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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