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Different properties of mutagen sensitive *musN* mutant, a member of the UvsC group, from *uvsC* mutant strains in *Aspergillus*

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*Aspergillus*의 UvsC group에 속한 돌연변이원 감수성 변이주 *musN*이 *uvsC* 돌연변이주와 다른 성질

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Mutangen sensitive hyper-rec type *musN* mutants were assigned into the UvsC group which contains genes involved in recombination and mutation. However, phenotypic properties of *musN* mutants were very different from those found in *uvsC* mutant strains which are rec- and lack UV-induced mutation. *musN* was not a mutator like *uvsC*. In addition, selenate resistant mutations in *musN* were induced similar to those in wild types by UV irradiation. Wild type levels of UV-sensitivity in dividing cells of *musN* also differ from the *uvsC* phenotypes. These indicate that the UvsC group has branched pathways.

돌연변이원 감수성 hyper-rec type *musN* 돌연변이주를 recombination과 mutation에 관여하는 유전자들이 포함된 UvsC group에 포함시켰다. 하지만, rec- 및 UV에 의한 돌연변이가 일어나지 않는 *uvsC* 돌연변이주와는 달리 *musN*은 매우 다른 성질을 나타냈다. *musN*은 *uvsC*와는 달리 mutator가 아니었다. 또한, UV 조사에 따른 selenate resistant mutation도 야생주와 비슷한 수준으로 유발되었다. 분열하는 *musN* 세포에서의 UV 감수성 또한 *uvsC*와는 달리 정상이었다. 이같은 결과는 UvsC group이 branched pathways를 갖고 있음을 나타낸다.

Key words : *uvs*, *musN*, DNA repair, *Aspergillus*

I. Introduction

The stability of DNA is continuously challenged by various physical and chemical agents which introduce a wide array of changes in DNA. Most mutations are the results of damage caused by these agents, e.g., heat causes deamination of bases and base loss by glycosylic hydrolysis; UV irradiation produces pyrimidine dimers, 6-4 photoproducts and strand breaks; ionizing radiation results in ring opening, base fragmentation, and single and double strand breaks (Singer and Kusmierek 1982). Some examples of chemical agents that damage DNA range from activated oxygen species generated during oxidative metabolism, common metabolites like glucose, inorganic and organic electrophiles including metals, alkylating agents and polycyclic aromatic hydrocarbons. To insure the stability of DNA, complex DNA repair mechanisms have

evolved that undo the damage caused to DNA. The mechanisms involved in repairing the damage are not simple straight forward biochemical pathways, rather they are more like repair networks or repair system (von Borstel and Hastings 1977).

The remarkable feats of DNA repair processes have first been identified in prokaryotes, especially in *E.coli* and its phages. By now over 50 genes and many enzymes are known in detail which contribute to the removal of damaged or inappropreate bases. In *E.coli,* various different repair mechanisms have been characterized and these are of four basic types (Sancar and Sancar 1988). Two of these occur in non dividing cells, namely 1) simple damage reversal, and 2) excision repair. Two others are postrepli- cation repair mechanisms, either 3) recombi- national repair, or 4) error-prone repair, leading to increased mutation. In eukaryotes, some evidence for the DNA repair processes corresponding to those of *E.coli* have been obtained, but none have yet been fully elucidated.

In *Aspergillus nidulans* the four epistatic groups of DNA repair include *uvs* mutations with similar properties (Kafer and Mayor 1986; Chae and Kafer 1993): namely, 1) the "UvsF" group of *uvs* mutations which increase UV mutagenesis and spontaneous mitotic recombination as found typically for excision repair types in *E.coli* and yeast; 2) the "UvsC" group of mutants which are sterile and abolish spontaneous mitotic recombination, but in addition increase spontaneous and practically lack UV-induced mutation (Jansen 1972; Kafer and Mayor 1986); 3) the "UvsB" group of mutations which greatly increase chromosomal aberrations and deletions, probably as a result of unrepaired chromosomal breaks, and therefore show low ascospore viability and increased mitotic recombination of a nonreciprocal type; and 4) the "UvsI" group of mutants which are defective in generating certain types of mutations.

Nine mutagen sensitive *mus* mutants have been tried to assign epistatic groups into the four "Uvs" groups (Kafer and Chae 1994). None of them exhibited epistatic interaction with UvsF or UvsB group mutant strains. Hyper-rec type of *musN* was assigned into the UvsC group based mainly on 4-NQO survivals. In this paper, we characterized *musN* further for phenotypic grouping. Also, we tried to show that the UvsC group contains genes work on branched or minor pathways in terms of mutation and recombination.

II. Materials and methods

1. Media, strains, and genetic works

Standard minimal and complete media and genetic procedures were those of Pontecorvo et al. (1953), Kafer (1977), and Scott and Kafer (1982). Strains for *uvs* and *mus* were those of Kafer and Chae (1994), and Chae and Kafer (1993) which are partially isogenic.

2. Determination of mutagen sensitivity

Treatment of MMS and irradiation of UV light were followed as previously described (Chae and Kafer 1993). UV survivals of dividing cells were calculated when plated conidia were irradiated after 4 h at 37°C.

3. Mutation assays

Mutants resistant to selenate were selected on nitrate-free MM containing 0.1 mM selenate, 0.02 mM D-methionine, and 5 mM urea (Arst 1968). For UV mutagenesis, conidia were plated onto appropriate media and irradiated imme- diately, using a GE germicidal 30W lamp at a dose rate of 1.6 J/m^2/sec. Plates were rotated at 33 1/2 rpm for even exposure.

III. Results and discussion

1. MMS test supporting the assignment of *musN* to the "UvsC" group

In addition to the results of Kafer and Chae

(1994), survival curves of conidia plated on MMS medium were obtained for double mutant strains combining *musN* with *uvsJ*, a member of the UvsF group. Synergistic interactions were observed in this case (Fig. 1). The result for *uvsJ* gives positive evidence for non-epistatic interaction of *musN* with members of the UvsF group. They support the more indirect evidence for the other two members of the UvsF group, which indicated lethality of *musN* double mutants with *uvsF*, and semi-lethality of *musN;uvsH* strains (Chae and Kafer 1993). Furthermore, when conidia were plated onto complete medium (CM) containing 0.005% MMS, triple mutants, *musN;uvsH;uvsI*, were shown to be clearly more sensitive than any of the three double mutant strains, namely *musN;uvsH*, *musN;uvsI*, or *uvsH;uvsI* (data not shown). This findings further demonstrated that these three mutants all belong to different epistatic groups. Combining the results for epistasis group based on the survival to 4-NQO (Kafer and Chae 1994) and to MMS, *musN* can be assigned into the "UvsC" group.

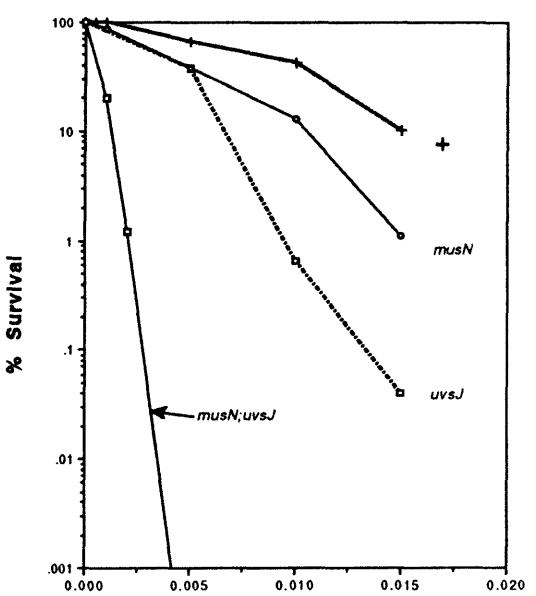

Fig. 1. MMS (metylmethane sulfonate) survivals of *musN* (open circles), *uvsJ* (closed circles), *musN;uvsJ* double mutants (open square).

2. UV-survival of *musN* mutants in dividing cells

The *musN* gene appeared to belong to the UvsC group. Typically, UvsC group genes, *uvsC*, *uvsE*, and *uvsA*, showed increases of sensitivity to UV light during growth compared to that measured with quiescent conidia (for *uvsC* and *uvsE*, Fortuin 1971; for *uvsA*, Jansen 1967). Thus, survival of *musN* strains to UV light was measured in growing cells. Different from other members of the UvsC group, *musN* mutant showed wild type levels of UV-sensitivity in growing cells (Fig. 2). In addition, no apparent interaction of *musN* with members of the three groups, UvsC, UvsI, and UvsB, was observed as expected, when UV-sensitivities of these double mutants were measured in dividing cells and compared to component single mutant strains (*musN;uvsF* double mutants are lethal; Fig. 2).

3. Spontaneous and UV-induced mutation in *musN* strains

It was known that both *uvsC* and *uvsE*, members of the UvsC epistatic group, show increased spontaneous mutation frequencies, i.e., they have mutator effects (Jansen 1972), while UV-induced mutations of these strains are much reduced compared to wild type (Kafer and Mayor 1986). Since *musN* are classified as a member of the UvsC group, it was of interest whether *musN* affects mutation frequencies spontaneously and induced by UV light. Selenate resistant mutation detection system was applied to measure the spontaneous and UV-induced forward mutation frequencies in *musN* mutant strains. It was found that *musN* by itself does not alter the frequencies of spontaneous, nor of UV-induced, selenate resistant mutations (Fig. 3 and Fig. 4).

In addition, *uvsC* is epistatic for spontaneous mutation in the double mutants with *musN*,

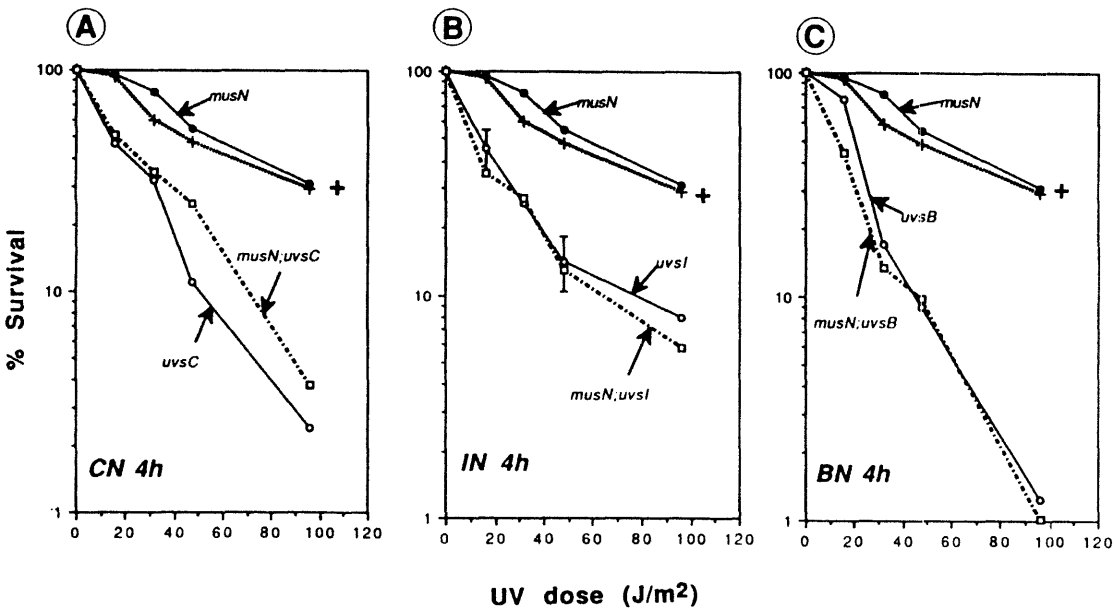

Fig. 2. Tests of *musN* for epistatic interactions with *uvs* of three groups using UV treatment of dividing (UV-survival of 4h preincubated, germinating conidia).
A, *musN* with *uvsC*; **B**, *musN;uvsI* double mutants; **C**, *musN;uvsB* double mutants

i.e., *musN;uvsC* strains are mutators like *uvsC* alone (Fig. 3). However, UV-induced mutation frequencies of the double mutant differ from the reduced levels typical for *uvsC* by showing more induced levels (Fig. 4). This results is of interest and unexpected, since the defect of *uvsC* in UV-induced mutagenesis was restored to a small extent by introducing *musN* mutation. Possibly, a small portion of premutagenic lesions produced by UV light in *uvsC;musN* genetic background may be chan-neled through other mutagenic repair path-way(s). On the other hand, premutagenic lesions generated after UV irradiation in *uvsC* strains may not be good substrate for such pathway(s). Thus, *uvsC* gene presumably is not responsible for all types of mutations. In the case of *uvsC;musN* double mutant strains, enhanced selenate resistant mutation frequencies after UV irradiation compared to those in *uvsC* strains were largely due to active *uvsI* gene products, since in triple mutants of *uvsC;musN* with *uvsI*, the mutation frequencies were reduced compared to those in *ucsC;musN* double mutant strains and

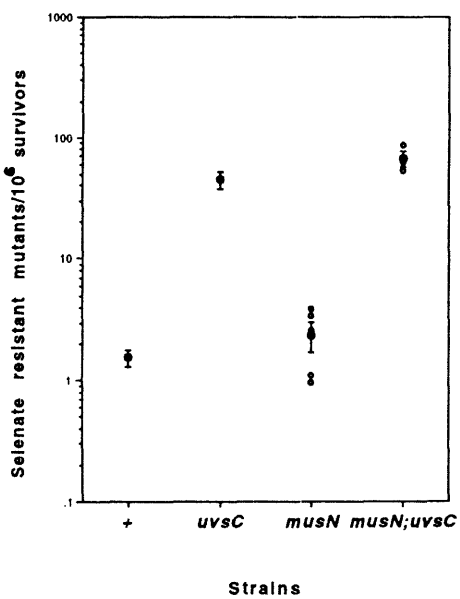

Fig. 3. Spontaneous mutation to selenate-resistance in *musN* and *musN;uvsC* double mutant strains. Individual frequencies of 3-5 independent experiments plotted (closed circles) and their averages with standard error bars indicated (open circles)

similar to those in *uvsC;uvsI* double mutant strains (data not shown).

Both spontaneous and UV-induced selenate resistant mutations were also analyzed in all other *mus* mutant strains. As found for *musN*, all these mutants, namely *musL, O, K, P, Q, R* and *musS*, showed wild type levels of spontaneous and UV-induced mutations (Fig. 5).

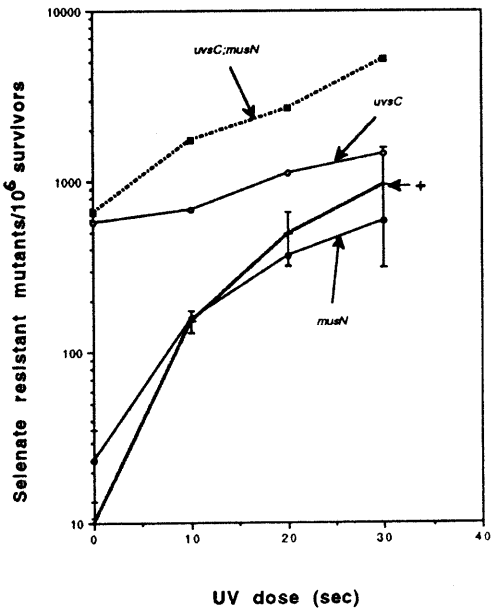

Fig. 4. UV-induced selenate-resistance mutation in *musN* and *musN;uvsC* double mutant strains. Frequencies are shown (average of 3 independent experiments for *musN, musN;uvsC*). For UV-survival, *musN, uvsC, musN;uvsC* strains are no more sensitive than wild type within the tested UV dose ranges (UV dose rate = 1.6 J/m^2/sec)

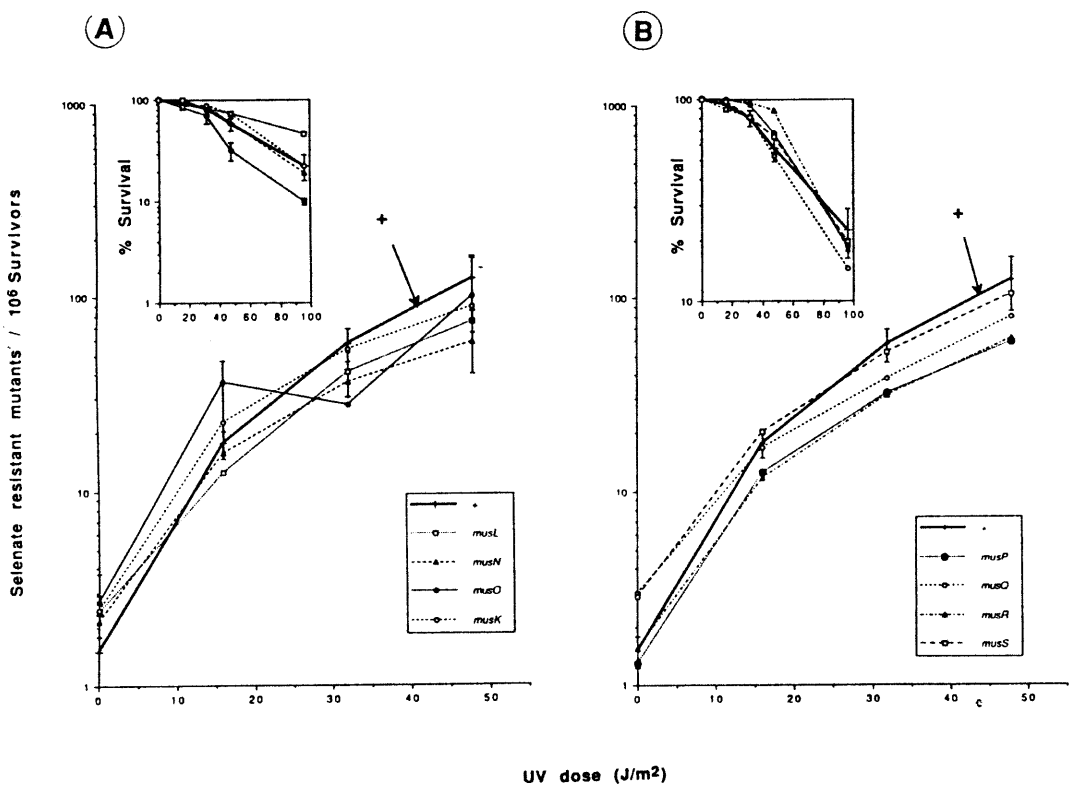

Fig. 5. UV-induced selenate-resistance mutation in *mus*+ control (+), and *mus* strains. **A**, *musK, musL, musN,* and *musO*; **B**, *musP, musQ, musR*, and *musS*. Symbols as indicated in

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