or -*ε *proofreading*

Masanori Ogawa, James Bugni, Laura Hays, Robert Goldsby, Brian Rubin, **Bradley Preston**

DNA polymerases δ and ε are essential nuclear proteins that function in normal DNA replication and repair. Both polymerases have an intrinsic 3′–5′ proofreading exonuclease (exo) activity that corrects misincorporation errors and contributes significantly to genome stability. Preston and colleagues previously showed that mice deficient for Pol δ exonuclease exhibit reduced survival ($t_{1/2} = 10$ months) with a 95% incidence of spontaneous cancers, primarily thymic lymphomas and skin squamous cell carcinomas (Goldsby et al., PNAS 99:15560, 2002). In order to determine the contribution of Pol ε exo to tumorigenesis, they have now generated mice with an inactivating mutation in the Pol ε exo domain (D272A/E274A) and characterized their mutator and cancer phenotypes.

Pol ε exo$^{-/-}$ mice ($n = 43$) died prematurely ($t_{1/2} = 18$ months) with a 95% cancer incidence. In contrast to Pol δ exo$^{-/-}$ mice, the Pol ε exo$^{-/-}$ animals developed very few thymic lymphomas (4%) and no squamous cell carcinomas. The most common tumors in the Pol ε exo$^{-/-}$ mice were adenomas/adenocarcinomas of the small intestine (25% incidence) and subdermal sarcomas (40%), neither of which were observed in Pol δ exo$^{-/-}$ mice. To assess whether loss of Pol ε exo causes a mutator phenotype, mutation rates at the *Hprt* locus were determined in mouse embryonic fibroblast (MEF) cell lines. Pol ε exo$^{-/-}$ and Pol δ exo$^{-/-}$ MEFs

showed similar mutator phenotypes (∼150-fold increase in base substitutions with no increase in microsatellite instability). Taken together, their results indicate that Pol δ and Pol ε proofreading play different, tissue-specific roles in tumor suppression and genome stability.

#### *1.3.3. Evolutionary conservation amongst DNA polymerases*

Ranga Venkatesan, Ern Loh, Bradley Preston, **Lawrence A. Loeb**

In accord with the universality of DNA replicative processes in organisms, DNA polymerases exhibit high levels of sequence and structural homologies at their active site. To create mutator mutants of DNA Pol δ, the Loeb laboratory exploited this homology by identifying mutants in *Escherichia coli* DNA polymerase I that alter the fidelity of DNA synthesis, and then introduced the corresponding mutations in *S. cerevisiae* DNA Pol δ. They screened large libraries that contained random substitutions in conserved motifs of *E.coli* DNA Pol I as well as random substitutions throughout the enzyme. Surprisingly, they observed that hot-spots for the generation of both mutator and anti-mutator enzymes were frequently found at sites distant from the catalytic domains. Based on the known mutator DNA Pol I phenotypes, they first analyzed substitutions at the highly conserved leucine residue (L612) in the motif A domain that forms part of the nucleotide-binding pocket in *S. cerevisiae* DNA Pol δ. Only a limited number of substitutions were tolerated; several of these substituents generated mutator polymerases. L612K, L612M and L612F substitutions enhanced mutation rates by 5–13-fold compared to the wild-type and exhibited a transient $G_2$/M arrest. L612G and L612N substitutions enhanced the mutation rate by 17–38-fold, displayed a prolonged S-phase, transient $G_2$/M arrest and were sensitive to hydroxyurea and methylmethane sulfonate. This study demonstrates the feasibility of recreating phenotypes based on homologies between different DNA polymerases and provides evidence that links mutations in replicative DNA polymerases to delays in the cell cycle caused by the activation of cell cycle checkpoints. The corresponding mutant Pol δ gene has been introduced in mice by allelic substitution; they are currently awaiting analyses of the mouse phenotypes.

## 2. Y-Family translesion DNA polymerases

### *2.1. DNA polymerases η and ι*

#### *2.1.1. Recent studies on Pol η: protein–protein interactions and POLH-deficient mice*

**Fumio Hanaoka**

Human DNA polymerase η (Pol η), the gene product responsible for XPV, efficiently and accurately bypasses the major UV-induced DNA lesion, cyclobutane pyrimidine dimer (CPD). There is no doubt that Pol η plays an important role in suppressing mutations caused by UV, thereby decreasing skin tumors. There are other polymerases that have translesion synthesis activity in mammalian cells besides Pol η, and it is not clear how these TLS polymerases are differentially utilized. In order to get insights on how these TLS polymerases behave in mammalian cells, Fumio Hanaoka's laboratory analyzed protein partners of human Pol η. They also generated Pol η-deficient mice to examine the redundancy or uniqueness of these TLS polymerases in vivo. They found that REV1, another Y-family polymerase could interact with Pol η in crude cell extracts and that REV1 co-localizes with CPD lesions induced by UV-irradiation through a micropore filter. This co-localization is dependent on the presence of Pol η. Hanaoka also reported the successful generation of Pol η-deficient mice. These knock-out mice are viable but are prone to skin cancer induced by UV exposure. The Hanaoka laboratory plans to use these mice as the XPV mouse model, and cross them with mice lacking other error-prone DNA polymerases to provide pivotal information about the physiological functions of each of these polymerases.

#### *2.1.2. Pol ι-dependent translesion replication of UV photoproducts*

Alexandra Vaisman, Kohei Takasawa, Shigenori Iwai, and **Roger Woodgate**

The spectrum of UV-induced mutations in synchronized wild-type S-phase cells reveals that only ∼25% of mutations occur at Thymine (T) residues, whilst 75% are targeted to Cytosine (C). The mutational spectra changes dramatically in XP-V cells, devoid of Pol η, where ∼45% of mutations occur at Ts and ∼55% at Cs. At the present time, however, it is unclear whether the large majority of C->T mutations that occur in either cell line, actually represents true misincorporations opposite C, or perhaps occur as the result of the correct incorporation of Adenine (A) opposite a C that had undergone deamination to Uracil (U). In order to assess the role that human Pol ι might play, if any, in the replicative bypass of UV-photoproducts, Woodgate et al. have analyzed the fidelity and efficiency of Pol ι-dependent bypass of T-T and T-U cyclobutane pyrimidine dimers (CPDs) in vitro. They find that bypass of the T-T CPD is inefficient and highly error-prone. In contrast, bypass of the T-U CPD is more efficient than the T-T CPD. Whilst Guanine (G) was frequently misincorporated opposite the 3′U of the T-U CPD, if one assumes that the U arose as a result of deamination of C, then the misincorporation would actually lead to error free replication, since a wild-type G:C base pair would be regenerated during the next round of semi-conservative synthesis. Based upon the reported in vitro properties of Pol η and Pol ι, they hypothesize that T->C mutations observed in a wild-type cell are largely due to Pol η-dependent misincorporation of G opposite the 3′T of a T-T CPD and that C->T mutations similarly

occur as a consequence of Pol η-dependent incorporation of A opposite U of a T-C CPD that underwent deamination. In contrast, Woodgate et al. speculate that the reduced number of C->T mutations observed in XP-V cells, is probably due to the Pol ι-dependent misincorporation of G opposite U of a deaminated T-C CPD, and that the concomitant increase in mutations observed at Ts, is due to error-prone bypass of T-T CPDs by pol ι.

### *2.1.3. Studies of the efficiency and fidelity of translesion DNA synthesis in vitro*

**Thomas A. Kunkel**

The human genome encodes 17 DNA polymerases, about half of which are thought to operate at a replication fork. When lesions in nuclear DNA stall replication by the major replicative polymerases, one or more switches occur to allow translesion synthesis (TLS) by specialized DNA polymerases. This is followed by another switch that allows normal replication to resume. Kunkel hypothesizes that these switches occur during transitions from preferential to disfavored use of damaged primer-templates, and that the polymerase and/or exonuclease called upon following each successive nucleotide incorporated is the one whose properties ultimately result in the highest efficiency and fidelity of bypass. In order to test this hypothesis, the Kunkel laboratory is quantifying the efficiency and fidelity of TLS by the major replicative and TLS polymerases, while working alone or in combination to copy a series of matched and mismatched primer-templates that mimic each incorporation needed to completely bypass a lesion. They observe a complementary pattern of efficient localized translesion synthesis by human Pol η, followed by mismatch excision and polymerization by Pol δ and Pol ε, that can account for efficient and accurate dimer bypass, as required to suppress sunlight-induced mutagenesis and skin cancer. They are using the same approach to study bypass of other DNA lesions relevant to genome stability and human disease.

### *2.1.4. 8-Hydroxyguanine in a mutational hotspot of the c-Ha-ras gene causes misreplication, 'action-at-a-distance' mutagenesis and inhibition of replication by Pol η and κ*

Pawel Jaloszyński, Ana Blanco Perez, Chikahide Masutani, Eiji Ohashi, Haruo Ohmori, Fumio Hanaoka, and **Susumu Nishimura**

Nishimura et al. have shown that the oxidative lesion, 8-OH-G, in the first or second position of codon 12 in the *c-Ha-ras* gene can induce transformation in NIH3T3 cells. Mutations were found not only at the site of the lesion but also in neighboring positions. They determined whether the error-prone DNA polymerases, Pol η and Pol κ, can misreplicate DNA with 8-OH-G in the sequence context of the *c-Ha-ras* gene. They found that Pol η incorporates dGMP as well as dCMP across from 8-OH-G in codon 12 of the human *c-Ha-ras* DNA fragment. In addition, replication catalyzed by Pol η resulted in misincorporation of dAMP, dTMP and dGMP opposite normal guanine, 3′-next to 8-OH-G in the template. Interestingly, two adjacent 8-OH-G residues, in the first and second position of codon 12, greatly relaxed the specificity of Pol η. This misincorporation was not observed with Pol α and β. Pol κ, on the other hand, inserted dAMP more efficiently than dCMP opposite 8-OH-G. Pol κ also catalyzed 'action-at-a-distance' mutagenesis. Interestingly, this misincoporation at a distance was greater on a mouse DNA template than on a human DNA template, indicating that neighboring sequences in, and surrounding the codon 12 influence the efficiency of misreplication by 8-OH-G. Their results suggest an important role of error-prone polymerases, such as Pol η and Pol κ in inducing hypermutability in codon 12 of the *c-Ha-ras* gene. Further, their observations are important for understanding the consequences of 8-OH-G being positioned within the mutational hot spots of oncogenes.

### *2.1.5. Possible roles of Y-family DNA polymerases in mutagenesis through incorporation of oxidized dNTPs*

Masatomi Shimizu, Petr Gruz, Hiroyuki Kamiya, Su-Ryang Kim, Francesca M. Pisani, Chikahide Masutani, Yan Xu, Hiroshi Sugiyama, Yusuke Kanke, Hideyoshi Harashima, Fumio Hanaoka, and **Takehiko Nohmi**

Altered oxidative metabolism with the generation of reactive oxygen species is a property of many tumor cells. The dNTP pool as well as DNA is continuously exposed to reactive oxygen species, and the incorporation of oxidized dNTPs into DNA is a potential source of spontaneous mutagenesis and carcinogenesis. Since the error rates of Y-family DNA polymerases are very high and they appear to have active sites large enough to accommodate bulky DNA adducts, Nohmi and colleagues postulated that they might also function in incorporating oxidized dNTPs into DNA. To address this question, they examined the incorporation specificity of archaeal *Sulfolobus* Y-family DNA polymerases and human DNA Pol η. These polymerases incorporated oxidized dNTPs (8-hydroxy-dGTP and 2-hydroxy-dATP) into nascent DNA in an error-prone manner. They almost exclusively incorporated 8-hydroxy-dGTP opposite template adenine (A), and 2-hydroxy-dATP opposite template thymine (T), guanine (G) and cytosine (C). In contrast, Klenow exo- and *Sulfolobus* B-family DNA polymerase incorporated 8-hydroxy-dGTP opposite template A and C, and 2-hydroxy-dATP opposite template T. Primers containing a 3′-8-hydroxy-GMP opposite a template A or 3′-2-hydroxy-dAMP opposite a template G, were efficiently extended by the Y-family DNA polymerases. These results suggest that Y-family DNA polymerases can promote mutagenesis through the misincorporation of oxidized dNTPs during DNA synthesis in addition to the well-characterized translesion synthesis across DNA lesions in the template.

### 2.1.6. Rad18, an essential coordinator for UV-induced translesion synthesis

**Masaru Yamaizumi**

Masaru Yamaizumi discussed the importance of PCNA ubiquitination and its interaction with the TLS polymerase, Pol η. In UV-irradiated mammalian cells, Rad18 and Rad6 are involved in PCNA mono-ubiquitination. Pol η preferentially binds to mono-ubiquitinated PCNA in vitro and in vivo. Since Rad18 constitutively binds to Pol η, it seems that Rad18 plays a crucial role in recruiting and loading Pol η at the stalling sites for TLS. Furthermore, Rad18 itself is mono-ubiquitinated in a Rad6 dependent manner. Mono-ubiquitinated Rad18 is mainly localized in the cytoplasm. Probably, this autoubiquitination system is important for the regulation of Rad18 localization in the nucleus.

## 2.2. DNA polymerase κ

### 2.2.1. Translesion DNA synthesis by mammalian DNA polymerase κ

**Haruo Ohmori**

Mammalian cells have multiple DNA polymerases suited for translesion DNA synthesis, which include four Y-family enzymes Pol η, Pol ι, Pol κ and REV1, as well as one B-family enzyme Pol ζ. The cellular function of each of these enzymes remains to be established. In order to clarify the function of Pol κ, Ohmori examined various DNA lesions as substrates in in vitro TLS reactions. In collaboration with the Shibutani laboratory (State University of New York at Stony Brook), they showed that Pol κ is able to bypass dG-*N*2 adducts by inserting the complementary dC opposite the lesions with different efficiencies. For example, Pol κ inserts dC opposite a dG-*N*2-BPDE (benzo[α]pyrene-diol-epoxide) at a 1% efficiency compared to non-damaged dG. Insertion of dC is two orders of magnitude higher than that of the other bases opposite the lesion. This is in contrast to Pol η that inserts dA opposite the 3′T of a T-T CPD at the same efficiency as it does with a non-damaged template. Even with such apparent low efficiencies of bypass in vitro, Ohmori speculated that Pol κ may play an important role in bypassing BPDE-adducts in vivo, because Pol κ-deficient murine embryonic stem (ES) cells show hypersensitivity to benzo[α]pyrene, generating more mutations than the parental cells.

More recently, Ohmori et al. found that Pol κ can efficiently bypass *N*2-[3-methoxyestra-1,3,5(10)-trien-6-yl]-2′-deoxyguanosine, by inserting C opposite the lesion with a 33% efficiency as non-damaged G. The DNA adduct was used in place of *N*2-(2-hydroestron-6-yl)-2′-deoxyguanosine, a product generated by the reaction between DNA and estrogen-2,3-quinone, a metabolite of estrogen. However, in preliminary experiments Ohmori et al. did not observe any obvious difference between parental cells and Pol κ-deficient mouse embryonic fibroblasts in their sensitivities to 17-β-estrodiol or its metabolites such as 2-hydroxyestradiol and 4-hydroxyestradiol.

### 2.2.2. DNA damage response mediated by translesion DNA synthesis

**Shunichi Takeda**, Mitsuyoshi Yamazoe, Aki Mizutani, and Eiichiro Sonoda

Tamoxifen (TAM) has anti-estrogen function, and is widely used for the treatment of breast cancer and for breast cancer prevention in high-risk women. However, TAM is carcinogenic in the human uterus and rat liver, highlighting the profound complexity of its effects. To explore the molecular mechanisms of its mutagenesis, Takeda et al. analyzed the effects of TAM treatment on gene-disrupted DT40 clones deficient in different DNA repair pathways. A short G1 phase and a lack of G1/S damage checkpoint in DT40 cells allowed them to detect defective recovery from replication block with high sensitivity. They found that gene-disrupted TLS mutants including *rad18*, *polκ* and *rev3* cells are hypersensitive to therapeutic concentrations of TAM, exhibiting an increase in chromosomal breaks. Interestingly, these mutants were also hypersensitive to a physiological metabolic product of estrogen, 4-hydroxyestradiol. Their data reveal a critical contribution of TLS to the prevention of chromosomal breaks by TAM and estrogen. They suggest that the employment of error-prone TLS polymerases during the copying of TAM-induced DNA lesions is likely to be responsible for the mutagenesis associated with clinical treatment by TAM and E2.

### 2.2.3. Enigmatic phenotypes of a Polκ mutant mouse strain

**Errol C. Friedberg**, Susana Velasco, and Lisa McDaniel

In order to reveal phenotypes in the absence of the translesion synthesis DNA polymerase, polymerase kappa (Pol κ), the Friedberg laboratory generated mice defective in the *Polκ* gene. The mice manifest several unexplained phenotypes. As reported previously, (Schenten, et al., Eur. J. Immunol. 32 3152, 2002) fibroblasts in culture from *polK*$^{-/-}$ embryos are abnormally sensitive to killing by UV radiation at 254 nm. However, purified Pol κ does not support TLS past the major photoproducts T<>T or [6–4] PP in vitro, suggesting that it is not required for TLS past such lesions in vivo. Independent studies have demonstrated efficient and accurate bypass of thymine glycol in vitro (Fischhaber et al., JBC, 277: 37604, 2002). Thus, it remains to be determined whether this property of the enzyme relates to the UV radiation sensitivity of mutant cells.

In the course of inbreeding Pol *κ* mice for multiple generations, Friedberg and colleagues identified multiple disease phenotypes. Many mice developed classical diabetes insipidus (DI). Others developed neurological defects, skeletal defects or vitiligo. Pedigree studies of the DI mice

showed that affected mice can be of any *polK* genotype (i.e., *Polκ*$^{-/-}$, *Polκ*$^{+/-}$ *or Polκ*$^{+/+}$), suggesting that the *Polκ* defect does not directly result in DI, but rather promotes the emergence of mutations elsewhere in the genome, some of which are revealed as DI (and other disease states). In support of this notion, direct analysis of mutations in the male germ line of *polK*$^{-/-}$ mice reveals a spontaneous mutator phenotype.

#### *2.2.4. Quantitative analysis of translesion DNA synthesis in mammalian cells*

**Zvi Livneh**, Sharon Avkin, Sheera Adar, Ziv Sevilya, Sigal Shachar, Noam Diamant, and Shay Covo

The main goal of the studies carried out by Livneh et al. was to elucidate the molecular mechanism of translesion synthesis, and in particular its operation program, including the regulatory elements which ensure that mutation frequency due to TLS DNA polymerases are kept under control to prevent excessive cancer risk. In order to achieve this goal, the Livneh laboratory developed a quantitative TLS assay system based on transient transfection of cultured mammalian cells with gapped plasmids, each carrying a site-specific lesion in the ssDNA region. They found that benzo[α]pyrene-G (BP-G) adducts were bypassed with an efficiency of 50% in mouse embryo fibroblasts, which is 20-fold higher than in *E. coli*. DNA Pol κ was found to be responsible for at least 2/3 of the TLS events across BP-G, and its bypass activity was two-fold more accurate than any other DNA polymerase. Strikingly, human cells were able to bypass a non-DNA segment (a hydrocarbon chain), despite the lack of any resemblance to DNA. These results suggest that (1) At least for some lesions, a specific TLS DNA polymerase performs the bypass reaction with higher efficiency and higher accuracy than any other DNA polymerase and (2) TLS is robust, and can replicate across lesions as extreme as non-DNA segments.

### *2.3. Rev1*

#### *2.3.1. Biochemical properties of the human Rev1 protein*

**Yuji Masuda**, Jinlian Piao, Kenji Kamiya

Cellular functions of the *REV1* gene are conserved from yeast to humans and appear important for maintaining genetic integrity through translesion DNA synthesis. Rev1 has two distinct functions in this regard: one is a deoxycytidyl transferase activity and the other is a less well-characterized non-catalytic activity. During biochemical characterization of the Rev1 protein, Masuda found that oligonucleotides that form higher order structures mediated by a guanine-rich sequence, specifically inhibit its transferase activity. Remarkably, other eukaryotic and prokaryotic DNA polymerases including the Klenow fragment of *E. coli* DNA Pol I and human DNA Pols α, β and η were found to be less inhibited by such oligonucleotides than the Rev1 protein. Masuda considered that this property of Rev1 may have a regulatory function in translesion DNA synthesis.

## 3. B family DNA polymerases

### *3.1. DNA damage tolerance mechanisms in S. cerevisiae*

Peter E.M. Gibbs, Hengshan Zhang, Roger Woodgate, John McDonald, and **Christopher W. Lawrence**

Christopher Lawrence et al. examined DNA damage tolerance mechanisms in *S. cerevisiae* in vivo, using transformation with plasmids carrying specifically located lesions. Pol η was only rarely involved in the bypass of a T-T (6–4) lesion or abasic site. They argue that Pol ζ, rather than Pol δ, probably incorporates nucleotides opposite the 3′ site of the UV lesion or abasic site, other than those inserted by Pol η or Rev1, because it appears to be the least accurate of the B-family enzymes, lacks proofreading activity, and because of its ability to extend from distorted termini. Bypass of these lesions is as low in strains lacking the Pol32 subunit of Pol δ as those deficient for Pol ζ or *REV1*, perhaps because Srs2 or proteins of the alternate sliding clamp cannot be recruited. Transformation of excision-repair deficient (*rad1*Δ) yeast strains with duplex plasmids carrying a T-T (6–4) photoadduct in each strand at staggered positions 28 bp apart showed that the error-free mechanism of DNA-damage tolerance in yeast depends on recombination between partially replicated sister strands. C-C mismatches opposite each photoproduct indicated whether plasmid DNA replication occurred by translesion synthesis or recombination processes. About 55% of the plasmids were fully replicated, and >95% of them achieved this by recombination. Sixty to 70% of the recombination events depended on *RAD18* and *RAD5* function, indicating that they were generated by the error-free DNA damage tolerance pathway; the remainder depending on *RAD52* function. These results constitute the first in vivo evidence indicating that the error-free DNA-damage tolerance process in yeast, and therefore perhaps in all eukaryotes, depends on recombination between partially replicated sister strands. The PCNA G178S substitution in *rev6-1* mutants abolishes both lesion bypass and the error-free recombination process, perhaps because it prevents ubiquitination of lysine 164 or the binding of factors required for damage tolerance activities.

### *3.2. Evidence for the role of specialized DNA polymerases in translesion synthesis past fork-blocking lesions in human fibroblasts*

**Veronica M. Maher**, Ziqiang Li, Xi-De Wang, Yun Wang, Kristin McNally, J. Justin McCormick, and Christopher W. Lawrence

Maher and colleagues are studying the mechanisms by which eukaryotic cells deal with fork-blocking lesions in their

DNA. As one aspect of these studies, they have been investigating whether the proteins coded for by the human homologs of the *S. cerevisiae Rev1* and *Rev3* genes play critical roles in translesion synthesis past fork-blocking lesions such as those induced by UV or benzo(α)pyrene diol epoxide (BPDE). To eliminate or greatly reduce the level of expression of one or other of these proteins in foreskin-derived human fibroblasts in culture, the Maher laboratory stably transfected the parental normal cell line with a plasmid carrying antisense against the target mRNA of interest along with a selectable drug resistance marker, or with a vector control plasmid. Antisense transfected cells were selected for drug resistance, and individual colonies (40–60 per target gene of interest) were isolated and characterized. Cell lines derived from independent colonies were assayed for the level of targeted protein (hRev1) and/or the level of antisense they expressed, and the level of targeted mRNA present. Cell strains exhibiting loss of the target mRNA or protein of interest were compared with their parental cell line and vector-control cell strains for sensitivity to the cytotoxic effects of UV and of BPDE and for the frequency of mutations induced in the *hprt* gene. Their results indicate that a substantial decrease in the expression of the human homologs of these two yeast genes, *REV1* and *REV3*, does not increase the sensitivity to the cytotoxic effect of these agents, but greatly decreases the frequency of mutants induced. Their data support the hypothesis that Rev1 and Rev3 are essential for translesion synthesis in human cells.

## 4. Other DNA polymerases

### *4.1. E. coli DNA polymerase I*

#### *4.1.1. DNA replication fork restart facilitated by DNA polymerase IV (DinB) in the reconstituted oriC plasmid replication system*

**Hisaji Maki**, Asako Furukori, and Myron F. Goodman

The inhibition or collapse of the DNA replication fork can result in loss of cell viability or an alteration of genome integrity. However, knowledge of how the DNA replication fork behaves when it encounters DNA blocking lesions in vivo is limited. In order to examine how the replication fork behaves when it encounters a single DNA blocking lesion, Maki et al. utilized a reconstituted system for oriC plasmid DNA replication in vitro. They found that an abasic DNA lesion on the lagging-strand template blocked DNA chain elongation during nascent Okazaki fragment synthesis but had no effect on the progression of the replication fork. In contrast, when the leading-strand template contained the lesion, the replication fork was severely inhibited. They are currently looking at the action of DNA polymerase IV (DinB protein) in rescuing blocked replication forks in vitro to help understand mechanisms underlying genome maintenance by translesion DNA polymerases.

### *4.2. DNA polymerase θ*

#### *4.2.1. Expression and function of Pol κ and Pol θ in human cancers*

**Jiyang O-Wang**

Translesion DNA polymerases are implicated in tolerance to both endogenous and exogenous DNA damage. To investigate their function in tumorigenesis, O-Wang examined the expression of these DNA polymerases in surgically resected, paired tumor and non-tumorous tissues from patients with lung, gastric and colon cancers. Among various polymerases examined, he found that Pol κ and Pol θ were frequently upregulated in human cancers. There was no correlation of elevated Pol κ expression with postoperative survival. Moreover, transgenic mice overexpressing Pol κ in T cells did not develop T cell lymphomas, suggesting that elevated Pol κ expression alone is not sufficient to induce tumor development. Interestingly, a significant association between Pol θ overexpression and a poor clinical outcome was observed. Since Pol θ is implicated in repair of DNA interstrand crosslinks, it is conceivable that tumors expressing high levels of Pol θ are more resistant to chemotherapy, which utilizes DNA crosslinking agents such as cisplatin and mitomycin C. These results suggest that certain low fidelity DNA polymerases might be involved in the initiation and/or progression of human cancer by protecting cells against endogenous and exogenous genotoxic stress.

## 5. Induction of lesions

### *5.1. Why don't you die when I sneeze? Biochemical analysis of hyper-mutational targeting by wild type and mutant AID*

Ronda Bransteitter, Phuong Pham, Peter Calabrese, and **Myron F. Goodman**

Changes in DNA sequence can be required for physiological responses. For example, the synthesis of high affinity antibodies requires activation-induced cytidine deaminase (AID) to initiate somatic hypermutation (SHM) and class-switch recombination (CSR), both of which result in DNA sequence diversity. In order to investigate AID-catalyzed deamination of C → U on single-stranded DNA and on actively transcribed closed circular double-stranded DNA, Goodman and colleagues used a *lacZ* expression assay in *E. coli* to detect the sequence context dependence and clonal distribution of AID-catalyzed C → T transition mutations. Mutations are initially favored at canonical WRC (W = A or T, R = A or G) SHM hotspot motifs, but over time mutations at neighboring non-hotspot sites increase creating random clusters of mutated regions, in a seemingly processive manner. N-terminal AID mutants R35E and R35E-R36D appear less processive and have altered mutational specificity compared to wild type AID. In contrast, a

C-terminal deletion mutant defective in CSR in vivo closely resembles wild type AID. A mutational spectrum generated during transcription of closed circular double-stranded DNA indicates that wild type AID retains its specificity for WRC hotspot motifs within the confines of a moving transcription bubble while introducing clusters of multiple deaminations predominantly on the non-transcribed strand.

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