nuvA, an Aspergillus nidulans gene involved in DNA repair and recombination, is a homologue of Saccharomyces cerevisiae RAD18 and Neurospora crassa uvs-2

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A 40 kb genomic clone and 23 kb EcoRI subclone that rescued the DNA repair and recombination defects of the Aspergillus nidulans nuvA11 mutant were isolated and the subclone sequenced. The subclone hybridized to a cosmid in a chromosome-specific library confirming the assignment of nuvA to linkage group IV and indicating its closeness to bimD. Amplification by PCR clarified the relative positions of nuvA and bimD. A region identified within the subclone, encoding a C3HC4 zinc finger motif, was used as a probe to retrieve a cDNA clone. Sequencing of this clone showed that the nuvA gene has an ORF of 1329 bp with two introns of 51 bp and 60 bp. Expression of nuvA appears to be extremely low. The putative NUVA polypeptide has two zinc finger motifs, a molecular mass of 48906 Da and has 39% identity with the Neurospora crassa uvs-2 and 25% identity with the Saccharomyces cerevisiae RAD18 translation products. Although mutations in nuvA, uvs-2 and RAD18 produce similar phenotypes, only the nuvA11 mutation affects meiotic recombination. A role for nuvA in both DNA repair and genetic recombination is proposed.

Keywords: Aspergillus nidulans, DNA repair, recombination, C3HC4 zinc finger motif, nuvA, RAD18

INTRODUCTION

The processes of DNA repair and recombination play a pivotal role in the proper segregation of chromosomes at meiosis as well as the maintenance of genome integrity and the generation of genetic diversity. To understand these processes in eukaryotes better, we have undertaken a study of Aspergillus nidulans as it is multicellular and biochemically and morphologically its mitotic cell-cycle resembles that of mammalian cells in that a substantial proportion of the vegetative cell-cycle is spent in G2 (Bainbridge, 1971; Bergen & Morris, 1983). This is important with respect to studies of mitotic recombination since it is believed that gene conversion and crossing-over are different outcomes of the same process, with gene conversion taking place during the G1 phase of the mitotic cell-cycle, whilst the G2 phase is associated with cross-over events (Roman & Ruzinski, 1990). Studies with Aspergillus therefore provide a valuable complement to the extensive studies in Saccharomyces cerevisiae which is single-celled and spends much of its time in G1.

In a previous paper (Osman et al., 1991) we reported the isolation of the A. nidulans nuvA11 mutation, a recessive, single gene defect on linkage group IV, originally identified as causing sensitivity to the DNA damaging agents N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline-1-oxide (4NQO). The nuvA11 mutants were shown to have a complex phenotype. Haploids are slow growing and have a 'crinkly' mor-
### Table 7. Strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype and relevant phenotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>F' recA1 endA1 gyrA96 thi-1 hsdR17 (r-, mcrA) supE44 relA1</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>DH1</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 (r-, mcrA) supE44 relA1</td>
<td>Bullock et al. (1987)</td>
</tr>
<tr>
<td>XL1-blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 (r-, mcrA) supE44 relA1</td>
<td>Stratagene</td>
</tr>
<tr>
<td>SOLRTM</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 (r-, mcrA) supE44 relA1</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>F1 helper phage</td>
<td>Interssistance-resistant helper phage containing amber mutations in genes I and II (5 kb, single-stranded)</td>
<td>Stratagene</td>
</tr>
<tr>
<td>ExAssistTM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. nidulans nuvA+ strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L20</td>
<td>wA3 pabaA1</td>
<td>Liverpool stock</td>
</tr>
<tr>
<td>A517</td>
<td>riboA1 yA2 adeE20; methG1 swC11adeE20 palC4 pabaB22 pyroA4 (a meiotic mapping strain for linkage group IV)</td>
<td>Fungal Genetics Stock Center (FGSC)</td>
</tr>
<tr>
<td>B608</td>
<td>paA1 wA3; niiAniaDAΔ608 yA2 laA1; niaD26 (A pair of strains carrying non-overlapping deletions in the niaD gene allowing selection for nitrate, utilizing products of intragenic recombination)</td>
<td>Tomsett &amp; Cove (1979)</td>
</tr>
<tr>
<td>CS387</td>
<td></td>
<td>Tomsett &amp; Cove (1979)</td>
</tr>
<tr>
<td>L600</td>
<td>pyrG89 yA2; niaD26 (previously referred to as TYS3870)</td>
<td>Osman et al. (1991)</td>
</tr>
<tr>
<td>L604</td>
<td>pyrG89 yA2 laA1; niaD26</td>
<td>This study</td>
</tr>
<tr>
<td>A. nidulans nuvAII strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L601</td>
<td>pyrG89 yA2; niaA1; niaD26 (previously referred to as TYS3871)</td>
<td>Osman et al. (1991)</td>
</tr>
<tr>
<td>L602</td>
<td>paA1 wA3; niaA1; niaD26 (previously referred to as B608nuvII)</td>
<td>Osman et al. (1991)</td>
</tr>
<tr>
<td>L603</td>
<td>yA2 laA1; niaA1; niaD26 (previously referred to as CS387nuvII)</td>
<td>Osman et al. (1991)</td>
</tr>
<tr>
<td>L605</td>
<td>(A pair of niaAII strains carrying non-overlapping deletions in the niaD gene allowing selection for nitrate, utilizing products of intragenic recombination) pyrG89 yA2 laA1; niaA1; niaD26</td>
<td>This study</td>
</tr>
</tbody>
</table>

Phylogeny, whereas nuvAII homozygous diploids have a normal growth rate but are very unstable. Mitotic recombination frequencies are significantly elevated in these diploids, yet meiotic recombination is virtually absent in sexual crosses between two nuvAII strains, even though the fertility and ascospore viability of such crosses appears to be unaffected. The nuvAII mutation also results in sensitivity to UV and γ irradiation and higher UV-induced mutation frequencies, but not γ-ray-induced mutation frequencies (S. May, this laboratory, unpublished observations). The nuvAII mutant phenotype was previously compared (Osman et al., 1991) with those of the S. cerevisiae RAD52 epistasis group mutants rad50 and rad52 which are defective in their repair of double-strand breaks (Game, 1983; Haynes & Kunz, 1981). Although similar, none of the phenotypes were identical to that caused by the nuvAII allele. We therefore aimed to clone the nuvA gene to understand more fully the role the gene product plays in DNA repair and recombination.

In this paper we describe the cloning of the nuvA gene by complementation of the nuvAII phenotype. We also describe the isolation of the nuvA cDNA sequence. The presumed location of the nuvA gene, close to hsp70 and msh1, on chromosome IV (Käfer & Chae, 1994) is also presented. The nucleotide and putative amino acid sequences are discussed and compared with the known DNA repair proteins UVS-2 of Neurospora crassa (Tomita et al., 1993) and RAD18 of S. cerevisiae (Jones et al., 1988).

### METHODS

**Strains, vectors and libraries.** Bacterial and fungal strains used in this study are described in Table 1. Both the cosmid library, containing 30–40 kb wild-type A. nidulans genomic DNA inserts...
in pWE15 (Brody et al., 1991), and the A. nidulans 24 h developmental cDNA library in iZAP (Aramayo & Timberlake, 1990) were obtained from the Fungal Genetics Stock Centre (FGSC), Kansas City, Kansas 66160-7420, USA. Vectors and libraries (Table 2) were propagated in E. coli XL1-blue using standard techniques and media (Sambrook, 1989). A. nidulans strains were grown in standard media (Cove, 1966) except mutagen-containing medium which was made up to pH 6.0. Mutagens were added to cooled molten agar or liquid media. MNNG was freshly made in ethanol but 4NQO was kept at -20 OC as a stock solution (1 mg ml-1), also in ethanol. Standard genetic manipulations were employed throughout this study (Pontecorvo, 1953).

### Assaying MNNG and 4NQO sensitivities

Mutagen sensitivities were assessed using the radial growth rates of point-inoculated colonies on agar plates containing various concentrations of MNNG (0.5-2.5 μg ml-1) and 4NQO (0.25-1.5 μg ml-1). Plates were incubated at 37 °C for 48 h prior to measuring.

### Recombination assays

The puA1 and wA3 markers on chromosome II and the yA2 and luA1 markers on chromosome I were used to assess both mitotic and meiotic intergenic recombination frequencies (Osman et al., 1991). Pairs of strains carrying heteroallelic deletions in the niaD gene were used to assay mitotic and meiotic intragenic recombination frequencies as described previously (Osman et al., 1991).

### Diploid stability and colony morphology

These were determined by visual assessment of diploid colonies on minimal base (MB) plates containing the appropriate supplements and nitrogen source. Test diploids were compared with homozygous wild-type diploids which are stable and morphologically homogeneous, and nua11 homozygous diploids which are extremely unstable and morphologically diverse.

### Fine mapping

Attempts were made to map the nua11 mutation meiotically within linkage group IV using the meiotic mapping strain AS17, employing standard genetic mapping techniques. A more accurate map position was obtained by hybridizing the cloned nua gene to the pWE15 cosmid library and identifying the physical map location of the positively hybridizing clones (R. Prade, University of Georgia, personal communication).

### DNA manipulations

Standard cloning techniques were used as described by Sambrook et al. (1989). Plasmids were prepared using an alkaline lysis method and Qiagen columns supplied by the manufacturer or by CsCl gradient purification (Sambrook et al., 1989). Double-stranded plasmid DNAs were sequenced using the dyeexynucleotide chain termination procedure (Sanger et al., 1977) with T7 DNA polymerase, according to the manufacturer's instructions (Pharmacia). PCR fragments were sequenced, after purification on an appropriate spin column (Pharmacia), on an Applied Biosystems 373A DNA Sequencer using the Taq DyeDeoxy Terminator Kit and protocol. Oligonucleotide sequencing and PCR primers were supplied by Pharmacia or synthesized on an Applied Biosystems 392 DNA/RNA synthesizer. Except where otherwise indicated,

### Table 2. Vectors and constructs used in this study

<table>
<thead>
<tr>
<th>Vector</th>
<th>Selectable marker*</th>
<th>Features</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Ap'</td>
<td>2.96 kb phagemid</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pBluescript SK —</td>
<td>Ap'</td>
<td>2.96 kb phagemid</td>
<td>Promega</td>
</tr>
<tr>
<td>pGEM-T System I</td>
<td>Ap'</td>
<td>Supplied as an EcoRV-cleaved pGEM-5ZF(+) vector treated with terminal transferase to produce 3' overhangs for direct PCR cloning</td>
<td></td>
</tr>
<tr>
<td>iZAP</td>
<td>Ap'</td>
<td>i insertion vector. In vivo excision releases cloned DNA in pBluescript SK —</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pWE15</td>
<td>Ap'</td>
<td>8.8 kb cosmid. Also carries the neomycin marker under the control of the SV40 promoter for use in eukaryotes</td>
<td>Stratagene</td>
</tr>
<tr>
<td>E. coli/A. nidulans</td>
<td>pyr4/Ap'</td>
<td>Cosmid containing the ant1 transformation-enhancing sequence of A. nidulans and a unique BamH1 cloning site</td>
<td>Ballance &amp; Turner (1985)</td>
</tr>
<tr>
<td>Constructs</td>
<td>pyr4/Ap'</td>
<td>pRG3 containing a 2.5 kb A. nidulans genomic DNA insert</td>
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</tr>
<tr>
<td>pSF20</td>
<td>pyr4/Ap'</td>
<td>pcAP2 containing a 40 kb A. nidulans genomic DNA insert</td>
<td>Fidel et al. (1988)</td>
</tr>
<tr>
<td>pNUV11A40</td>
<td>pyr4/Ap'</td>
<td>pCAP2 containing a 40 kb A. nidulans genomic DNA insert</td>
<td>Osman et al. (1991)</td>
</tr>
<tr>
<td>pEA14</td>
<td>pyr4/Ap'</td>
<td>pRG4 containing a 2.5 kb A. nidulans genomic DNA insert</td>
<td>This study</td>
</tr>
<tr>
<td>pLW100</td>
<td>Ap'</td>
<td>pBluescript SK — containing the nua cDNA insert</td>
<td>This study</td>
</tr>
<tr>
<td>pLW101</td>
<td>Ap'</td>
<td>pGEM-T containing the 5' end of the nua gene</td>
<td>This study</td>
</tr>
<tr>
<td>pW17F02</td>
<td>Ap'</td>
<td>pWE15 clone of A. nidulans genomic DNA that hybridized the pEA14 insert</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Ap, ampicillin.
Fig. 1. Diagram of the 9.5 kb bimDnuvA region of chromosome IV. All the BamHI (B) and SstI (S) sites specific to this region and found on the cosmid clones pNUV11A40 and pW17F02 are marked, as are the EcoRI (E) sites used to subclone the 2.3 kb nuvA17-complementing fragment of pEA14. The location of the PCR primer pairs in this region and the size of their PCR products used to verify the contiguity of the various nuvA sequences and the orientation of the nuvA and bimD genes are shown above the restriction map. The sections covered by the nuvA genomic clone pEA14, the 'zinc finger' encoding region used as a probe, the 5'-truncated cDNA clone pLW100, and the RT-PCR product along with its sub-products are shown below the map.

PCR amplifications were performed on a Perkin Elmer DNA Thermal Cycler 480 using Gibco Tag DNA polymerase with the buffer and MgCl₂ solutions supplied by the manufacturer. The oligonucleotide sequences of the PCR primers used in this study, most of which were originally designed for sequencing, are given below. ³²P-labelled DNA probes were prepared essentially as described previously (Feinberg & Vogelstein, 1983) using a random-primed DNA labelling kit (Boehringer Mannheim). Chromosomal DNA from A. nidulans was prepared by the Raeder & Broda (1985) method. Plaque and colony hybridizations and Southern transfers were on to Gene Screen Plus nylon-based membranes (DuPont) using the manufacturer's recommended protocols. Hybridizations were carried out in a Techne hybridization oven using Blotto hybridization solution (Sambrook et al., 1989).

**PCR Primers.** The sequences of the DNA primers used were as follows. M13-20, GTAAAACGACGGCCAGT; M13-reverse, GGAAACAGCTATGACCATG; bimD1, CATCTATCGTGAGTGCTGCGGG; bimD2, CTGGAACCGGGGATGCAGG; bimD3, CCTGCATCCCCGGTTCCAG; iwa1, GAATTCCGCTTGGGAAGCTTGG; iwa2, GAATTCTGGCGTTGAGG; iwa5, CCCAAGCCTCTGCAACGCCAG; LI1, GAGCGTACCATGACATTC; LI5, GACCATCTGTGCCAG; LI13, AACGTCCCTCCGCTGCCA; CD1, CCCTGTTGCAGGTCTAG; CD2, CTATGACTGAAAGACAGG; CD3, GTGGAACGCGCAATTTGT; CD4, CACAATGCGGTTCAC; CD5, GCTATGACAATAGAACAG; CD8, CAAGAGCGCCCTTGGTG; CD10, CAAAGTGGACGAGTCCGG.

**RNA manipulations.** A. nidulans mRNA was purified from freshly grown and frozen mycelia using Dynabeads oligo(dT)₅₀–₅₅ (Dynal) according to the manufacturer's instructions. Northern blots were performed as described by Sambrook et al. (1989), but using 0.22 M formaldehyde in both the buffer and the agarose gel (Sylvers & Beresten, 1993). RNA size markers were used according to manufacturer's instructions (Gibco). Reverse transcriptase (RT) reactions were performed with Tth DNA polymerase according to the manufacturer's instructions (Boehringer Mannheim). Modifications to buffer and EGTA prior to subsequent PCR of the RT product were also done according to manufacturer's recommendations, except that RNase H (2.7 U) (Gibco) was included in the PCR mix and the samples were incubated at 37 °C for 5 min prior to the 95 °C denaturing step.

**Modified 'RACE no more' RT PCR.** This approach to finding the 5'-end of a transcript was first described by Weis (1994). In this...
modified version both the first strand synthesis and the subsequent PCR was carried out using the single niaA-specific primer, CD4, shown in Fig. 1. Unlike the Weis version, in this protocol a single enzyme, Tth DNA polymerase (Boehringer Mannheim), was used to perform both the RT part of the procedure (65 °C for 20 min) and the subsequent PCR reaction (denature for 2 min at 95 °C followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s, 72 °C for 60 s, finishing with 72 °C for 5 min) following the RNase H treatment (37 °C for 5 min). These modifications enabled the RT reaction to be carried out at a higher temperature, thereby reducing any secondary structure problems in the RNA templates and removing the requirement for a phenol/chloroform extraction and ethanol precipitation to change buffers for the PCR reaction.

**Transformations.** *A. nidulans* transformations were as described by Ballance & Turner (1985). DNA samples for transformations were prepared following standard alkaline lysis mini-preparation procedures (Sambrook et al., 1989), then cleaned with Stratagene resin (Stratagene) according to manufacturer's instructions. *E. coli* was transformed using the heat-shock-induced method described by Sambrook et al. (1989) or by electroporation-induced transformation, according to the method recommended by the manufacturers of the electroporation apparatus (Bio-Rad).

**DNA sequence analyses.** DNA sequences and predicted amino acid sequences were analysed using programs contained within the Genetics Computer Group, Wisconsin Package, Version 8.0 (1994). In particular, programs TFASTA and FASTA, which compare the query sequence with those contained in the GenBank/EMBL database, were used to identify homologies to other genes and proteins. The GAP program (selecting the default parameters) was utilized in the alignment of the NUVA, RAD18 and UVS2 putative amino acid sequences.

**RESULTS AND DISCUSSION**

**Rescue of the nuaA11 phenotype by the genomic clone, pNUV11A40**

A 40 kb *A. nidulans* genomic DNA clone, pNUV11A40 (Fig. 1), that restored the MNNG and 4NQO sensitivities of the nuaA11 mutant strain, L601, was described previously (Osman et al., 1991). The resistance of strain L601 transformed with pNUV11A40 was shown to be the same as that of an equivalent wild-type strain, L600, transformed with just the vector, pCAP2, whilst L601 remained hypersensitive to both agents when transformed with vector alone. There were several possible explanations for the observed increase in resistance of the pNUV11A40 transformants, including over-expression of an unrelated DNA repair gene due to duplication. Suppression of the MNNG and 4NQO sensitivities was not therefore considered conclusive evidence that pNUV11A40 carried the nuaA gene. Confirmation that the clone was directly restoring the mutant allele was obtained by determining its effects on other aspects of the nuaA11 phenotype. To determine its effects on recombination, pNUV11A40 was used to transform the nuaA11 mutant strain L605 which contains the yA2 and luA1 markers on chromosome I and can be used for assessing intergenic recombination frequencies. It also carries the niaD26 deletion on chromosome VIII, one of the two niaD alleles used in intragenic recombination assays (Osman et al., 1991). Control transformants with the vector alone in L605 and also in the isogenic wild-type strain, L604, were created. The MNNG and 4NQO sensitivities of these haploid transformants were tested and found to be the same as the original transformants (Osman et al., 1991) (data not shown). Sexual crosses were carried out and diploids made with the new transformants and the nuaA11 strain, L603, which contains the linkage group II markers wA3 and puA1 plus the niaD allele, niiAniaDa608, the other deletion required for the intragenic recombination assays (Osman et al., 1991). The mitotic and meiotic, intergenic and intragenic, recombination frequencies were then determined as described previously (Osman et al., 1991). Mitotic intragenic (Table 3) and intergenic (Table 4) recombination frequencies in nuaA11/nuaA11 homozygous diploids made with the pNUV11A40 transformants were reduced to levels comparable to wild type as observed in pCAP2-transformed

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
<th>Mitotic recombination frequency (%)</th>
<th>Meiotic recombination frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B608/CS387</td>
<td>nuaA+ / nuaA+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B608/L603</td>
<td>nuaA+ / nuaA-</td>
<td>112</td>
<td>136</td>
</tr>
<tr>
<td>B608/L605:pCAP</td>
<td>nuaA+ / nuaA-::pCAP</td>
<td>119</td>
<td>112</td>
</tr>
<tr>
<td>B608/L605:pNUV11A40</td>
<td>nuaA+ / nuaA::pCAP</td>
<td>96</td>
<td>80</td>
</tr>
<tr>
<td>B608/L605:pEA14</td>
<td>nuaA+ / nuaA::pEA14</td>
<td>120</td>
<td>56</td>
</tr>
<tr>
<td>L602/L603</td>
<td>nuaA+ / nuaA-</td>
<td>870</td>
<td>5</td>
</tr>
<tr>
<td>L602/L605:pCAP2</td>
<td>nuaA+ / nuaA-::pCAP</td>
<td>660</td>
<td>5</td>
</tr>
<tr>
<td>L602/L605:pNUV11A40</td>
<td>nuaA+ / nuaA::pCAP</td>
<td>95</td>
<td>72</td>
</tr>
<tr>
<td>L602/L605:pEA14</td>
<td>nuaA+ / nuaA::pEA14</td>
<td>92</td>
<td>88</td>
</tr>
</tbody>
</table>
Table 4. Mitotic and meiotic intergenic recombination frequencies

Mitotic intergenic recombination was measured by marker segregation in benlate-induced haploids arising from diploids constructed using the transformants, compared with wild-type and nuvA mutants. Mitotic recombination frequencies were measured in map intervals between yA2 and luA1 (68 cM apart on chromosome I) and also between wA3 and puA1 (>200 cM apart on chromosome II) (Clusterback, 1994) in crosses constructed with transformants, compared with the control wild-type and nuvA homozygous and heterozygous diploids. The values indicate the numbers of separate haploid colonies arising. Meiotic intergenic recombination frequencies were measured in map intervals between yA2 and luA1 (68 cM apart on chromosome I) and also between wA3 and puA1 (>200 cM apart on chromosome II). Mitotic and meiotic intergenic recombination frequencies in the wild-type strain L20 were not fully restored to wild-type levels by pNUVllA40 (data not shown), although both were much reduced. A satisfactory explanation for this anomaly has yet to be determined, although it may be due to the continued expression of the defective copy of the nuvA gene. Southern blots of all transformants were consistent with a single homologous integration event, which would result in the presence of both a wild-type and mutated copy of the nuvA gene (data not shown).

\[
\begin{array}{ll|ll|ll|ll|ll|ll}
\text{Strain} & \text{Genotype} & \text{Mitotic recombination} & \text{Meiotic recombination} \\
 & & \text{Linkage group I yA2- luA1} & \text{Linkage group II wA3-puA1} & \text{Meiotic recombination (cM)} \\
 & & \text{Recombinant} & \text{Parental} & \text{Recombinant} & \text{Parental} & \text{Linkage group I yA2- luA1} & \text{Linkage group II wA3-puA1} \\
B608/CS387 & nuvA+/nuvA+ & 0 & 118 & 0 & 179 & 41-20 & 42:50 \\
B608/L603 & nuvA+/nuvA- & 0 & 133 & 0 & 191 & 44-40 & 41:60 \\
B608/L605:pCAP & nuvA+/nuvA- & 2 & 152 & 1 & 117 & 37-70 & 52:70 \\
B608/L605: pNUVllA40 & nuvA+/nuvA- & 0 & 123 & 0 & 141 & 41-60 & 43:21 \\
L602/L603 & nuvA+/nuvA- & 122 & 43 & 101 & 123 & 29-80 & 47:80 \\
L602/L605: pCAP2 & nuvA+/nuvA- & 66 & 92 & 72 & 86 & 31-86 & 45:40 \\
L602/L605: pNUVllA40 & nuvA+/nuvA- & 0 & 76 & 1 & 93 & 41-20 & 53:40 \\
L602/L605: pEA14 & nuvA+/nuvA- & 1 & 134 & 3 & 134 & 34-10 & 56:50 \\
\end{array}
\]

**pEA14, the smallest fragment able to restore nuvA11 to wild type**

\(EcoRI\) and K\(pnI\) restriction enzyme digests of the 40 kb pNUV11A40 \(A.\ niddans\) genomic DNA insert were performed and the resultant fragments ligated into the multiple cloning site of the \(Escherichia\ coli\)/\(A.\ niddans\) shuttle vector pRG4. These subclones were then used to transform the \(nuvA11\) mutant strain L603. Several of the uridine and uracil prototrophic colonies from each of the transformations, including those transformed with the vector alone, were tested for sensitivity to MNNG and 4NQO (data not shown). Only one of the subclones, pEA14 (Fig. 1), which contained a 2.3 kb EcoRI insert, consistently produced resistant transformants, Southern blots of which were consistent with a homologous integration of the plasmid into the nuvA locus (data not shown). The ability of this subclone to rescue the \(nuvA11\) mitotic and meiotic (Tables 3 and 4) recombination defects was tested and found to be comparable to that of the larger clone, pNUV11A40, pEA14, like pNUV11A40, also failed to rescue fully the morphological defects and diploid instability associated with the \(nuvA11\) phenotype. From these observations it was deduced that the nuvA gene was contained, at least in part, on the 2.3 kb EcoRI fragment. This fragment was subjected to DNA sequencing and restriction enzyme analysis to determine the extent and nature of the nuvA gene. The results of these analyses, when compared to the DNA sequence of a cDNA clone (described below), demonstrated that the pEA14 subclone contained only the 5' end of the nuvA coding region (Figs 1 & 2). A series of PCRs, using primers directed against both the genomic and cDNA clones, were performed with a chromosomal DNA template from the \(A.\ niddans\) wild-type strain L20. The PCR fragments obtained (Fig. 1) were of the predicted sizes and were consistent with both the genomic and the cDNA clones being derived from contiguous sequences.
The **nuvA** gene of *Aspergillus nidulans*

1. 1071
   2338 MT TCG GAT ACG CCG CGA AGC GCC CAA CTC GTT GAT CAG CCT ATC

2. 2236

3. 2134

4. 1981 CTA CTG AAA GAT ACG GTG CTA CGA AAG AAA

5. 1930 CTA GCA GCC ACG GGA

6. 1810 CTG AAG CCA GCC GCT

7. 1651 GAG GAC TAT ATG CCG G GTAGGT "TT CAACGCGTTC ATCCGTGGTA TCCACTAACT

8. 1397 CAG GAG CTG GTG GAG GGG TTT AAG AAT

9. 1346 ACA

10. 1021 GCAACGCCAT TTCTTTCCCC AATCGAGAGA CGCCATCCTG AATCCGCCCA AGCTCTCACG

11. 1244 GTC TGC AAG GAC TTT TTC GAC AAC CCT GTG ATC ACG TCG

12. 860

13. 817

14. 664 CTA

15. 404

16. 361 CAAATGTGGA TTTTGCATCT GTAGCGCTAG TTAGTGACTA TTAGAGGCAT GGCCGCCCGT

17. 301 CATTGAGATC CTGCAGTATG GTAGGTPGCA

18. 901 ATAGCCATCC TTTCGACGAA mTOOCOCC GTTGTTGTAT ATCGGAGATG CAATGAGCAG

19. 121 GGTCGTACAC CTGTTCTCCT C'TGTTCGACT TCAAGTCAGC AAGACAAGGC TTACATGATA

20. 181 GmTCTACA TGTAGACGAG CGTACCATGA CATTCTGATT 'TGAACATCCC ACTGCTTCGT

21. AAT TCQ O&T

22. TOO TCa CCCm COT OU TTA Qu:

23. Leu Trp

24. TPO TOO

25. Asn

26. CTA m AM

27. Leu Lys Pro

28. TIT

29. Asp

30. TTT

31. Ser

32. Gln Glu Leu

33. CTA TCT GAT CAG GAG CTG AAG

34. Thr Gly Ser Leu

35. GCT GTA TGT GGA CGG AGO ATG AAA

36. Val Ile

37. GCT GTA TGT GGA CGG AGO ATG AAA

38. Val Ile

39. Leu Leu Gln Arg

40. CCA CCG

41. Leu

42. Ala

43. CTA GTG CCG

44. Val

45. Gln Glu Leu Lys Leu

46. AGC TCT GAT CAG GAG CTG AAG

47. Asp

48. OAT TOO CTC

49. Ala

50. AM

51. Asp

52. TTT

53. Ser

54. Gln Leu

55. Arg

56. CTG GTG GGC ATA CCA

57. Val Asp Ala

58. AAC

59. Asp

60. CTA

61. Arg

62. AAA

63. Met

64. AGA CTT CCT GTT ATA AAT TAT TCT

65. Gln Glu Leu Val Thr Ser Leu Arg Cys Gin

66. CTA TTA AAA TTC CCG TCG TCT CAA CTC

67. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

68. CTA TTA AAA TTC CCG TCG TCT CAA CTC

69. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

70. CTA TTA AAA TTC CCG TCG TCT CAA CTC

71. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

72. CTA TTA AAA TTC CCG TCG TCT CAA CTC

73. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

74. CTA TTA AAA TTC CCG TCG TCT CAA CTC

75. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

76. CTA TTA AAA TTC CCG TCG TCT CAA CTC

77. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

78. CTA TTA AAA TTC CCG TCG TCT CAA CTC

79. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

80. CTA TTA AAA TTC CCG TCG TCT CAA CTC

81. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

82. CTA TTA AAA TTC CCG TCG TCT CAA CTC

83. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

84. CTA TTA AAA TTC CCG TCG TCT CAA CTC

85. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

86. CTA TTA AAA TTC CCG TCG TCT CAA CTC

87. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

88. CTA TTA AAA TTC CCG TCG TCT CAA CTC

89. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

90. CTA TTA AAA TTC CCG TCG TCT CAA CTC

91. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

92. CTA TTA AAA TTC CCG TCG TCT CAA CTC

93. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

94. CTA TTA AAA TTC CCG TCG TCT CAA CTC

95. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

96. CTA TTA AAA TTC CCG TCG TCT CAA CTC

97. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

98. CTA TTA AAA TTC CCG TCG TCT CAA CTC

99. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

100. CTA TTA AAA TTC CCG TCG TCT CAA CTC

101. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

102. CTA TTA AAA TTC CCG TCG TCT CAA CTC

103. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

104. CTA TTA AAA TTC CCG TCG TCT CAA CTC

105. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

106. CTA TTA AAA TTC CCG TCG TCT CAA CTC

107. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

108. CTA TTA AAA TTC CCG TCG TCT CAA CTC

109. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

110. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

111. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

112. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

113. Ser Gin Leu Arg Thr Ser Thr Arg Cys Ser His Thr Glu Gly Cys Pro

Fig. 2. Nucleotide sequence of the genomic DNA containing the **nuvA** gene (numbered 1–2336) compared to its cDNA sequence (bold type; numbered 1–2222) with the deduced amino acid sequence for the coding region. The limits of the sequence covered by the RT-PCR product are indicated whilst the 5’ start of the truncated cDNA clone plWf100 is indicated by △. The genomic sequence from 1 to 2337 was obtained from clone pE14A; the 3’ limit of this sequence is identified by double underlining. The genomic sequence from 2134 to 3269 was obtained from cosmid plW17F02 by PCR using primers CD3 and CD8. The limits of this sequence are denoted by dashed underlining.
in the *A. nidulans* genome and therefore the presumed overlap between them was justified. Also, the presence of multiple 'nuvA-like' sequences in the *A. nidulans* genome is unlikely, since Southern blots of *A. nidulans* chromosomal DNA probed with the pEA14 2·3 kb EcoRI fragment did not reveal any unexpected bands (data not shown).

**Cloning the nuvA cDNA**

Computer homology searches with the predicted amino acid sequences of the *nuvA11* rescuing clone, pEA14, revealed significant homologies to the *N. crassa nuv-2* (Tomita et al., 1993) and the *S. cerevisiae RAD18* (Jones et al., 1988) gene products. The most striking homology between all three amino acid sequences occurred in a region that contained a C3HC4 type of zinc finger motif. Whilst a suitable candidate for the *nuvA* ORF had yet to be confirmed, a decision was taken to use this region of the pEA14 clone to probe an *A. nidulans* 24 h developmental cDNA library based on *AZAP* (Aramayo & Timberlake, 1990). The probe was prepared by PCR amplification of the 333 bp fragment of pEA14 containing the zinc finger coding region using the PCR primers (Fig. 1) L15 and L113 (30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, finishing with 72 °C for 10 min). The probe was used in a plaque hybridization. Only two independent, putative, hybridization-positive clones were identified from over 50 000 plaques screened. In retrospect this result was not too surprising since recent attempts to estimate transcript size, and study the levels of expression, of *nuvA* using Northern blots probed with the same 'zinc finger' fragment repeatedly failed to detect any message when used to probe mRNA samples from either 24 h cultures, similar to those used to create the cDNA library, or from cultures exposed to the DNA damaging agents MNNG or 4NQO. All of the blots consistently produced the expected results when reprobed with the control gene, *y-actin* gene (Fidel et al., 1988) (data not shown), and we conclude that the *nuvA* gene is expressed at very low levels in vegetative tissue. Since restriction enzyme analysis and partial DNA sequencing demonstrated that the two cDNA clones were identical, DNA sequencing proceeded using only one of them, pLW100. This revealed that the clone had been truncated at the 5' end of the gene (Fig. 2), resulting in the loss of part of the 5' coding and untranslated regions. To overcome this problem a modified version of the 'RACE no more' RT PCR method was adopted (Weis, 1994), as described in Methods. The products of the RT-PCR were detected as two faint bands of approximately 1.5 kb and 900 bp on a 0·6 % low melting point agarose (Bio-Rad) gel (data not shown). The bands were recovered and subjected to further PCR analysis. It was assumed that the *nuvA*-specific primer, CD4, had somehow become attached to the 5' end of the RT-PCR product, as proposed by Weis (1994), thereby enabling the amplification to occur with just a single primer, creating a product with CD4 sites at both ends. For the PCR analysis of the RT-PCR products, specific primer pairs were chosen to test this prediction and confirm that they were derived from *nuvA* transcripts (Fig. 1). Pair A comprised CD2, derived from the known cDNA sequence, and the genomic-sequence-derived primer LI13 which should amplify the zinc finger coding region to give a 416 bp product; primer pair B comprised CD1, whose sequence was complementary to the CD2 primer, and the RT-PCR primer CD4. According to the known cDNA sequence, the predicted product of primer pair B would be 362 bp and comprise one end of the RT-PCR product; the equivalent genomic sequence would be larger due to the presence of introns. Primer pair C, CD2 and CD4, was therefore used for PCR of the opposite end of the RT-PCR fragments. This pair was predicted to give an approximately 540 bp product from the smaller of the two RT-PCR fragments and approximately 1140 bp from the larger fragment. PCR products were only obtained from the smaller RT-PCR fragment, but they were all of the expected sizes implying that they were derived from the *nuvA* transcript and that CD4 primers were located at both ends of the RT-PCR product. The 362 bp product of primer pair B was sequenced directly using CD1 and CD4 as sequencing primers. The sequence obtained corresponded to that already determined for the cDNA clone pLW100. A similar sequencing strategy for the CD2/CD4 PCR product was more problematical, and it was therefore cloned into the PCR cloning vector pGEM-T (Promega) and sequenced using the M13 (−20) and M13 reverse sequencing primers. The sequence derived was as expected based on the genomic sequence for that region of the *nuvA* gene encoding the putative C3HC4 type zinc finger motif.

**Fine mapping of nuvA**

The assignment, by mitotic mapping experiments (Osman et al., 1991), of *nuvA11* to linkage group IV could not be confirmed by conventional meiotic mapping. However, the positive hybridization of the 2·3 kb pEA14 *A. nidulans* genomic DNA insert (Fig. 1) to pW17F02, a chromosome-IV-specific clone from the pWE15 *A. nidulans* genomic library (Brody et al., 1991) identified its site. No linkage was detected to any of the chromosome IV markers present on the meiotic mapping strain A517. Since a physical map of linkage group IV had been constructed (Wang et al., 1994), it was possible to assign the *nuvA* gene to an approximate physical map position based on the known chromosomal location of cosmid pW17F02. Using this information, *nuvA* was predicted to be close to *bimD* on the left arm of chromosome IV, since *bimD* also hybridized to this cosmid (R. Prade, University of Georgia, personal communication). A series of PCR experiments was then performed in an attempt to orientate *nuvA* with respect to *bimD* (Fig. 1). PCR primers directed against the published sequence for *bimD* (accession number L03200) were used in conjunction with available *nuvA* primers with PCR buffer 1 in the Expand Long Template PCR System (Boehringer) according to the manufacturer's recommendations (denature for 2 min at 94 °C, followed by 10 cycles of 94 °C for 10 s, 62 °C for 30 s and 68 °C for 8 min, then 20 cycles of 94 °C for 10 s, 62 °C for 30 s and 68 °C for 8 min, increasing by 20 s each
cycle, followed by 68 °C for 7 min and kept at 4 °C overnight). From these experiments we were able to determine that both genes have the same direction of transcription and that the 3' end of the bimD gene lies approximately 2 kb upstream of the start of the nuvA gene. This orientation was consistent with the expected locations of BamHI and SstI restriction enzyme sites in this region, predicted from the DNA sequence of bimD and a previously constructed restriction enzyme map of the equivalent region in pNUV11A40, the original nuvA11 rescuing cosmid (Fig. 1).

The genetic map for chromosome IV contains very few markers in the vicinity of bimD (Clutterbuck, 1994; Käfer & Chae, 1994), resulting in difficulty in meiotic mapping. The only other marker, the uvsH gene, is 3 cM from bimD (Käfer & Chae, 1994). This is not inconsistent with the physical distance between nuvA and bimD, which together with the broad similarity of the nuvA and uvsH mutant phenotypes (sensitivity to UV and increased mitotic recombination) suggests that they could be the same gene. There are, however, significant differences in the meiotic phenotypes of nuvA and uvsH mutant alleles, for while nuvA homozygous crosses show an absence of meiotic recombination, yet are otherwise phenotypically normal, no effect on meiotic recombination is observed for uvsH homozygous crosses. This is so even in the very poorly fertile crosses involving the extreme allele uvsH77 (Käfer & Mayor, 1986). However, the low fertility could be due to aberrant meiotic recombination. Early in our study, diploid complementation assays were performed to test nuv mutations against known uvs mutations. In those tests, nuvA11 and uvsH alleles complemented (Osman et al., 1991) and we concluded that they were therefore non-allelic, since there were also differences in their mutant phenotypes. Because of our recent physical mapping data however, we cannot exclude the possibility that this represents intragenic complementation, perhaps at a complex locus.

The nuvA nucleotide and predicted amino acid sequences

The nucleotide sequences of both strands of the genomic clone pEA14, the cDNA clone pLW100, the RT-PCR clone pLW101 and RT-PCR fragment from primer pair B have been determined (Fig. 2) and the contiguity of these fragments in the A. nidulans genome has been confirmed (Fig. 1). In addition, the sequence of the genomic DNA defined by primers CD3 and CD8 has been determined by PCR amplification from pW17F02. In the genomic DNA sequence preceding the ATG codon of the nuvA ORF at position 1151, there are two TATA-like boxes at positions 334–340 and 588–592, and at position 431–433 there is a CAAT-like box. These sites are a long way from the coding region and their functional validity has yet to be verified. A comparison of the genomic DNA sequence with the cDNA sequence has revealed the presence of two introns, one of 51 bp and one of 60 bp. The consensus 5' and 3' splice junction sequences (5' -GT and 3' -AG) are conserved in intron 1 (1620–1634) but not in intron 2 (1827–1886). The genomic DNA sequence is complete for the coding region of the gene, and terminates 209 bp before the end of the cDNA sequence. Due to difficulties in sequencing the beginning of the RT-PCR product, even after cloning, only 4 bp of the cDNA sequence that is presumed to represent 5' untranslated sequence is available. The nuvA ORF comprises 1329 bp of the total 2.2 kb of cDNA sequence, whilst the remaining 900 bp is assumed to be 3' untranslated sequence. The large extent of 3' untranslated sequence and the observation that it contains other potential ORFs is perhaps an indication that the nuvA message can undergo alternative splicing reactions and if uvsH and nuvA prove to be allelic, perhaps accounts for the intragenic complementation. Future studies of nuvA expression will test this hypothesis. The nuvA ORF encodes a 443 amino acid protein, molecular mass 48.9 kDa, containing two zinc finger motifs near the N terminus. The first of the zinc finger motifs is termed a C3HC4 or ring finger motif and has been found in a variety of proteins involved in recombination and differentiation including the human V(D)J recombination activating protein, RAG-1 (Shatz et al., 1989), the UVS-2 protein of N. crassa (Tomita et al., 1993) and the RAD18 protein of S. cerevisiae (Jones et al., 1988). The second motif has the consensus sequence C/H-X2-C/H-X,-,-C/H (Berg, 1990).

Comparison with UVS-2 and RAD18

Database searches with the putative nuvA translation product identified significant homologies between NUVA and the translation products of the uvs-2 gene of N. crassa (Tomita et al., 1993) and the RAD18 gene of S. cerevisiae (Jones et al., 1988). More specifically, using the Genetics Computer Group Program GAP program (1994), 39% of the residues were found to be identical and 57% similar to those of the UVS-2 protein, with 25% identical and 46% similar to the RAD18 protein (Fig. 3). In a comparison between UVS-2 and RAD18, 25% of the residues were identical and 68% homologous (Tomita et al., 1993). The highest homology exists around the two zinc finger motifs; there is 100% conservation of nine amino acid residues from the histidine of the C3HC4 zinc finger. A second interesting feature is the high level of conservation in the region covered by the NUVA residues 272–280 inclusive; these residues end the tract of significant homology between the three proteins. The putative nucleotide binding site of RAD18, the core sequence of which shares no apparent homology with either NUVA or UVS-2, is located in the C-terminal region of least homology. This may represent an important difference between the three proteins or may imply that the similarity of the RAD18 sequence to a nucleotide binding site is purely coincidental.

The phenotypes that result from mutations in these three genes are very similar. All cause an extreme sensitivity to UV light and other DNA damaging agents and show increased levels of UV-induced mutation (nuvA, unpublished results; uvs-2, Tomita et al., 1993; RAD18, Cassier-Chauvat & Fabre, 1991), which in the case of
RAD18 is thought to be due to the stimulation of the error-prone repair pathway. RAD18 mutants, like those of *nuvA*, show an increase in mitotic recombination frequencies. They also show an increase in the frequency of UV- or other DNA damaging-agent-induced mitotic recombination, an effect which has been attributed to the channelling of recombinogenic structures into the RAD52 recombination repair pathway. Due to results from an excision repair assay in *N. crassa* (Worthy & Epler, 1973), *uvz-2* was originally thought to be required for excision repair of DNA damage. However, more recent experiments have shown that it is not required for the excision of thymine dimers (Ishii *et al.*, 1991) and is therefore unlikely to be involved in excision repair of these lesions. RAD18 is a member of the RAD6 epistasis group and is presumed to act, in consort with RAD6 and complexed with RAD6, in an error-free post-replication repair pathway, whereas REV1 and REV3, other members of the RAD6 epistasis group, are thought to be required for the mutagenic post-replication pathway (Prakash *et al.*, 1993). One suggestion is that the ssDNA binding property of RAD18 is used to target RAD6 to areas of DNA damage where the separate ubiquitin-conjugating function of RAD6 could be used to modify the stalled replication machinery (Bailly *et al.*, 1994). Unlike *uuvA11*, none of the mutants of either *uvz-2* or RAD18 have been shown to have any effects on either meiosis or meiotic recombination, although RAD18 transcript levels are increased during meiosis (Jones & Prakash, 1991) and rad1 rad18, rad2 rad18 and rad3 rad18 double mutants suffer from a drastic reduction in spore viability. From these observations and the similarity to NUVA, it seems likely that RAD18 will, using different experimental conditions, be found to be involved in meiotic recombination since the effects seen in the *nuvA11* mutant are most dramatic when the markers are very close, as in the intragenic recombination assay.

Further work is required to establish how mutations in *nuvA* might have opposing effects on recombination during meiosis and mitosis and how these are related to its effects on DNA repair. Given their juxtapositions on chromosome IV and the similar responses to DNA damaging agents, it will be interesting to find out the relationship, if any, between *uvzH*, *bimD* and *nuvA*.

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**NOTE ADDED IN PROOF**

The recent publication of the *Aspergillus uvsH* gene sequence (Yoon *et al.*, 1995, *Mol Gen Genet* 248, 174–181) confirms that *nuvA* and *uvsH* are the same gene.

**REFERENCES**


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