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The Effects of *uvsH* Gene in *Aspergillus nidulans* on Mitotic Recombination Behabiour

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*Aspergillus nidulans*에 있어서 *uvsH* 유전자가 Mitotic Recombination에 미치는 영향

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Abstract: The strain of *Aspergillus nidulans* carring a *uvsH* mutation which had been shown to be absolutely required for UV or 4-NQO induced mutagenic processes was studied on mitotic recombinational behaviour. Although the effect of *uvsH* locus on spontaneous mitotic crossing over between *fpB37* and centromere was not considerable, UV-induced intergenic recombination did not occur in *uvsH/uvsH* homozygotic diploid. In case of gene conversion at riboflavin locus between a pair of non-complementary alleles, *riboA1* and *riboA3*, the *uvsH* mutation was not concerned with that process occurred spontaneously or induced by UV irradiation. When the cells were irradiated by UV light, high degrees of aneuploid productions were detected in diploid homozygous for *uvsH* as compared with wild type, while much difference was not found during normal growth.
Key words: *Aspergillus nidulans*, 4NQO and UV-sensitive inwtant, mitotic recombination.

One of the most interesting and least understood basic genetic processes is recombination. Genetic recombination seems to be of universal occurrence in living organisms. The knowledge of its mechanism is of basic importance because it is the process by which segments of corresponding chromosomes of paternal and maternal origin are exchanged. Combined genetic and biochemical analysis, especially in prokaryotes, has revealed a complex interrelationship of recombination with DNA repair and with mutation (Kimball, 1978 ; Radding, 1978). Many of the enzymes involved in recombination and repair are nucleases of various types (Radding, 1982). While the detailed biochemical reactions in most cases are still unknown and often can not be identified from enzyme properties and reactions *in vitro*, evidence for *in vivo* function can in some cases be deduced from the properties of mutants. Numerous mutants in *Ustilago maydis* (Holliday, 1965), *Neurospora crease* (Schroeder, 1975) and *Aspergillus nidulans* (Shanfield and Kafer, 1969) ; Jansen, 1970 ; Fortuin, 1971) are exceptionally sensitive to UV light. Several of these mutants are altered in properties of recombination, whether meiotic or mitotic, and of mutation.

Recently, several genes that are involved in the rapair process of *Aspergillus nidulans*

have been identified in our laboratory. Among them, the *uvsH* gene was absolutely required for mutagenic processes induced by UV or 4-NQO (Han et al., 1983). In view of these observations, it became of interest to determine the role of the *uvsH* gene in UV-induced recombination. In this paper, the induction of mitotic crossing over and gene conversion by UV irradiation were compared in *uvsH/uvsH* homozygotic diploid and in wild type.

MATERIALS AND METHODS

Strains

The haploid strains and the diploid strains which were constructed for the present study are listed in Table 1. In order to investigate gene conversion, heteroallelic mutant over *riboA*1 genetic marker was isolated from FGSC 154. The R3 strain was suitable for this purpose and its heteroallelic site was designated *riboA*3.

Media

Culture media were prepared as described earlier (Han et al., 1983a). p-Fluorophenylalanine (PFP) was added to a final concentration 100mg/m*l* into the minimal agar medium.

UV irradiation

Cells were harvested with 0.08 Tween 80, washed twice with 0.05M sodium citrate buffer (pH6.0) and resuspended in same buffer at cell density of 2×10^7cells/m*l*. Portions of 7m*l* were irradiated with a UV lamp (UVP INC.(CA. 91778 U.S.A. lamp No. 3400801)) at a dose rate 16erg/mm²/sec in an open Petridish with mild magnetic stirring.

Estimation of mitotic recombination

Conidiospores of diploid strain plated on a minimal medium supplemented with PFP can not grow unless a somatic crossing over between centromere and *fpB*37, or a mutation of the wild allele of *fpB*37, has occurred. Segregants were scored as well-sporulating colonies growing on media supplemented with PFP after plating ten thousands of diploid conidia. Spontaneous and UV-induced mitotic segregants arising from intragenic mitotic recombination were identified as growing colonies on minimal media without riboflavin. Genetic markers on the linkage

Table 1. *Strains*

HAPLOIDS		
FGSC 154	adE20, biA1; wA3, cnxE16; sC12; methG1; nicA2; lacA1; choA1; chaA1	
FGSC 163	suA1adE20, yA2, adE20; AcrA1; phenA2; pyroA4; lysB5; sB3; nicB8; coA1	
FGSC 168	suA$^{+}_{2}$adE20, adE20, biA1; sB3, choA1; chaA1	
FSSC 475	fpB37, galD5, suA1adE20, riboA1, anA1, pabaA1, yA2, adE20, biA1; sD85, fwA2	
R3	adE20, riboA3, biA1; wA3, cnxE16; sC12; methG1, nicA2; lacA1; choA1; chaA1	
UVSH*	suA1adE20, adE20, biA1; sB3; choA1; chaA1; uvsH	
UVS1-111	fpB37, galD5, suA1adE20, riboA1, anA1, pabaA1, yA2, adE20, biA1; sB3; uvsH	
UVS1-814	riboA3, biA1, adE20; AcrA1; sB3; pyroA4; lysB5; nicB8; chaA1; uvsH	
DIPLOIDS		
D400	+/+	FGSC168×FGSC 475
D411	uvsH/uvsH	UVSH×UVS1-111
Dr40	+/+	R3×FGSC 475
Dr11	uvsH/uvsH	UVS1-814×UVS1-111

Requirements: ad; adenine, paba; p-aminobenzoic acid, bi; biotine, pyro; pyridoxine, nic; nicotinic acid, cho; choline, ribo; riboflavin, phen; phenylalanine, meth ; methionine, lys; lysine, an; aneurine
Conidial colors: cha; chartreuse, y; yellow, w; white
Inability to utilize: s; sulfite, cnx; nitrate, gal; galactose, lac; lactose
Resistance: Acr; acriflavin, fp; p-fluorophenylalanine
Adenine suppressor: suA1adE20, Compact colony: co
*It was described as UVS1 in former article. (Han et al., 1983a; b)

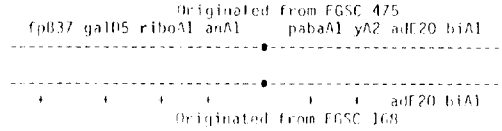

Fig. 1. *Genetic map of linkage group I in D400*

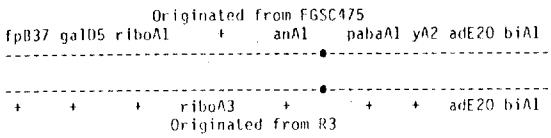

Fig.2. *Genetic map of linkage group I in Dr40.*
The riboA3 is a heteroallelic locus of the riboA1.

group 1 of D400 and Dr40 were shown in Fig.1 and Fig.2.

RESULTS

UV-sensitivity at Gl cell stage

As shown in Fig.3, the *uvsH/uvsH* homozygotic diploid was also sensitive to UV irradiation as well as the haploid UVSH, although

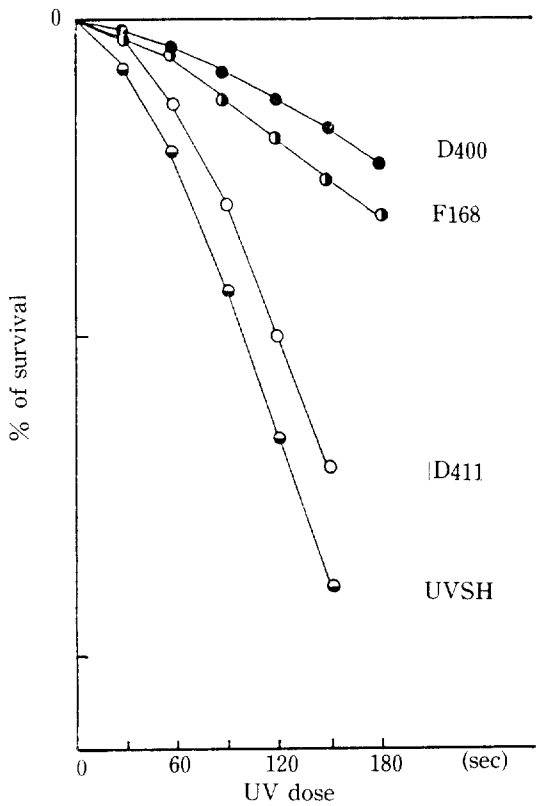

Fig. 3. *UV survival curves of diploid D411 homozygous for uvsH, D400 wild type, haploid UVSH and FGSC 168 wild type. Cells were irradiated by a 16erg/mm²/sec dose rate UV lamp.*

Table 2. *Frequencies of spontaneous mitotic crossing over.*

Strain	Mean no. of cells/plate	Mean no. of fpa^{r}* colonies/plate	Frequency of fpa^{r} colonies
FGSC168	7.8×10^{6}	0	1.28×10^{-7}
UVSH	8.7×10^{6}	0	1.15×10^{-7}
D400	2.9×10^{4}	14.6	5.12×10^{-4}
D411	9.3×10^{4}	54.4	5.91×10^{-4}

*p-fluorophenylalanine resistant

diploids were more resistant than haploids. Since it was recently being recognized that cell stage was of importance in both mutagenesis and recombination (Barale et al., 1982 ; Fabre, 1978 ; Roman and Fabre, 1983 ; Han and Kang, 1985), the following experiments were carried out which only Gl stage-arrested conidiospores.

Effect of *uvsH* locus on spontaneous intergenic and intragenic mitotic recombination

The level of spontaneous intergenic recombination between *fpB37* and centromere was nearly the same in the strain homozygous for *uvsH* mutation as that of in the wild type (Table 2). As shown in Table 3, the effect of *uvsH* site on the frequency of gene conversion as well as of intergenic recombination was not considerable in *uvsH/uvsH* homozygotic diploid.

Effect of *uvsH* mutation on UV-induced intergenic recombination

Fig.4 shows curves for UV-incued mitotic crossing over in wild type and in diploid homozygous for *uvsH*. The frequency of induced

Table 3. *Frequencies of spontaneous gene conversion*

Strains	Mean no. of cells/plate	Mean no. of $ribo^{+}$ colonies/plate	Frequency of $ribo^{+}$ colonies
FGSC475	7.7×10^{7}	0	1.3×10^{-8}
R3	4.8×10^{7}	0	2.1×10^{-8}
UVS1-111	8.3×10^{7}	0	1.2×10^{-8}
UVS1-814	5.2×10^{7}	0	1.9×10^{-8}
Dr 40	6.2×10^{7}	81	1.3×10^{-7}
Dr 11	5.4×10^{7}	62	1.1×10^{-7}

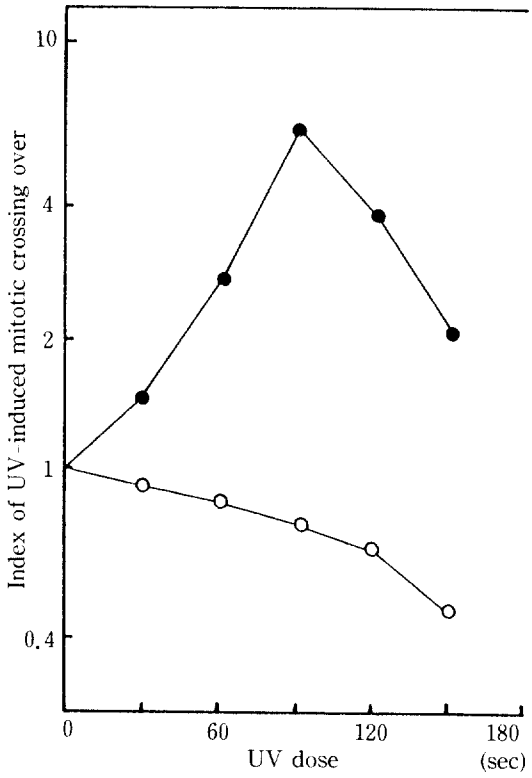

Fig. 4. *Frequencies of mitotic crossing lover between fpB37 and centromere induced by 16 erg/mm²/sec UV light in diploid D411(○—○) homozygous for uvsH and D400 wild type(●—●). Induction levels are expressed as index for the spontaneous mitotic crossing over frequencies.*

mitotic intergenic recombination was found not to increase together with the increasing UV dose in *uvsH/uvsH* homozyotic diploid. In wild type, the decline in recombination frequences at high doses can be interpreted on the basis that killing and recombination are stochastically dependent processes, i.e. at high doses, the probability of survival of recombinant clones is less than that of nonrecombinants.

Effect of *uvsH* mutation on UV-induced mitotic gene conversion

Gnen conversion is generally measured by the appearance of revertants to prototrophy at heteroallelic loci in diploid strains. UV-induced intragenic recombination at riboflavin locus between a pair of non-complementary alleles, *riboA*1 and *riboA*3, was tested in diploid homozygous for *uvsH* and wild type. The *uvsH* mutation prevented induction of reversion (Han and Kang, 1985). In wild type strain Dr40, therefore, both spontaneous and UV-induced ribo⁺ prototrophs can be generated by reversion as well as by conversion, while in *uvsH/uvsH* homozygotic diploid Dr11 by conversion only. The frequency of reversion was found to be one or two magnitude lower than that of conversion in both spontaneous and UV-induced ribo⁺ prototrophs, which suggested that the contribution of reversions to the level of ribo⁺ prototrophs in the strain with wild phenotype be rather small. However, the spontaneous gene conversion frequency was so low that the UV-induced gene conversion in wild type could not be distinguished from the UV-induced reversion of *riboA*3. This may be due to the *riboA*1 locus which seems to be caused by deletion. As shown in Table 4, UV-inducibility of gene connversion was not significantly affected by *uvsH* mutation.

Mitotic aneuploid from *uvsH/uvsH* homozygotic diploid

Mitotic segregants of the aneuploid type were isolated by the method of selection for aneuploid-looking, irregular, sectoring colonies over spontaneous and UV-induced production. When the cells were irradiated for 90 sec with a 16erg/mmi/sec dose rate UV lamp, almost 8-fold increase of aneuploid type has been detected in D411 homozygous for *uvsH* as compared with D400 wild type,

Table 4. *UV-Induced gene conversion frequencies.*

Strain	720 erg/mm	1440 erg/mm	2160 erg/mm
FGSC475	1.2×10^{-8}	1.5×10^{-8}	2.3×10^{-8}
R3	2.9×10^{-7}	3.8×10^{-6}	9.1×10^{-6}
UVS1-111	8.6×10^{-8}	2.1×10^{-8}	2.5×10^{-8}
UVS1-814	1.3×10^{-8}	1.9×10^{-8}	1.6×10^{-8}
Dr 40	1.2×10^{-7}	3.5×10^{-6}	4.8×10^{-6}
Dr 11	9.7×10^{-7}	3.1×10^{-6}	4.2×10^{-6}

Table 5. *Frequencies of spontaneous and UV -induced aneuploid production.*

Strain	Spontaneous aneuploid production	UV-induced aneuploid production	
		480 erg/mm²	1440 erg/mm²
D400 (+/+)	0.1×10^{-2}	3.4×10^{-2}	5.1×10^{-2}
D411 (*uvsH/uvsH*)	1.3×10^{-2}	1.8×10^{-1}	4.1×10^{-1}

while much difference was not found during normal growth (Table 5).

DISCUSSION

UV-sensitive mutants have been isolated and used extensively to gain insight into recombination mechanisms. Its effectiveness may be dut to the close relationship of DNA repair and recombination. We investigated here the effects of uvsH mutation on spontaneous and UV-induced mitotic recombination. Intergenic and intragenic mitotic recombination occured spontaneously during the growth of *uvsH/uvsH* homozygotic diploid at a normal frequency. Further more, spontaneous meiotic recombination was not concerned with *uvsH* locus (Han *et al.*, 1983b). Esposito (1968) showed that the frequency of mitotic recombination could be stimulated by a variety of treatments, including UV light, ionizing radiation and chemical recombinagens. Although gene conversion and associated reciprocal recombination are thought to be results of mismatched bases caused by recombinagens and their mismatch repair (review: Whitehouse, 1982), the recombination mechanisms are still obscure. Recently, Roman and Fabre (1983) demonstrated that gene conversion and associated reciprocal recombination were separable events in vegetative cells of *Saccharomyces cerevisiae* with respect to the stage in the cell cycle when each occured and possibly with respect to mechanism. We obtained a similar result with diploid homozygous for *uvsH* on the events of mitotic recombination in *Aspergillus nidulans*. The data presented here showed that *uvsH* locus was responsible for the UV-induced mitotic intergenic crossing over, not gene conversion, at G1 cell stage. This result suggests that the enzyme involved in UV-induced gene conversion is somewhat different from that of intergenic crossing over, while *uvsH* locus was found to be not a regulatory site (Han, 1986). The frequency of mitotic intergenic crossing over was not increased by UV radiation in *uvsH/uvsH* diploid. In *Saccharomyces cerevisiae*, an error-prone recombinational mode of DNA damage tolerance has been proposed (Haynes and Kunz, 1981). The fact that *uvsH*, which was known to be concerned with error-prone repair defectiveness (Han and Kang, 1985), blocked the UV-induced mitotic crossing over entirely suggests that the mitotic crossing over induced by UV radiation between homologous chromosomes is under the control of *uvsH* gene and thus this process may be responsible for error-prone repair pathways. Analysis of all types of mitotic segregants from diploids of *Aspergillus nidulans* with non-selective methods showed that aneuploids resulting from mitotic nondisjunction occured with a relatively high frequency of 1-2% (Kafer, 1961). From the results of spontaneous aneuploid production in Dr40 wild type and Dr11 homozygous for *uvsH*, both of them were not deviated from Kafer's results. But aneuploids were produced quite frequently in *uvsH/uvsH* homozygotic diploid when irradiated by UV light. It seems interesting to find out the interrelationship between DNA repair and chromosomal nondisjunction during exposed to mutagens. Actually, the cell lethally of *uvsH/uvsH* diploid for UV radiation was reduced in compensation for the aneuploid production. Therfore, we propose that sister chromatid exchanges which were not detected genetically may play a certain role in enhancing cell survivality as a tool of DNA repair. After unrepaired damage sites on both sister chromatids are arranged into one sister chromatid which is finally ruled out, there probably mitotic nondisjunction occurs.

적 요

*Aspergillus nidulans*에서, UV나 4-NQO에 의한 돌연변이 유발에 있어 절대적으로 필요한 ***uvsH*** 돌연변이를 가지고 있는 변이주를 이용하여 mitotic recombination 현상을 조사하였다. 비록 ***uvsH*** locus는 ***fpB*** 37과 centromere 사이에서의 자발적인 mitotic crossing over에는 영향을 주지 않았지만 ***uvsH/uvsH*** 동형이배체에서 UV에 의한 intergenic recombination은 일어나지 않았다.

또한 서로 상보적이 아닌 ***riboA*** 1과 ***riboA*** 3 유전학적 표지에서의 riboflavin에 대한 gene conversion에 있어서 ***uvsH*** 돌연변이는 자발적이든 UV로 유발시켰던 이 과정에 관여하고 있지 않았다. 비록 정상적인 성장에서는 거의 차이가 없었지만, 세포들을 UV로 조사하였을 때 야생주에 비해 ***uvsH*** 동형 이배체에서의 aneuploid 발생이 높은 빈도로 나타났다.

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