The Isolation of Mutagen-Sensitive *nuv* Mutants of *Aspergillus nidulans* and Their Effects on Mitotic Recombination

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**ABSTRACT**

More than 200 mutants of *Aspergillus nidulans* were isolated as hypersensitive to the monofunctional alkylating agent MNNG and/or UV-irradiation (designated *nuv* mutants). Of these, 23 were selected for further characterization. All were markedly hypersensitive to both MNNG and the quasi-UV-mimetic mutagen 4-NQO. The hypersensitive phenotype of each mutant was shown to result from mutation of a single gene. The *nuv* mutants exhibited a diverse range of growth responses on solid media containing various concentrations of MNNG or 4-NQO. This suggested that they represented many nonallelic mutations. Analysis to determine the dominance/recessiveness of the *nuv* mutations with respect to hypersensitivity revealed that most were fully recessive, although several appeared to be semidominant. A novel system to assay homologous mitotic recombination using simple plating tests was developed. The system was exploited to determine the effects of the *nuv* mutations on mitotic recombination. Of the 25 mutations tested, 10 caused a hypo-recombination phenotype and 13 a hyper-recombination phenotype, while 10 appeared to have no effect on recombination. The hypo-rec effect of one of the mutations, *nuv-117*, appeared to be semidominant. Transcomplementation analysis between seven of the *nuv* mutations defined at least six nonallelic loci.

Despite its fundamental importance for a wide variety of biological processes, little is known of the underlying molecular mechanisms involved in homologous genetic recombination in eukaryotic cells. Much of our knowledge has relied on studies in lower eukaryotes, particularly a few species of fungi that have genetic systems amenable for detecting, analyzing and assaying recombination events (Whitehouse 1982; Orr-Weaver and Szostak 1985; Hastings 1988).

Studies on the genetic control of homologous recombination depend on the isolation and characterization of mutants defective in the recombination pathway(s). DNA metabolic pathways in cells, including replication, transcription, recombination, repair and mutagenesis, are not distinct biological processes but are overlapping and, in part, under the same genetic control. The isolation and characterization of mutants hypersensitive to DNA damaging agents in many organisms continues to provide a diverse source of mutations defective in these processes, as well as revealing the complex interrelationships between them. Hypersensitive mutants have been a particularly useful source of mutants defective in homologous recombination in both prokaryotes (e.g., How ard-Flanders and Theriot 1966; Clark 1973) and eukaryotes (e.g., Holliday 1967; Baker and Smith 1979; Prakash et al. 1980). By far the best studied lower eukaryote with respect to the mutational analysis of homologous recombination is the budding yeast *Saccharomyces cerevisiae*. A relatively large number of *S. cerevisiae* mutants affecting DNA repair and/or recombination have been isolated and characterized and many of the corresponding genes have been cloned (Haynes and Kunz 1981; Game 1983; Cooper and Kelly 1987; Friedberg 1988, 1991). Work with filamentous fungi has progressed much more slowly such that only a relatively small number of such mutants have been isolated and characterized. In *Aspergillus nidulans* only nine UV-sensitive (*uvs*) mutants have previously been extensively characterized for effects on recombination (reviewed by Ka fer and Mayor 1986).

There may be fundamental differences between DNA repair and recombination in *S. cerevisiae* and in *A. nidulans*. Like mammalian cells but unlike *S. cerevisiae*, germinating *A. nidulans* cells spend a substantial part of their vegetative cell cycle in G2 (Bainbridge 1971; Bergen and Morris 1983). During G2 there are two copies of each chromosome existing as sister chromatids, which means there are greater opportunities for homologous recombination in haploid cells of *A. nidulans* than *S. cerevisiae*. Recombinational repair pathways in *A. nidulans* may therefore be more significant than in *S. cerevisiae*. This is the case with *Schizosaccharomyces pombe*, which also spends a large part of its vegetative cell cycle in G2 (Phipps, Nasim and Miller 1985). The pleiotropic phenotypes and epistatic grouping of *A. nidulans* mutants defective in DNA repair/recombination (Kafer and Mayor,
1986) more resemble those of repair/recombination mutants of other eukaryotes than do those of S. cerevisiae, implying that the genetic control of recombination in A. nidulans may be more typical. Indeed, recently cloned S. pombe rad genes thought to be involved in recombination (Lehman et al. 1991; Subramani 1991) show no extensive homology to any cloned S. cerevisiae recombination genes.

In a previous paper (Osman et al. 1991) we described the isolation and characterization of an A. nidulans mutant, nuv11, hypersensitive to N-methyl-N' -nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline-1-oxide (4-NQO), and with effects on meiotic and mitotic recombination. We now describe the isolation of a further 23 mutants hypersensitive to these damaging agents and, using simple plating tests, the characterization of their effects on spontaneous mitotic intragenic recombination.

MATERIALS AND METHODS

A. nidulans strains and media: A. nidulans strains used in this work carried markers in general use that have been described previously (Clutterbuck 1974). The routine genetic techniques used were modified after Pontecorvo et al. (1953). Standard media have been described previously (Cove 1966). "Difco" Bacto-Agar (Difco Laboratories, Detroit, Michigan) was used in all media. Minimal base (MB) consisted of a carbon source, salts and trace elements. Minimal medium (MM) consisted of MB plus a nitrogen source containing media were made up at pH 6.0.

Isolation of MNNG- and/or UV-sensitive mutants: Mutants were isolated from three different parental strains, each of which was phenotypically wild-type with respect to mutagen sensitivity. Conidial (asexual uninucleate haploid spore) suspensions of the three parental strains, B608 (pabAl, wuA3, niaD608), CS887 (yA2, luA1, niaD26) and L20 (pabAl, wuA3), were each treated with MNNG (0.5 μg/ml final concentration) to give less than 10% survival. Master plates were prepared byoverlaying mutagenized conidia at a density of approximately 100 surviving conidia per plate onto MM (to eliminate auxotrophic mutants) containing 0.08% sodium deoxycholate (SD) to induce compact colonies. Minimal medium (MM) consisted of MB plus a nitrogen source and supplements for auxotropic markers. Mutagen-containing media were made up at pH 6.0.

Quantifying MNNG and 4-NQO sensitivity: A heavy suspension of conidia was spread onto a MM plate using a sterile glass spreader and incubated for 24 hr at 37° to produce a nonsporulating mycelial "mat." Mycelial "plugs" were cut out from the mats using the wide end of a sterile Pasteur pipette. The plugs were transferred in duplicate onto MM plates containing various concentrations of MNNG or 4-NQO and incubated for 48 hr at 37°. The diameters of the resulting colonies were measured under a stereoscopic microscope to the nearest 0.1 mm using a micrometer. Radial growth was expressed as percentage of average diameter of colonies on zero-dose plates. This technique avoids the problem of delayed germination found with some mutagen treatments and ensures that the true exponential phase growth rate is measured.

RESULTS

Isolation of nuv mutants: By the end of the mutant isolation program, 18 putative mutagen-hypersensitive mutants had been identified from screening about 4,800 mutagenized L20 colonies, over 80 from screening about 8,000 mutagenized B608 colonies, and over 100 from screening about 10,000 mutagenized CS887 colonies. The mutant strains were designated L20nuv, B608nuv and CS887nuv, respectively, followed by a unique distinguishing number.

After two rounds of screening, 25 of the mutants were retained for further investigation on the basis that they were the most hypersensitive to MNNG: it had been found previously in both S. cerevisiae (Brendel, Khan and Haynes 1970) and A. nidulans (Kaffer and Mayor 1986) that hypersensitivity to monofunctional alkylating agents was especially pronounced in mutants that affect recombination. The 23 mutants selected were as follows: nine mutants isolated in B608—nuv-1, nuv-2, nuv-4, nuv-6, nuv-7, nuv-8, nuv-10, nuv-12, nuv-20; 12 from CS887—nuv-102, nuv-103, nuv-107, nuv-109, nuv-110, nuv-111, nuv-112, nuv-114, nuv-115, nuv-117, nuv-129, nuv-121; two in
the L20 background—nuv-329 and nuv-334. All of these mutants were hypersensitive to both MNNG and 4-NQO.

**Mutant nuv allele segregation:** Each of the nuv mutant phenotypes was shown to result from mutation of a single gene by sexually crossing each mutant strain with a strain phenotypically wild-type with respect to mutagen-sensitivity. Segregation of the nuv-10 phenotype was analyzed by the parasexual cycle, which involved diploid construction and haploidization, since sexual crosses heterozygous for nuv-10 produced no viable hybrid sexual spores. Randomly chosen progeny from each cross were screened for hypersensitivity to MNNG and 4-NQO by wire inoculation of conidia into mutagen-containing media. In each case there was a 1:1 mutant/wild-type phenotypic segregation with respect to hypersensitivity. Analysis of the sensitivities of the progeny did not reveal any new phenotypic class, all progeny being clearly of one parental type or the other, and with hypersensitivity to MNNG and 4-NQO always segregated together.

**Comparison of hypersensitivities of nuv strains to MNNG and 4-NQO:** The hypersensitive phenotype of each nuv allele was tested in both B608 and CS387 genetic backgrounds, relative to the parental strains, by measuring the hyphal extension of germinating mycelial plugs on solid minimal media containing various concentrations of MNNG or 4-NQO. To make direct comparisons between the different nuv strains, the determinations were carried out as two large experiments, one for sensitivity to MNNG and the other for sensitivity to 4-NQO. In most cases the growth response of diploids heterozygous and homozygous wild-type diploids. None of the mutants may have been defective in nitrate reductase, the nitrate assimilation genes. The two strains were deficient in nitrate reductase, the niaD gene product.

The growth responses of several of the diploid strains are represented graphically in Figure 3. In all cases, except for nuv-20, the homozygous nuv diploid was markedly hypersensitive to MNNG and 4-NQO relative to the corresponding heterozygous nuv and homozygous wild-type diploids. An interesting result was that the diploid homozygous for nuv-20 did not appear to be hypersensitive to either MNNG or 4-NQO. The homozygous nuv-20 diploid was haploidized on benlate-containing media (Hastie 1970) and the haploid segregants tested for hypersensitivity to MNNG and 4-NQO. All segregants were hypersensitive confirming that the nuv-20 hypersensitive phenotype was haploid-specific.

In most cases the growth response of diploids heterozygous for the nuv mutations was similar to that of homozygous wild-type diploids, suggesting complete recessiveness of the nuv allele to its wild-type allele. All of the mutations appeared to be recessive except three: nuv-2, appeared to be semi-transdominant for hypersensitivity to both MNNG and 4-NQO; nuv-8 and nuv-111 appeared to be semi-transdominant only for hypersensitivity to 4-NQO. In these cases the heterozygous diploid was intermediate in hypersensitivity relative to that of the corresponding homozygous nuv and homozygous wild-type diploids. None of the mutants appeared to be completely dominant.

**Establishment of a novel assay for spontaneous mitotic intragenic recombination:** Mitotic intragenic recombination between heteroalleles is most conveniently measured in the case of mutations conferring auxotrophy. This allows rare prototrophic recombinants to be selected on medium lacking the appropriate nutrient. The mitotic recombination assay developed is based on the two A. nidulans strains, B608 (waA4, puuA2, niiA/mid608) and CS387 (ya2, luA1, niaD26), each carrying a non-overlapping hetereoallelic deletion within the niaD gene on chromosome VIII. The ends of the deletions had been previously determined by fine-structure mapping (Tomsett and Cove 1979). They are shown schematically in Figure 4, together with the structural organization of the nitrate assimilation genes. The two strains were deficient in nitrate reductase, the niaD gene product.
Hypersensitivity to nuu-2 mutation. The growth response of germinating mycelia of nuu-2 mutant strains in comparison with parental strains inoculated onto solid media containing various concentrations of MNNG or 4-NQO. The percentage hyphal growth represents the relative hyphal extension of strains growing in the presence of MNNG or 4-NQO in comparison with controls growing in the absence of MNNG or 4-NQO. It should be noted that the linear growth response of multinucleate hyphae to MNNG and 4-NQO was determined in these experiments, not conidial survival.

They could not therefore utilize nitrate as sole nitrogen source. However, commercial agar contains traces of reduced nitrogen, which allows nitrate nonutilizing strains to grow on nitrate-containing solid media with a characteristic residual growth phenotype, namely sparse, nonsporulating mycelial growth. This phenotype was exploited to qualitatively assay mitotic recombination within the nuAD gene in the diploid B608/CS387.

Centrally inoculated B608/CS387 diploid colonies initially grew with the characteristic residual growth phenotype on nitrate-containing media. After 4–5 days incubation, sporulating subcolonies were observed to arise within the sparse mycelial growth, Figure 5, plates A. As controls, heteroallelic diploids were constructed between each test strain and strains carrying an overlapping deletion (see Figure 4); a diploid was constructed between B608 and B144 (pabaAl, fuAl, niiAniaD526), and between CS387 and B317 (biAl, niiAniaD564). In plating tests with these
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Figure 3.—Dominance/recessiveness of nau mutations with respect to hypersensitivity to MNNG and 4-NQO. This was determined by measuring the growth response of germinating mycelia of diploids heterozygous and homozygous for the nau mutations in comparison with the homozygous wild-type parental diploid.

diploids no subcolonies arose, Figure 5, plates B and C.

The subcolonies were genetically analyzed and shown to be still diploid and true nitrate-utilizing recombinants. They were subcultured onto nitrate plates and grew with a normal nitrate-utilizing phenotype. All those tested were shown to be diploid by haploidization on benlate plates (Hastie 1970). Each produced nitrate-utilizing and nitrate-nonutilizing haploid segregants. Nitrate-utilizing segregants were sexually crossed with either nitrate-utilizing or nonutilizing strains, and the progeny analyzed. The phe-
notypic ratios of the progeny obtained were consistent with the conclusion that the haploid segregants were true *niaD* recombinants: crosses with nitrate-utilizing strains produced all nitrate-utilizing progeny; the numbers of nitrate utilizers and nonutilizers from crosses with nitrate-nonutilizing strains were not significantly different (P = 70–50%) from a 1:1 ratio. Preliminary genetic analysis of the haploid segregants from the nitrate-utilizing recombinants (L. IWANEJKO, unpublished data) revealed that they could arise by conversion.

Effect of *nuv* mutations on mitotic recombination: The assay described above was exploited to qualitatively determine the effect of heterozygous or homozygous *nuv* mutations on spontaneous mitotic recombination in B608/CS387 diploids. Each diploid was tested on three separate plates. The results were consistent and reproducible. This assay was especially effective at identifying *nuv* mutations causing a hypo-rec phenotype. Examples of plates from which the results were obtained are shown in Figure 5, plates D–G. Three classes of *nuv* mutations were identified on the basis of the mitotic recombination phenotype of the homozygous *nuv* diploid: rec*"* types (*nuv*-1, *nuv*-10, *nuv*-12, *nuv*-20, *nuv*-103, *nuv*-107, *nuv*-109, *nuv*-115, *nuv*-120, *nuv*-121), hypo-rec types (*nuv*-2, *nuv*-4, *nuv*-6, *nuv*-7, *nuv*-8, *nuv*-10, *nuv*-112, *nuv*-114, *nuv*-117, *nuv*-329) and hyper-rec types (*nuv*-102, *nuv*-111, *nuv*-334). Rec*"* types displayed a similar recombination phenotype to wild-type diploids. Hyper-rec types produced a several-fold increase in the number of *niaD* recombinants, the effect of *nuv*-102 being the most marked followed by *nuv*-117 and *nuv*-334. The hypo-rec types produced a several-fold decrease in the number of recombinants compared with wild-type. They could be classified into three groups: diploids homozygous for *nuv*-2, *nuv*-7, *nuv*-110, *nuv*-112 and *nuv*-329 produced a greater than 10-fold decrease; *nuv*-4 and *nuv*-114 had an intermediate effect, a less than 10-fold but greater than fourfold reduction; *nuv*-6, *nuv*-8 and *nuv*-117 caused a less than fourfold decrease. The *nuv*-117 mutation appeared to have a semidominant effect; the heterozygous *nuv*-117 diploid also displayed a hypo-rec phenotype but not to the extent of the homozygous *nuv*-117 diploid, Figure 5, plates F and G. The other *nuv* mutations affecting recombination appeared to be fully recessive, in each case the heterozygous *nuv* diploid displaying a similar recombination phenotype to the homozygous wild-type diploid.

Complementation analysis with some of the *nuv* mutations: Complementation analysis is necessary to determine the number of genes defined by the *nuv* mutations. An initial study was carried out with the previously described mutation *nuv*11 (OSMAN et al. 1991) and six of the *nuv* mutations with effects on mitotic recombination from this study: *nuv*-2, *nuv*-4, *nuv*-102, *nuv*-110, *nuv*-114 and *nuv*-117. Diploids heterozygous in trans for two *nuv* mutations were constructed for all possible pairwise combinations. Allelism was determined by testing these heterozygous diploids for hypersensitivity to MNNG and 4-NQO, and for mitotic recombination phenotype using the simple plating assay. The responses were compared with that of the singly heterozygous *nuv* diploids (as positive controls) and the homozygous *nuv* diploids (as negative controls). The results are shown in Table 1. The results for trans complementation with respect to hypersensitivity and to recombination phenotype were consistent. They showed that the seven *nuv* mutations analyzed defined at least six nonallelic loci. The *nuv*-102 and *nuv*117 mutations were noncomplementing for hypersensitivity, and the *nuv*-102/*nuv*117 diploid had the same hypo-rec phenotype as the homozygous *nuv*-117 diploid. They thus appeared to be allelic despite their different effects on mitotic recombination. The diploids heterozygous for *nuv*-117 and each of the other *nuv* mutations displayed a hypo-rec phenotype comparable to that of the *nuv*117/wild-type diploid. This confirmed the semidominant hypo-rec phenotype of *nuv*-117. Diploids heterozygous with *nuv*-2 were all hypersensitive to MNNG and 4-NQO compared with wild-type, confirming its semi-transdominance for this phenotype.

Several of the *nuv* mutations were assigned to linkage groups (whole chromosomes) by mitotic analysis (MCCULLY and FORBES 1965): *nuv*-2 to chromosome V; *nuv*-4 to chromosome II; *nuv*-102 and *nuv*-114 to chromosome VIII. Despite exhaustive attempts the *nuv*-117 mutation could not be mapped to a specific chromosome. This was also the case with some previously isolated *us* mutations (EVSEEVA and KAMENeva 1977). None of the previously characterized mutations causing hypersensitivity to DNA damaging agents, the *us* (KAFER and MAYOR 1986) and *sag*
A. nidulans Recombination Mutants

Figure 5.—A simple assay for mitotic recombination. Sporulating subcolonies arose spontaneously within the characteristically sparse mycelium of centrally inoculated B608/CS387 heteroallelic diploid colonies growing on nitrate-containing medium (Plates A). These subcolonies were shown to be nitrate-utilizing \(niaD^+\) recombinants, and to have arisen either by crossing over or gene conversion. As controls, heteroallelic diploids were constructed between each test strain and a strain carrying an overlapping \(niaD\) deletion. In plating tests with these diploids, B608/B114 (Plate B) and B317/CS387 (Plate C), no nitrate-utilizing subcolonies arose. Recombination-defective \(nuv\) mutants were identified by comparing the frequency of nitrate-utilizing subcolonies arising from B608/CS387 diploids heterozygous and homozygous for a \(nuv\) mutation. Plates demonstrating the recessive hyporec phenotype of \(nuv-2\) and the semi-transdominant hypo-rec phenotype of \(nuv-117\) are also shown: Plates D, B608\(nuv2\)/CS387; Plates E, B608\(nuv2\)/CS387\(nuv117\); Plates F, B608/CS387\(nuv117\); Plates G, B608\(nuv117\)/CS387\(nuv117\).

(Swirski et al. 1988) mutations, mapped to chromosome II. The \(nuv-4\) mutation therefore probably defines a new locus in \(A.\) nidulans. The \(nuv-2\) mutation was analyzed for allelism with the \(wdsI\), \(wusE\) and \(wusf\) mutations also located on chromosome V. Complementation analysis was performed by scoring for hy-
TABLE 1

Trans complementation analysis of nuv mutants

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Complementation was determined by scoring: (i) sensitivity of germinating mycelial plugs of heterozygous diploids to growth on solid media containing 2.0 μg/ml MNNG or 0.75 μg/ml 4-NQO; (ii) the mitotic intragenic recombination phenotype of heterozygous diploids. + = complementation; − = noncomplementation.

persensitivity of diploids to MNNG and 4-NQO. The results (data not shown) showed that nuv-2 was non-allelic to all of these uvs mutations. The nuv-2 mutation therefore also defines a new locus in A. nidulans.

DISCUSSION

The mutant isolation program was successful in isolating a large number of mutants hypersensitive to MNNG and/or UV-irradiation. Of the 23 nuv mutants chosen for further characterization, all were hypersensitive to both MNNG and 4-NQO and in each case this phenotype appeared to be due to single chromosomal gene mutation. The wide diversity of growth responses to MNNG and 4-NQO exhibited by the nuv mutants was desirable since this meant that they were more likely to be mutations in different genes. Initial complementation analysis seems to bear this out.

From what is known from studies in other model organisms (e.g., FRIEDBERG 1988), mutagen-hypersensitive mutants can result from mutations inactivating structural genes for DNA repair activity, or those that affect the regulation of expression of DNA repair genes. For eukaryotic organisms, mutations in genes involved in chromatin structure and regulation of the cell cycle can also produce a hypersensitive phenotype (HARRIS and BOYD 1987; WEINERT and HARTWELL 1988). It is also important to consider that some mutations may cause hypersensitivity due to indirect consequences. For example, sensitivity of cells to MNNG has been previously shown to be affected by intracellular thiol content (SEDGWICK and ROBINS 1980) and pH (DELC, HOPWOOD and FRIEND 1970). It can also be envisaged that a hypersensitive phenotype could be due to mutations affecting cell permeability, or causing enhanced damage to some essential cellular component other than DNA.

The tests for hypersensitivity of diploids heterozygous and homozygous for the nuv mutations relative to wild-type were important for identifying recessive and semi-transdominant nuv alleles. Mutant alleles exhibiting dominance are of particular interest because they are candidates for specifying genes involved in regulation of repair functions. Alternatively they could specify structural genes for repair enzymes, which exhibit a gene dosage effect in diploids. The semidominance of nuv-8 and nuv-111 to 4-NQO but not to MNNG is most readily explainable if it is postulated that the function encoded by the wild-type genes is a major regulatory or structural component for repair of lesions caused by 4-NQO, but only a minor component for the repair of MNNG-induced lesions. The apparent haploid-specific sensitivity of nuv-20 is interesting but not readily explainable in molecular terms.

A test system has been developed in A. nidulans, which is novel for this organism and which can assay for mitotic intragenic recombination rapidly and effectively using simple plating tests. It directly measures recombinant subcolonies arising from independent events within the diploids, giving an accurate measure of differences in the frequency of recombination events in different strains. Traditional assays do not give such a direct measure of recombination frequency. They rely on harvesting conidia from heteroallelic diploid colonies growing on nonselective media and, hence, a recombinant cell can give rise to a clonal population distorting the frequency (JANSEN 1970; FORTUIN 1971).

In this study we have investigated the effects on mitotic recombination of mutations, which affect the ability to repair general DNA damage. It seems likely that those mutations that do show effects on the intragenic recombination measured in our assay will not only affect recombination in the niaD region but will also affect recombination in other regions of the genome. Among the mutants initially isolated as hyperek to DNA damaging agents, a number of Rec phenotypes were observed. Of the 23 nuv mutations characterized in our assay, 10 appeared to have no marked effects on mitotic recombination. These nuv mutations were phenotypically similar to the previously characterized uvsA mutation (JANSEN 1967). Ten nuv mutations appeared to be hypo-rec, similar to the previously characterized uvsC and uvsE mutations (FORTUIN 1971; JANSEN 1970). Three appeared to be hyper-rec, as were the uwsB, uwsD, uwsF, uwsH and uwsJ mutations (LANIER, TUVESON and LENNOX 1968; SHANFIELD and KAFER 1969; JANSEN 1970; WRIGHT and PATEMAN 1970; FORTUIN 1971). nuv-117 had a semidominant hypo-rec phenotype, which could imply a gene dosage effect for its product or a mutation within a regulatory gene or sequences.

The possible defects of the nuv mutations affecting mitotic recombination and the underlying functions of the gene products can to some extent be postulated by extrapolation from what is known from mutational studies in other organisms. Mutations causing a hypo-
rec phenotype for spontaneous homologous chromosomal recombination are the most likely to be defective in structural or regulatory genes directly involved in recombination. For example, the characteristic phenotype of the S. cerevisiae RAD52 group mutants, which are thought to be defective in recombination processes, is recombination deficiency (Haynes and Kunz, 1981; Game, 1983; Cooper and Kelly, 1987; Friedberg, 1988, 1991). Mitotic hyper-rec mutants could also be defective in primary recombination functions, as is the rad50 mutant of S. cerevisiae (Malone et al., 1990). However, such a phenotype could also be the result of secondary effects caused by defects in genes involved in other aspects of DNA metabolism. For example, these mutations could be defective in a repair mechanism, which causes the accumulation of recombinogenic lesions, or lesions that induce the expression of recombination enzymes. Some eukaryotic mutants characterized as defective in excision repair or error-prone replicative repair have been shown to be hyper-rec for spontaneous mitotic chromosomal recombination. For example, the rad3 and rad6 mutants of S. cerevisiae, which are deficient in excision repair and error-prone repair, respectively, are also hyper-rec for spontaneous mitotic recombination (Kern and Zimmerman, 1978).

Trans complementation analysis between at least seven of the nuv mutations showed that they defined at least six nonallelic loci. The remaining nuv mutants are currently being assigned to specific chromosomes and tested for complementation between themselves and with the previously characterized uvs and sgs mutants. The nuv-102 and nuv-117 mutations appear to be noncomplementing for both hypersensitivity and mitotic recombination. If nuv-102 and nuv-117 do define a single locus, two allelic mutations have been isolated with very different effects on hypersensitivity and mitotic recombination. Similar differential effects for allelic DNA repair and recombination defective mutants are common in both eukaryotes and prokaryotes. For example, whereas most S. cerevisiae rad52 mutants were hypo-rec for mitotic recombination, the leaky rad52-2 mutation displayed a mitotic hyper-rec phenotype (Malone et al., 1988). The availability of several different mutants in the same gene is useful in establishing the range of possible phenotypic effects, and in identifying those that are typical for a specific gene. Phenotypic differences between allelic mutants are most likely to arise with genes encoding multifunctional proteins or regulatory functions.

In conclusion, there is little doubt that a set of mutants has been isolated that define genes important in DNA metabolism, with the prospect that some may be involved in regulation and control. The nuv-2 and nuv-4 mutations have already been shown to define new loci in A. nidulans, increasing the number of genes known to be involved in DNA repair and recombination in this organism. The further phenotypic characterization of these mutants together with assignment to epistatic groups will be important in determining their functions. The availability of a large number of mutants should facilitate the cloning of the relevant genes by complementation and, consequently, purification and biochemical characterization of the gene products. The correlation of mutant phenotype to biochemical activity will be the critical criterion by which the molecular mechanisms of homologous recombination will be elucidated. The number of mutants now available in A. nidulans make it attractive as a complementary system to S. cerevisiae. It is likely that some of the nuv mutants will define genes of types not previously characterized, although the availability of any homologous genes from two evolutionarily distinct fungi should help in the isolation of homologues from higher eukaryotes.

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LITERATURE CITED


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