Questions for Mutagenic DNA Repair Pathways in *Aspergillus nidulans*: Characterization of *uvsJ*^{RAD6} and DNA Polymerase ζ Consisted of *uvsI*^{REV3} and *revA*^{AEV7}

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The RAD6 pathway plays a crucial role in post-replicative DNA repair in eukaryotic cells. Rad6-Rad18 complex required for replication through DNA damage sites. In this process, at least three Rad6-Rad18 dependent pathways operate, which include mutagenic translesion synthesis (TLS) by polymerase ζ (zeta, Rev3-Rev7 complex), error-free translesion synthesis by Polymerase η (eta, Rad30), and postreplication repair of discontinuities by a Rad5 dependent pathway. Mutations caused from unrepaired DNA lesions require active gene actions. In *S. cerevisiae*, Rev3 and Rev7 form DNA polymerase ζ that is able to replicate damaged templates but generates errors. Such translesion DNA synthesis (TLS) was required for most of damage-induced mutagenesis in yeast. More recently, the Y-family of DNA polymerases, UmuC/DinB/Rev1/Rad30 superfamily of mutagenesis proteins, appeared to be crucial to TLS. However, the machinery and its regulation remain elusive. In *Aspergillus nidulans*, four epistatic groups of DNA repair genes, *i. e.*, UvsF, UvsC, UvsI, and UvsB have been classified. Genes in the epistasis groups has been cloned and summarized in Table 1.

I. The *radB* gene of *Aspergillus nidulans*, an allele of *uvsJ*, encodes a homolog of yeast *RAD6*

In *Aspergillus nidulans*, lack of mutagen-induced mutations has been observed in mutants of two different epistasis groups, UvsI and UvsC. In yeast, Rad6 protein is indispensable to generate mutations. However, the function of Rad6 is mostly unknown except its ubiquitin conjugation activity. To investigate whether the *RAD6*-dependent mutation pathway is also operated in *Aspergillus nidulans*, we have been cloned a *Rad6* homolog and characterized its null mutant. A Rad6 homolog of *A. nidulans*, named as *radB* was isolated from a chromosome specific genomic DNA library by a PCR based sib-selection method. Determination of genomic DNA and cDNA sequences of *radB* revealed an open reading frame of 456 bp, interrupted by three introns, encoding a polypeptide of 151 amino acids with high amino acids similarities to the corresponding proteins of *N. crassa* (MUS-8), *S. pombe* (rhp6), and *S. cerevisiae* (Rad6).

Since the cosmid clone having the *radB* gene was assigned on the left arm of the chromosome V, a test for complementation to *uvsJ* which was mapped on the left arm of the chromosome V was

carried out. The 2.9 kb of *Eco*RI/*Bam*HI DNA fragment from the cosmid clone was able to complement to *uvsJ* in terms of MMS-sensitivity. To confirm an allelism of *radB* to *uvsJ*, a mutation site by sequencing of several PCR products from *uvsJ1* mutants was identified. We also found that *uvsJ1* was a temperature sensitive mutant showing the same level of mutagen-sensitivity to wild type at the permissive temperature (25°C) but demonstrating high sensitivity to MMS and UV-radiation at 37°C similar to *uvsJ* null mutants. Disruption of *uvsJ* caused a slow-growth phenotype on an agar plate, indicating its requirement on normal growth. Such a phenotype was not seen in *uvsJ1* mutant carrying a single point mutation at 58th amino acid histidine. In contrast to yeast *rad6* mutants, Δ*uvsJ* as well as *uvsJ1* mutants exhibited increased UV-induced mutation frequencies in the system detecting selenate resistant forward mutations when compared with that for wild type. However, UV-induced reversions of *choA1* mutant alleles in Δ*uvsJ* and *uvsJ1* mutants were not detected. Forced over-expression of UvsJ-[C88A], an E2 enzyme active-site mutant protein, in $uvsJ^{+}$ background resulted in the change of colony growth, indicating a dominant-negative effect of the mutant protein.

uvsJ expression was induced after MMS-treatment demonstrated by Northern analysis. UvsJ proteins also clearly shown to be inducible after MMS-treatment. Without treatment of mutagens, only a little expressions of *uvsJ-p::(sgfp)2* and *uvsJ::(sgfp)2* fusions were detected through the whole fungal body when monitored in the pTuvsJ-p::(sgfp)2 and pTuvsJ::(sgfp)2 transformants. On the other hand, when the cells of the pTuvsJ-p::(sgfp)2 transformant was subject to mutagenic DNA-damage rendered by MMS, the level of dimeric sGFP increased both in vegetative and asexually differentiated cells. Surprisingly, UvsJ::(sGFP)2 protein was specifically accumulated in the nuclei of conidiospores under the conditions of continuous MMS-treatments.

Using the yeast two-hybrid assay system, we were able to show $UvsJ^{Rad6}$-$UvsH^{Rad18}$ interactions in similar to Rad6-Rad18 in yeast. Interestingly, UvsJ1 mutant protein exhibited reduced affinity to UvsH at 37°C, while UVSJ-[C88A] was able to interact with UvsH. Such interactions were confirmed *in vitro* by GST-pull down assay. For further analysis of *uvsJ*, we isolated a protein, JipA that interacted with UvsJ by the yeast two-hybrid system. JipA was able to interact with mutant UvsJ proteins, UvsJ1 and the E2 active-site cysteine mutant UvsJ-C88A. The N- and the C-terminal regions of UvsJ required for the interaction with UvsH were not necessary for the JipA and UvsJ interaction. About 1.4 Kb *jipA* transcript was detected in northern analysis and its amount was not significantly increased in responses to DNA-damaging agents. Sequence determination of genomic and cDNA of *jipA* revealed an ORF of 893 bp interrupted by 2 introns, encoding a putative polypeptide of 262 amino acids. JipA has 40% amino acid sequence identity to Tip41 of *S. cerevisiae* that negatively regulates the TOR signaling pathway by interacting with Tap42. *JipA* null mutant was constructed by targeted gene disruption. *ΔjipA* mutant showed similar levels of MMS-sensitivity to wild type. Null mutation of *jipA* conferred resistance to rapamycin, a specific inhibitor of TOR kinases. However, *ΔuvsJ* exhibited a similar level of rapamycin sensitivity to wild type. We are currently under investigating a possible role of UvsJ in respect to JipA ubiquitination on the TOR signaling pathway.

II. Isolation and characterization of *uvsI*^{rev3} and *revA*^{rev7} in *Aspergillus nidulans*, an possible error-prone DNA polymerase ζ.

Polymerase ζ is an error-prone DNA polymerase of yeast, consisting of at least two subunits: Rev3 of the catalytic component and Rev7 of unknown function in yeast. This complex is responsible for translesion DNA synthesis (TLS) to replicate past certain types of DNA lesions. In *Aspergillus nidulans*, the *uvsI* gene has been cloned and the 9.1 kb DNA fragment containing the ORF was sequenced. UvsI carried well conserved the hexapetide motifs among DNA polymerases in the C-terminus and showed a high amino acid similarity to Rev3. In addition, UvsI protein was able to interact with yeast Rev7 tested based on the yeast two-hybrid assay system, indicating that UvsI is a functional homolog of Rev3. About 5.3 kb *uvsI* transcript was detected in northern analysis and its transcription amount was increased in response to UV and MMS. A null mutant having the deletion of the entire *uvsI* ORF was constructed. The phenotypes of Δ*uvsI* mutants were not deviated from those reported in *uvsI501* mutants by showing high UV-sensitivity and reductions of spontaneous and UV-induced reversions of certain mutant alleles. However, frequencies of forward mutations selecting selenate resistant mutants were similar to that for wild type. No growth defect was exhibited in *ΔuvsI* differing from the embryonic lethality of mouse *rev3I* null mutants. Among genes responsible for mutation induction in *Aspergillus nidulans*, synergistic interactions between Δ*uvsI*^{rev3} and Δ*uvsC*^{rad51}, and between Δ*uvsJ*^{rad6} and Δ*uvsC*^{rad51} were observed. Sequencing of an upstream region of the *uvsI* gene revealed another ORF of 1,401 bp without a putative intron in opposite direction. This ORF starts 365 bp upstream from the *uvsI* start codon and encodes a putative polypeptide exhibiting a high amino acid similarity to SLU7 involving in the second step of pre-mRNA splicing in *S. cerevisiae.* 1.5 kb Transcripts of the ORF increased in response to mutagen treatments.

We also isolated a yeast Rev7 homolog, RevA by using the truncated UvsI protein as a bait in the yeast two-hybrid system. UvsI-RevA interaction was further confirmed in vitro using a GST-pull down assay. A cosmid clone carrying the *revA* gene was identified by the PCR based sib-selection method from the chromosome specific genomic library. The *revA* gene is located at the *Aspergillus nidulans* chromosome VII. DNA sequencing of the cDNA as well as genomic DNA of *revA* revealed one open reading frame of 813 bp, encoding a polypeptide of 270 amino acids. No intron was found in the coding region. The 1.4Kb *revA*transcript was detected and the amount of transcript was increased after MMS-treatment examined by Northern analysis. RevA has a HORMA domain (for Hop1p, Rev7p and MAD2) which has been implied to recognize chromatin states causing from DNA adducts, double stranded breaks or non-attachment to the spindle and to act as an adaptor recruiting other proteins. *revA* null mutant was generated by targeted gene replacement. *ΔrevA* mutant was not sensitive to MMS and UV. In addition, forward mutation frequencies were similar to wild type. In conclusion, DNA polymerase ζ MMS-induction of the *revA* transcript was dependent on *uvsD,* a homolog of *rad26* in *S. pombe,* but was normal in *uvsB*^{Rad3} mutant background. In conclusion, DNA polymerase ζ was not the major protein in producing mutations in *Aspergillus nidulans.*

Table 1. Epistatic groups and genes of DNA repair in *Aspergillus nidulans*

Epistasis groups	Genes	Possible Functions
UvsF	*uvsF*	*uvsF = RFC1 homolog (Kafer and May, 1997)*
	uvsH	*uvsH = RAD18 homolog (Yoon et al., 1995)*
	uvsJ	*uvsJ = RAD6 homolog (Jang and Chae, This study)*
UvsC	*uvsC*	*uvsC = RecA, RAD51 homolog (Seong et al., 1997)*
	uvsE	*musN = recQ homolog (Hoffman and Harris, 2001)*
	uvsA	*radA = RAD52 homolog (Ka and Chae, unpublished data)*
	musN	
	radA	
UvsI	*uvsI*	*uvsI = REV3 homolog (Han et al, 1998)*
	musR	*revA = REV7 homolog (Cha et al., This study)*
	revA	
UvsB	*uvsB*	*uvsB = ATR, Rad3 homolog (Hoffman and Harris, 2000)*
	uvsD	*uvsD = rad26 in S. pombe (De Souza et al., 1999)*