Tests for epistasis of *bimD* with UV repair genes representing four epistatic groups and their putative types of DNA repair in *Aspergillus nidulans*

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bimD6, a ts mitotic mutant sensitive to UV only in dividing cells and defective in recombination, was tested for epistasis with members of the four Uvs groups of *A. nidulans*. UV survival of quiescent and germinating conidia was compared. While details varied between tests, results from UV-treated germinating conidia consistently showed additive or synergistic interaction. This suggested a new type of function for *bimD* and perhaps indirect effects on DNA repair and recombination, in line with recent evidence for defects in chromosome cohesion and compaction in mutants of *bimD* and its homologs (van Heemst *et al*. 2001 PNAS **98**:6267-6272).

The ts mutant, *bimD6*, which is blocked in mitosis at 42°, was found to be meiotic-defective and hypersensitive to UV and MMS (methyl-methane sulfonate) during growth at low temperature. When cloned its sequence showed no homology to any known gene (Denison *et al*. 1992 Genetics **134**:1085-1096). We therefore started a project testing *bimD* for epistasis with *uvs* gene members of the four Uvs groups identified in Aspergillus (Chae and Kafer 1993 Curr. Genet. **24**:67-74). Some of these groups contain genes with mutants of very similar phenotypes, suggestive of functional grouping, as known for epistatic groups of yeast *RAD* genes involved in radiation repair. Similarities and differences of the Aspergillus Uvs groups compared to the budding yeast RAD groups and repair types are increasingly becoming documented (see below).

In Aspergillus, conidia are dormant non-metabolizing spores, basically in G_0, and the first mitosis occurs only after several hours of exposure to growth conditions. Certain repair-defective mutants are found to be UV sensitive when quiescent conidia are treated; presumably in such cases repair can occur in haploid non-dividing cells, as typically occurs in excision and possibly in other types of error-free repair. Alternatively, mutants may show sensitivity only when growing cells are treated, as was found for *bimD6* and is typical for mutants defective in recombinational repair. The latter type of treatment was therefore more likely to give meaningful results but we used both methods, because synergism in double mutants has occasionally also been found when one or both of the single mutants did not show sensitivity (e.g., in MMS tests of *uvsA101* and *uvsI501*, neither of which is hypersensitive to MMS, the double mutant showed high sensitivity; Chae and Kafer 1993 ref. cit.).

Single and double mutant strains of *bimD6* and *uvs* mutations from each of the four epistatic groups were compared for UV survival using parallel platings of UV treated conidia, irradiated either immediately (data not shown) or after preincubation (4 examples in Fig. 1). Overall, the results of tests using dividing cells showed additive or synergistic interaction of *bimD6* with all of the tested mutations. However, in a few cases the difference between double and single mutants were barely additive; namely for *uvsD153* (Fig. 1C), and especially for *musN227* which is not UV sensitive (Fig. 1D). In addition, since for such tests null mutations were not available (especially for *bimD* and *uvsF* which are essential genes) these results have to be interpreted with caution.

However, conclusions can be reached with some confidence when the null phenotype is known to be identical to that of the mutants used. This appears to be likely for *uvsI501*, and has recently been shown for the cell cycle checkpont genes *uvsB* and *uvsD*, after sequencing and construction of disruption alleles (De Souza *et al*. 1999 Mol. Biol. Cell **10**:3661-3674). This is however not the case for *musN227* which will need further testing. (*musN227* had produced inconsistent results which could well have been due to partial function; namely, while *musN227* apparently showed typical epistatic interaction with *uvsC114* in tests for MMS survival, non-epistatic interaction, i.e., significantly increased UV-induced mutation was obtained for the *musN227;uvsC114* double mutant strain; Kafer and Chae 1994 Curr. Genet. **25**:223-232; Chae 1993 Ph.D. Thesis, McGill Univ. Montreal Canada). In fact, the original *musN227* mutant was recently shown to differ from *musN?* types which have poor growth and high sensitivity to MMS and UV, in line with the identified homology of *musN* to *E. coli*, fungal and human *recQ* helicases and their extreme mutant phenotypes (A. Hofmann, personal communication).

In several cases, the findings after pregermination were confirmed by at least additive interaction also after treatment of quiescent conidia; namely, for *uvsF201* and *uvsI501* results were almost identical (shown for *uvsI* in Fig. 1 A) and for *uvsB110* the double mutant with *bimD6* showed synergism in either test but considerably more after pregermination. On the other hand, for mutants with dormant conidia that are not UV sensitive, i.e., *uvsE182*, a recombination-deficient type similar to the *recA* homolog *uvsC114*, and also for *musN22*, treatment of quiescent spores produced overlapping survival curves of all mutant and control strains. Such results differed clearly from those obtained after pregermination (Figs 1B and 1D).

The overall conclusion, therefore, is that *bimD* most likely is not a member of one of the four identified epistasis groups of UV repair genes in Aspergillus. This agrees with recent findings which suggest a different primary function for *bimD* from that proposed for genes of the four Uvs groups (see below). While genetic analysis of *bimD6* showed extreme reduction of spontaneous mitotic recombination in diploids, similar to that found for the *recA* homolog *uvsC114*, intrachromosomal conversion in *bimD6* duplications was practically at wild type levels but almost absent in *uvsC114* (van Heemst *et al*. ref. cit.). Furthermore, detailed cytoimmunological analysis localized the abundant BIMD protein on the chromosomes at most stages of the meiotic and mitotic cell cycle, as found also for its Sordaria homolog, *SPO76* (van Heemst *et al*. 1999 Cell **98**:261-271). It was therefore concluded that *bimD* is unlikely to be enzymatically involved in recombination, DNA repair or cell cycle progression, but rather functions in mitotic and meiotic chromosome morphogenesis, possibly affecting chromosome cohesion and compaction. This conclusion is supported by analysis of *bimD6* suppressors (e.g., Holt and May 1996 Genetics **142**:777-787) and also by results in yeast and human homologs (reviewed by van Heemst and Heyting 2000 Chromosoma **109**:10-26).

Such *bimD* function differs from that of members in the four Uvs epistatic groups of *A. nidulans* which increasingly is becoming better defined [superceding even recent reviews; e. g., Kafer and May 1998 In Nickoloff and Hoekstra (eds), DNA Damage and Repair, vol.1, Humana, Totowa NJ, p. 477-502]. Current analysis also reveals more clearly similarities and differences to the model provided by budding yeast. Namely, yeast *RAD* genes can be classified into three essentially non-overlapping groups by epistasis and by types of radiation-repair function: 1) RAD3 group, involved in nucleotide excision repair (NER; mutants mainly UV sensitive); 2) RAD6 group, active in error-free and mutagenic postreplication repair (mutants UV and X-ray sensitive); 3) RAD52 group, required for homologous recombination and double strand break repair (mutants mainly X-ray sensitive).

While comparison of *A. nidulans* epistatic UV repair groups to yeast groups (2) and (3) reveals interesting partial correspondence, no information about group (1), i.e., excision repair, is available right now. The hypothesis is that in Aspergillus details of excision processes and mutants differ from those described for budding yeast, but correspond to the situation in Neurospora (and also in fission yeast); more specifically, that two types of excision repair are active, one being specific for UV dimers (Yajima *et al*. 1995 EMBO J **14**:2393-2399) the other resembling yeast and human NER (Hatekayama *et al*. 1998 Curr. Genet. **33**:276-283). Provided both processes can partially substitute for each other, only the double mutant, but neither type of single mutants is very UV sensitive, which accounts for the lack of analysis of NER genes in Aspergillus (even though some of them are known to exist and be active).

Interpretation of current results with respect to post-replication repair, i.e., function of yeast RAD6 group genes, reveals fairly close correspondence with Aspergillus genes of two groups, “UvsF” and “uvsI”.

A) The “UvsF”or better “UvsH or UvsJ” epistatic group, includes so far three genes:

a) *uvsF*, a homolog of human and yeast *RFC1*, is an essential gene and codes for the largest component of DNA replication factor C (Kafer and May 1997 Gene **191**:155-159); *uvsF* is therefore is not specifically a repair gene. Apparently, a point mutation in *uvsF201* interferes solely with its repair function (S-K Chae, personal communication) resulting in defects resembling those found for mutants in excision repair genes while retaining normal growth [similar cases have been reported for other mutated replisome components in yeast; e. g., *pol30-46* is repair defective resulting from a point mutation in PCNA (=proliferating cell nuclear antigen) which interacts with RFC and is essential for DNA replication (Xiao *et al*. 2000 Genetics **155**:1633-1641)].

b) The two other genes of this group, *uvsH* and *uvsJ*, are homologs of *RAD18* and *RAD6*, the two major DNA repair genes of the yeast RAD6 group. Both genes, which interact, are required for important error-free as well as all mutagenic postreplication repair. *uvsH* mutants resemble closely their *rad18* homologs (Yoon *et al*. 1995 Mol. Gen. Genet. **248**:174-181) being highly UV and X-ray sensitive, with increases in spontaneous and UV-induced mutation. *uvsJ1* and *uvsJ?* share a similar phenotype, but their resemblance with *rad6* is less close (Chae 1993 ref. cit. and personal communication).

B) *uvsI*, the gene that defines the “UvsI” group, is a homolog of *REV3* (Han *et al*. 1998 FEMS Microbiol. Lett. **164**:13-19) which codes for the catalytic subunit of the mutagenic bypass DNA polymerase ζ. *REV3* belongs to a RAD6 subgroup and equivalent function of Aspergillus *uvsI* seems likely. Such primary polymerase function had indeed been postulated first based purely on genetic analysis of mutation specificity, after *uvsI501* was found to be defective in reversion only of certain types of point mutations (Chae 1993, ref. cit.; Chae and Kafer 1997 Mol. Gen. Genet. **254**:643-653).

C) Similarly, there is good correspondence for recombinational repair, i.e., between genes of the yeast RAD52 group and genes of the Aspergillus “UvsC” group. The latter includes *uvsC*, a *recA/RAD51* homolog (van Heemst *et al*. 1997 Mol.Gen. Genet. **254**:654664) which interacts with *radC*, a homolog of *RAD52* (S-K.Chae; personal communication). Mutants of both genes are especially sensitive in growing cells, to MMS and UV rather than X-ray, and neither disruption is lethal. Two further genes, *uvsE and musL*, have similar rec$^-$ mutant phenotypes. All these Aspergillus genes are also meiotic defective and show increased chromosome malsegregation, similar to yeast *rad52* and mutants of other genes of the RAD52 group.

D) The two genes in the fourth epistatic Aspergillus group, “UvsB”, have been identified as homologs to fission yeast cell cycle check-point control genes, *uvsB* showing homology to *rad3* and the related human ATM/ATR genes, and *uvsD* to *rad26* (De Souza *et al*. 1999 ref cit.). These findings explain their unusual features which differ not only from mutants of known yeast UV repair genes, but also from mutants of the budding yeast homologs, *MEC1* and *PIE1*. These latter genes, but not those of fission yeast or Aspergillus, are essential genes presumably with additional functions (Desany *et al*. 2001 Genes Dev. **12**:2956-2970; Wakayama *et al*. 2001 Mol. Cell. Biol. **21**:755-764).

Both, the Aspergillus *uvsB* and *uvsD* mutants, also show high levels of genomic instabilities. However, since mitotic levels of intra- and intergenic homologous recombination are clearly increased, such instabilities are not likely to result from defects in recombination, as found for rec$^-$ mutants of Aspergillus *uvsC and uvsE* (Jansen 1970 Mutat. Res. **10**:33-41; Fortuin 1971 Mutat. Res. **11**:265-277; Kafer and Mayor 1986 Mutat. Res. **161**:119-134) and also is known for Rad51 homo- and paralogs in a variety of organisms (e. g., Takata *et al*. 2001 Mol. Cell. Biol. **21**:2858-2866).

Acknowledgement: The diligent assistance by Tania Fan with the various UV sensitivity tests is very much appreciated.

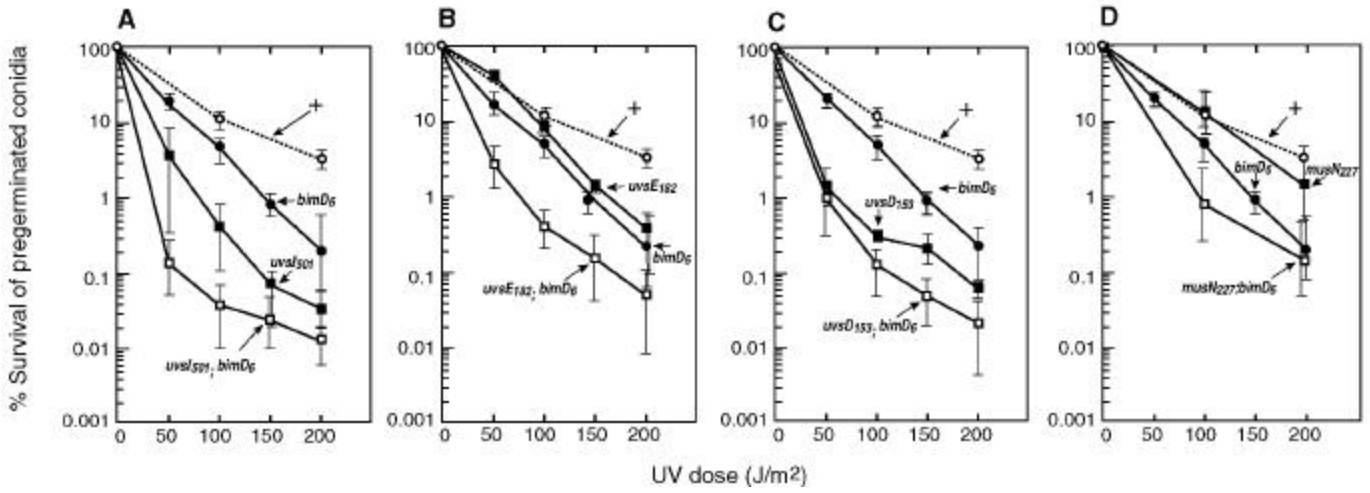

Fig. 1: UV survival of *bimD6* and various UV-sensitive mutants, compared to their congenic double mutant strains (as indicated) and to the repair-proficient (+) control. Conidia were plated, pregerminated by incubation at 30° for 5 1/2 hours, and irradiated on the plates for various times up to a UV dose of 200 J/m2, using a dose rate of 1.6 J/m2/s.