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

Lesley Iwanejko,† Catherine Cotton,‡ Gary Jones,§ Brian Tomsett and Peter Strike

Author for correspondence: Peter Strike. Tel: +44 151 794 3620. Fax: +44 151 794 3655.
e-mail: strike@liv.ac.uk

Department of Genetics and Microbiology, The University of Liverpool, Donnan Laboratories, Liverpool L69 3BX, UK

A 40 kb genomic clone and 2.3 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 48 906 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

† **Present address:** Department of Cancer Genetics, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 9BX, UK.

‡ **Present address:** Department of Biological and Chemical Science, White-lands College, Roehampton Institute of Higher Education, Sutherland Grove, London SW15 35N, UK.

§ **Present address:** School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, UK.

Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; 4NQO, 4-nitroquinolene-1-oxide; RT, reverse transcriptase.

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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-nitroquinolene-1-oxide (4NQO). The *nuvA11* mutants were shown to have a complex phenotype. Haploids are slow growing and have a 'crinkly' mor-

Table 1. Strains used in this study

Strain	Genotype and relevant phenotype	Source or reference
<i>E. coli</i>		
DH1	F' <i>recA1 endA1 gyrA96 thi-1 hsdR17</i> (r_k^- , m_k^-) <i>supE44 relA1</i> λ^-	Hanahan (1983)
XL1-blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17</i> (r_k^- , m_k^+) <i>supE44 relA1 lac</i> [F' <i>proAB lacI^q</i> ZAM15 Tn10 (<i>tet^r</i>)]	Bullock <i>et al.</i> (1987)
SOLR TM	<i>sbcC recB recJ uvrC umuC::Tn5</i> (<i>kan^r</i>) <i>e14-(mcrA),Δ(mcrCB-hsdSMR-mr^r)171</i> [F' <i>proAB lacI^q</i> ZAM15] <i>endA1 gyrA96 thi-1 lac relA1 λ^r</i> , Su ⁻ (non-suppressing)	Stratagene
F1 helper phage		
ExAssist TM	Interference-resistant helper phage containing amber mutations in genes I and II (5 kb, single-stranded)	Stratagene
<i>A. nidulans nuvA⁺</i> strains		
L20	<i>wA3 pabaA1</i>	Liverpool stock
A517	<i>riboA1 yA2 adE20; methG1 suC11adE20 palC4 pabaB22 pyroA4</i> (a meiotic mapping strain for linkage group IV)	Fungal Genetics Stock Center (FGSC)
B608	<i>puA1 wA3; niiAniaDΔ608</i>	Tomsett & Cove (1979)
CS387	<i>yA2 luA1; niaD26</i> (A pair of strains carrying non-overlapping deletions in the <i>niaD</i> gene allowing selection for nitrate, utilizing products of intragenic recombination)	Tomsett & Cove (1979)
L600	<i>pyrG89 yA2; niaD26</i> (previously referred to as TYS3870)	Osman <i>et al.</i> (1991)
L604	<i>pyrG89 yA2 luA1; niaD26</i>	This study
<i>A. nidulans nuvA11</i> strains		
L601	<i>pyrG89 yA2; nuvA11; niaD26</i> (previously referred to as TYS3871)	Osman <i>et al.</i> (1991)
L602	<i>puA1 wA3; nuvA11; niiAniaDΔ608</i> (previously referred to as B608 <i>nuv11</i>)	Osman <i>et al.</i> (1991)
L603	<i>yA2 luA1; nuvA11; niaD26</i> (previously referred to as CS387 <i>nuv11</i>) (A pair of <i>nuvA11</i> strains carrying non-overlapping deletions in the <i>niaD</i> gene allowing selection for nitrate, utilizing products of intragenic recombination)	Osman <i>et al.</i> (1991)
L605	<i>pyrG89 yA2 luA1; nuvA11; niaD26</i>	This study

phology, whereas *nuvA11* 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 *nuvA11* strains, even though the fertility and ascospore viability of such crosses appears to be unaffected. The *nuvA11* 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 *nuvA11* 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 *nuvA11* 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 *nuvA11* phenotype. We also describe the isolation of the *nuvA* cDNA sequence. The presumed location of the *nuvA* gene, close to *bimD* and *wsH*, 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

Table 2. Vectors and constructs used in this study

Vector	Selectable marker*	Features	Source or reference
<i>E. coli</i>			
pBluescript SK —	Ap ^r	2.96 kb phagemid	Stratagene
pGEM-T System I	Ap ^r	Supplied as an <i>EcoRV</i> -cleaved pGEM-5Zf(+) vector treated with terminal transferase to produce 3' overhangs for direct PCR cloning	Promega
λ ZAP	Ap ^r	λ insertional vector. <i>In vivo</i> excision releases cloned DNA in pBluescript SK —	Stratagene
pWE15	Ap ^r	8.8 kb cosmid. Also carries the neomycin marker under the control of the SV40 promoter for use in eukaryotes	Stratagene
<i>E. coli/A. nidulans</i>			
pCAP2	pyr4/Ap ^r	Cosmid containing the <i>ans1</i> transformation-enhancing sequence of <i>A. nidulans</i> and a unique <i>Bam</i> HI cloning site	Ballance & Turner (1985)
pRG4	pyr4/Ap ^r	pUC19-based vector derived from pRG3. Contains the pBluescript SK — polylinker	Waring <i>et al.</i> (1989)
Constructs			
pSF20	pyr4/Ap ^r	pRG3 containing a γ -actin gene fragment	Fidel <i>et al.</i> (1988)
pNUV11A40	pyr4/Ap ^r	pCAP2 containing a 40 kb <i>A. nidulans</i> genomic DNA insert	Osman <i>et al.</i> (1991)
pEA14	pyr4/Ap ^r	pRG4 containing a 2.5 kb <i>A. nidulans</i> genomic DNA insert	This study
pLW100	Ap ^r	pBluescript SK — containing the <i>nuvA</i> cDNA insert	This study
pLW101	Ap ^r	pGEM-T containing the 5' end of the <i>nuvA</i> gene	This study
pW17F02	Ap ^r	pWE15 clone of <i>A. nidulans</i> genomic DNA that hybridized the pEA14 insert	This study

* Ap, ampicillin.

in pWE15 (Brody *et al.*, 1991), and the *A. nidulans* 24 h developmental cDNA library in λ ZAP (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 *et al.*, 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°C 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 ($0.5\text{--}2.5\text{ }\mu\text{g ml}^{-1}$) and 4NQO ($0.25\text{--}1.5\text{ }\mu\text{g ml}^{-1}$). 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 *nuvA11* homozygous diploids which are extremely unstable and morphologically diverse.

Fine mapping. Attempts were made to map the *nuvA11* mutation meiotically within linkage group IV using the meiotic mapping strain A517, employing standard genetic mapping techniques. A more accurate map position was obtained by hybridizing the cloned *nuvA* 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 dideoxynucleotide 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,

Fig. 1. Diagram of the 9.5 kb *bimD/nuvA* region of chromosome IV. All the *Bam*HI (B) and *Sst*I (S) sites specific to this region and found on the cosmid clones pNUV11A40 and pW17F02 are marked, as are the *Eco*RI (E) sites used to subclone the 2.3 kb *nuvA11*-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 *Taq* DNA polymerase with the buffer and $MgCl_2$ 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. ^{32}P -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, GTAAACGACGGCCAGT; M13-reverse, GGAAACAGCTATGACCATG; bimD1, CATCTATCGTCGATGCTGCGGG; bimD2, CTGGAACCGGGGATGCAGG; bimD3, CCTGCATCCCCGGTTCCAG; iwa1, GAATTCGCTTGGAAGCTTGG; iwa2, GAATTCTGGCGTTGCAGAGG; iwa5, CCCAAGCCTCTGCAACGCCAG; LI1, GAGCGTACCATGACATTC; LI5, GACCATCTGTGCC-

GAC; LI13, AACGTCCCTCCGCTGCCA; CD1, CCCTGTT-CAGGTCATAG; CD2, CTATGACCTGAACAGGAG; CD3, GTGGAACGCCAATTGTG; CD4, CACAATTGGC-GTTCCAC; CD5, GCTATCGACAATAGAACAG; CD8, CAAGAGCCGCTCTTGGTG; CD10, CAAAGTGGACGAGATCGG.

RNA manipulations. *A. nidulans* mRNA was purified from freshly grown and frozen mycelia using Dynabeads oligo(dT)₂₅₋₃₀ (Dyna) 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 protocol (Boehringer Mannheim). Additions of PCR 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 *nuvA*-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 Strataclean 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 *nuvA11* 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 *nuvA11* 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 *nuvA* gene. Confirmation that the clone was directly restoring the mutant allele was obtained by determining its effects on other aspects of the *nuvA11* phenotype. To determine its effects on recombination, pNUV11A40 was used to transform the *nuvA11* 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 *nid26* deletion on chromosome VIII, one of the two *nid* 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 *nuvA11* strain, L603, which contains the linkage group II markers *wA3* and *puA1* plus the *nid* 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 *nuvA11/nuvA11* homozygous diploids made with the pNUV11A40 transformants were reduced to levels comparable to wild type as observed in pCAP2-transformed

Table 3. Mitotic and meiotic intragenic recombination frequencies in parental and transformed strains obtained using diploids and sexual crosses, respectively

nid⁺ recombinants were scored, as described in Methods. The B608/CS387 (*nuv*⁺/*nuv*⁺) recombination frequencies (46.69×10^{-3} for mitotic and 1.07×10^{-3} for meiotic) were taken as 100%.

Strains	Genotype	Mitotic recombination frequency (%)	Meiotic recombination frequency (%)
B608/CS387	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁺	100	100
B608/L603	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻	112	136
B608/L605:pCAP	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::pCAP	119	112
B608/L605:pNUV11A40	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	96	80
B608/L605:pEA14	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	120	56
L602/L603	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻	870	5
L602/L605:pCAP2	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::pCAP	660	5
L602/L605:pNUV11A40	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	95	72
L602/L605:pEA14	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	92	88

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 *nuvA11* 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) (Clutterbuck, 1994) in crosses constructed with transformants, compared with the control wild-type and *nuvA11* homozygous and heterozygous crosses.

Strain	Genotype	Mitotic recombination				Meiotic recombination (cM)	
		Linkage group I <i>yA2-luA1</i>		Linkage group II <i>wA3-puA1</i>		Linkage group I <i>yA2-luA1</i>	Linkage group II <i>wA3-puA1</i>
		Recombinant	Parental	Recombinant	Parental		
B608/CS387	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁺	0	118	0	179	41·20	42·50
B608/L603	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻	0	133	0	191	44·40	41·60
B608/L605:pCAP	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::pCAP	2	152	1	117	37·70	52·70
B608/L605:pNUV11A40	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	0	123	0	141	41·60	43·21
B608/L605:pEA14	<i>nuvA</i> ⁺ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	0	116	4	127	43·80	46·85
L602/L603	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻	122	43	101	123	29·80	47·80
L602/L605:pCAP2	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::pCAP	66	92	72	86	31·86	45·40
L602/L605:pNUV11A40	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	0	76	1	93	41·20	53·40
L602/L605:pEA14	<i>nuvA</i> ⁻ / <i>nuvA</i> ⁻ ::p <i>nuvA</i> ⁺	1	134	3	134	34·10	56·50

nuvA11/+ heterozygous diploids. Recombination in *nuvA11*/*nuvA11* homozygous diploids made with the pCAP2 transformants remained high. Similarly, both intergenic (Table 4) and intragenic (Table 3) meiotic recombination frequencies in *nuvA11* homozygous crosses with the pNUV11A40 transformants were restored to wild-type values. The morphological variation of *nuvA11* mutants and the instability of *nuvA11*/*nuvA11* homozygous diploids were not fully restored to wild type by pNUV11A40 (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 *nuvA11* 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).

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

EcoRI and *KpnI* restriction enzyme digests of the 40 kb pNUV11A40 *A. nidulans* genomic DNA insert were performed and the resultant fragments ligated into the multiple cloning site of the *Escherichia coli*/*A. nidulans* shuttle vector pRG4. These subclones were then used to transform the *nuvA11* mutant strain L605. 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. nidulans* 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

1 GAATTCTGGC GTTGCAGAGG GTTGGGTGGT TCCTCGTTCT TGATTTCAGC TTTTCCGGGT
61 TGTAGAGGGT CAGATTGGGC AGGCTCTTTT CGTGGCACCG TACTCGAAGG GGGAGGTGGT
121 GGCTGCACAC CTGTTTCTCT CTGCTTGACAT TCAAGTCAGC AAGACAGACG TTATCATATG
181 GTTTTCTTACA TGTAGACAGC GTGATTACGA CATTTCTGAT TGACACATCC ACTCGTTGGT
241 ACTCAGGATG ATCCCTCGTG CATGATGGTAA CCAGCATCTC AGACGTACGA TCAGTGAGCA
301 CATTGAGATC TCGCAGTATG GATGGTTGCA CGT TAAZAT GTATATAGAA GATTATGACT
361 CAAATGTAGT TTTTGGCATCT GTAGCGCTAG TTAGTGACTA TTAGAGGCAT GCGCGCCGGT
421 CACGGTGGAG CAATCATGTC CTTCCTGAGT TTCTTCCCGC TCCGACAGCA GCGACGATAC
481 AGGACCTTGG TCCGATTCAG TTTCCGGGTT GGGCAGCGAG CTGCTGAATG AATCAAGTCT
541 CGGCATTATA TCCATTCTCG TGTACACGTT GCCATTGCGA TCGCTCTCGT TCTACAGGGT
601 TCGACGGGCT TGGGACACCC CGTGAAGACG ATATGTTGGG TCGACCTGTC TCGGTTTCTT
661 GGAGCTTTCA TTGAGTGAGCA ACCATAGAGC CATTCTGTCC TACATTAATA TCACTTTAAA
721 TTTTATGAT TATGAGACT ACCTCTGCTC GAGCGTCTG TGTACTCTCA ATCCGCGCC
781 CAGCGACGCA TATGTAACAT GTTGCTCAATG CAGGTAAAGA ATGTAGCTCG AATTTTCAGC
841 TACTAGACCTG AGCATGTTCA TAAAGACAGT CTTCCGCCGA AAAATCTTCT ATGATCAGCG
901 ATGAGCCATCT TTTTCGACGA TTTTGGGCCG GTTGTTGTAT ATCCGAGATG CAATGAGCAG
961 GCACCTTGAT CGAGCGGTGA CGCGAAGCCA CTTTACCGCT ATCAGTCAGC CAGCTTTGGC
1021 GCAACGCCAT TCTTTTCCCG AATGACAGCA CGCCATCTGT AATCGGCCA ACCTCTTCAG
1081 ATATCAGGAT ATAGAACAGA TCTTCCGAGG AAGTCTGAT GGAAGTGGC GATGATACGC

1141 GTTTTGTACTG ATG GAG CCG ACA TTT GAC ATC CCG GAC TCG ACC GAT TGG CTC
1ACTG ATG GAG CCG ACA TTT GAC ATC CCG GAC TCG ACC GAT TGG CTC
Met Glu Pro Thr Phe Asp Ile Pro Asp Ser Thr Asp Trp Leu

1193 GAC ACG CCG TTA ACG CTT CTC GCG CTT TTT GAA ACG TCC CTC CCG TGC CAG
47 GAC ACG CCG TTA ACG CTT CTC GCG CTT TTT GAA ACG TCC CTC CCG TGC CAG
Asp Thr Pro Leu Thr Leu Leu Ala Pro Phe Glu Thr Ser Leu Arg Cys Gln

1244 GTC TCG AAG GAC TTT TTC GAC AAC CTT GTG ATC ACG TCG TGC AGC CAC ACA
98 GTC TCG AAG GAC TTT TTC GAC AAC CTT GTG ATC ACG TCG TGC AGC CAC ACA
Val Cys Lys Asp Phe Phe Asn Pro Val Ile Thr Ser Cys Ser His Thr

1295 TTT TCG TCG CTA TGT ATT CCG CCG TGT CTG AGT ACA GAG GGA AAA TGC CCT
149 TTT TCG TCG CTA TGT ATT CCG CCG TGT CTG AGT ACA GAG GGA AAA TGC CCT
Phe Cys Ser Leu Cys Ile Arg Arg Cys Leu Ser Thr Glu Gly Lys Cys Pro

1346 ACA TCG CCG AGC TCT GAT CAG GAG CTG AAG TTG COT CGC AAT TCG GTG GTG
200 ACA TCG CCG AGC TCT GAT CAG GAG CTG AAG TTG COT CGC AAT TCG GTG GTG
Thr Cys Arg Ser Ser Asp Gln Glu Leu Lys Leu Arg Arg Asn Trp Val Val

1397 CAG GAG CTG GTG GAG GGG TTT AAG AAT GCT CGA CCG AGC ATA TTA CAG CTA
251 CAG GAG CTG GTG GAG GGG TTT AAG AAT GCT CGA CCG AGC ATA TTA CAG CTA
Gln Glu Leu Val Glu Gly Phe Lys Asn Ala Pro Ser Ile Leu Gln Leu

1448 GCT AGG ATG GCA CAA ACT GGC ACG GAT GAT AGT GGA GAT TTG GCT GCG GAA
302 GCT AGG ATG GCA CAA ACT GGC ACG GAT GAT AGT GGA GAT TTG GCT GCG GAA
Ala Arg Met Ala Gln Thr Gly Thr Asp Asp Ser Gly Asp Leu Ala Ala Glu

1499 GAG CCT GCA TCG AAA AAG CCG AAA ATA GAG CAG AAT GCC ATT GTC GGC
353 GAG CCT GCA TCG AAA AAG CCG AAA ATA GAG CAG AAT GCC ATT GTC GGC
Glu Pro Ala Ser Lys Lys Arg Lys Ile Glu Pro Asn Ala Ile Val Gly Thr

1550 GAT GGT CTT CCT GAG GAG GGT ATT CCG ACC CGA TCA CAG AGT CGA GGA GCT
404 GAT GGT CTT CCT GAG GAG GGT ATT CCG ACC CGA TCA CAG AGT CGA GGA GCT
Asp Gly Pro Glu Glu Gly Ile Arg Thr Arg Ser Gln Ser Glu Arg Ala

1601 AGT CCG CAG CCA CAA GCT ACT CTT GTT CAG GTC ATA GAT GAT GGT AAT GAT
455 AGT CCG CAG CCA CAA GCT ACT CTT GTT CAG GTC ATA GAT GAT GGT AAT GAT
Ser Arg Gln Pro Gln Ala Thr Pro Val Gln Val Ile Asp Asp Gly Asn Asp

1651 GAG GAC TAT ATG CCG G GAGGTATTTT CAACCGTTC ATCCGTTGGTA TCCACTAAT
506 GAG GAC TAT ATG CCG G GAGGTATTTT CAACCGTTC ATCCGTTGGTA TCCACTAAT
Glu Asp Thr Tyr Met Pro Cys 6 Intron 1

1707 TTTGTTTGGCA G C AC GCG TTG GTG CCG TGC CCT GTA TGT GGA CCG AGG ATG AAA
521 AC GCG TTG GTG CCG TGC CCT GTA TGT GGA CCG AGG ATG AAA
Ser Gly Leu Val Pro Cys Pro Val Cys Gly Arg Met Lys

1759 GAA GAA GCC GTG TTC CGT CAT CTT GAC TCC TGT ACT GGG ACT GCA GAG GAA
562 GAA GAA GCC GTG TTC CGT CAT CTT GAC TCC TGT ACT GGG ACT GCA GAG GAA
Glu Glu Ala Val Phe Arg His Leu Asp Ser Cys Thr Gly Thr Ala Glu Glu

1810 CTG AAG CCA GCG GCT TT GGGTAAGATT CCATACCGCT AGTGCACAGT TGCATATACAT
613 CTG AAG CCA GCG GCT TT GGGTAAGATT CCATACCGCT AGTGCACAGT TGCATATACAT
Leu Lys Pro Ala Ala Ph Intron 2

1867 AGTGCTAACT CAGTGCCTTA T GGG TCG TTA GCA CCT GGC CCG GCG AAA TGC TTC
630 T GGG TCG TTA GCA CCT GGC CCG GCG AAA TGC TTC
e Gly Ser Leu Ala Pro Gly Pro Arg Lys Ser Phe

1930 CTA GCA GCC ACG GGA AAA CCA CCG GAA AGA CTT CTT GTT ATA AAT TAT TCT
664 CTA GCA GCC ACG GGA AAA CCA CCG GAA AGA CTT CTT GTT ATA AAT TAT TCT
Leu Ala Ala Thr Gly Lys Pro Pro Glu Arg Leu Pro Val Ile Asn Tyr Ser

1981 CTA CTG AAA GAT ACG GTG CTA CGA AAG AAA TTG AAG GAT CTG GGC ATA CCA
715 CTA CTG AAA GAT ACG GTG CTA CGA AAG AAA TTG AAG GAT CTG GGC ATA CCA
Leu Leu Lys Asp Thr Val Leu Lys Ala Lys Leu Lys Asp Leu Gly Ile Pro

2032 AAC TGG GCG CCG CGA GCT TTG CTG Lys AAG CGA CAT ACT GAG TGG TTT AAC
766 AAC TGG GCG CCG CGA GCT TTG CTG Lys AAG CGA CAT ACT GAG TGG TTT AAC
Asn Trp Gly Pro Arg Ala Leu Leu Gln Arg Arg His Thr Glu Trp Trp Lys Asn

2083 TTG TGG AAC GCC AAT TGT GAC TCT AGG ACA CCG AAA CCG AAA CGA GAG TTA
817 TTG TGG AAC GCC AAT TGT GAC TCT AGG ACA CCG AAA CCG AAA CGA GAG TTA
Leu Trp Asn Ala Asn Cys Asp Ser Arg Thr Pro Lys Pro Lys Arg Glu Leu

2134 CTG CGT GAA TTA CAG GTC TGG GAG CCG ACC CAA GGA GGA AAT TCA GTC ACT
868 CTG CGT GAA TTA CAG GTC TGG GAG CCG ACC CAA GGA GGA AAT TCA GTC ACT
Leu Arg Glu Leu Asp Val Trp Glu Arg Thr Gln Gly Gly Asn Ser Val Thr

2185 CCC ACC GAC CCA ACG AAC GCT GTA ATG AAT AAG GAT TTT AAT ACG GAA GAG
919 CCC ACC GAC CCA ACG AAC GCT GTA ATG AAT AAG GAT TTT AAT ACG GAA GAG
Pro Thr Asp Pro Thr Lys Ala Val Met Asn Asp Asp Phe Asn Thr Glu Glu

2236 TGG TCG GCG AAC TAT GAC ACA GAT TTC AAG GCA TTG ATC GCG AAT GCG GCA
970 TGG TCG GCG AAC TAT GAC ACA GAT TTC AAG GCA TTG ATC GCG AAT GCG GCA
Trp Ser Ala Asn Asn Tyr Asp Thr Asp Phe Lys Ala Leu Ile Ala Asn Ala Arg

2287 AAG AAA AAC GAT GCT GTT ATT CCG TCA ACA ATA CCG AAT GCT TCC CAA GCG
1021 AAG AAA AAC GAT GCT GTT ATT CCG TCA ACA ATA CCG AAT GCT TCC CAA GCG
Lys Lys Asn Asp Ala Val Ile Arg Ser Thr Thr Ile Pro Asn Ala Ser Gln Ala

2338 AAT TCG GAT ACG CCG CGA AGC GCC CAA CTC GTT GAT CAG CCT ATC GAA GCT
1075 AAT TCG GAT ACG CCG CGA AGC GCC CAA CTC GTT GAT CAG CCT ATC GAA GCT
Asn Ser Asp Thr Pro Arg Ser Ala Gln Leu Val Asp Gln Pro Ile Glu Ala

2389 TCG CTA ACT CCG CAA GAT GTG GAG GAA AAG CTA ATG AAT CCT CAG GAT
1123 TCG CTA ACT CCG CAA GAT GTG GAG GAA AAG CTA ATG AAT CCT CAG GAT
Ser Leu Thr Pro Gln Asp Val Asp Glu Lys Asp Thr Met Asn Pro Gln Asp

2440	GCT	ATC	GAC	AAT	AGA	ACA	GAG	GTT	CCC	CCG	GTA	CCA	GAT	CCT	CCC	CAA	GCA
1174	GCT	ATC	ANC	AAT	AGA	ACA	GAG	GTT	CCC	CCG	GTA	CCA	GAT	CCT	CCC	CAA	GCA
	Ala	Ile	Asp	Asn	Arg	Thr	Glu	Val	Pro	Pro	Val	Pro	Asp	Pro	Pro	Gln	Ala
2491	TTA	AGC	GGG	ATA	GAT	CGT	GCG	GTG	AAT	TCG	CCG	ATG	AAG	AAT	GTG	'ACC	GAG
1225	TTA	AGC	GGG	ATA	GAT	CGT	GCG	GTG	AAT	TCG	CCG	ATG	AAG	AAT	GTG	'ACC	GAG
	Leu	Ser	Gly	Ile	Asp	Arg	Ala	Val	Asn	Ser	Pro	Met	Leu	Asn	Val	Thr	Glu
2542	GGC	GAT	GCC	CAG	GCG	ATT	CCC	ATC	TCC	AGC	AGT	GCG	TCC	ACC	CAT	AAA	GCG
1276	GGC	GAT	GCC	CAG	GCG	ATT	CCC	ATC	TCC	AGC	AGT	GCG	TCC	ACC	CAT	AAA	GCG
	Gly	Asp	Ala	Gln	Ala	Ile	Pro	Ile	Ser	Ser	Ser	Ala	Ser	Thr	His	Leu	Thr
2593	CGC	CAT	TGAATCTGTC	AAACGGCTCTC	GTACGACGAGA	CCGACATTTCA	GACAGTTCGG										
1327	CCG	CAT	TGAATCTGTC	AAACGGCTCTC	GTACGACGAGA	CCGACATTTCA	GACAGTTCGG										
	Pro	His															
2649	AAC	TCCAGCA	ATGGCTAGGA	CTCGCCGGCG	CCCTCAGTAC	CTCTGTGTTT	AATTGACGAA										
1383	AAC	TCCAGCA	ATGGCTAGGA	CTCGCCGGCG	CCCTCAGTAC	CTCTGTGTTT	AATTGACGAA										
2709	GAGTGAAC	TGGGAACTGC	GATATTACCT	CAAGCTCCCTT	CTTCGACCGA	CATCTGGGAT											
1443	GAGTGAAC	TGGGAACTGC	GATATTACCT	CAAGCTCCCTT	CTTCGACCGA	CATCTGGGAT											
2769	AGCTTTTCCA	TGCTGTCTCT	CAAGCGAGAA	TCGAGCTGTT	GCCCCATG	TGCTTTCCGG											
1503	AGCTTTTCCA	TGCTGTCTCT	CAAGCGAGAA	TCGAGCTGTT	GCCCCATG	TGCTTTCCGG											
2819	ATAGCCCCCT	TGGTPTCCAG	ACCAAGGTGG	TCGAGCTCGG	CGAGCTGCTT	CTTGTTTCCC											
1563	ATAGCCCCCT	TGGTPTCCAG	ACCAAGGTGG	TCGAGCTCGG	CGAGCTGCTT	CTTGTTTCCC											
2879	TCGCGCTCCT	GCTCTTCCCT	CGCTTTCCGT	CTCTTGAACT	CCAGCCCTTC	GAGCTCTCTG											
1623	TCGCGCTCCT	GCTCTTCCCT	CGCTTTCCGT	CTCTTGAACT	CCAGCCCTTC	GAGCTCTCTG											
2939	TCAATCTCAG	TCTCATCAAT	CTGTGCGCCA	GCTTCACCCA	TGATGTTTCC	GATCTCGTCC											
1683	TCAATCTCAG	TCTCATCAAT	CTGTGCGCCA	GCTTCACCCA	TGATGTTTCC	GATCTCGTCC											
2999	ACTTTGAGCA	TTTCTCTCGG	CAGCTCATAG	ACGACATCTT	GAGATTTTGA	GAGCATGCGC											
1743	ACTTTGAGCA	TTTCTCTCGG	CAGCTCATAG	ACGACATCTT	GAGATTTTGA	GAGCATGCGC											
3059	AGTTGAGCAT	TGAGGACCTG	AAGGGGCCCC	GTCTGTGATT	CCATCACTTT	GAGGTACTCG											
1803	AGTTGAGCAT	TGAGGACCTG	AAGGGGCCCC	GTCTGTGATT	CCATCACTTT	GAGGTACTCG											
3119	ACATGCTCG	TGCGCTGTTC	GATCTTGAGC	TGGGTTTGCT	CAAGCTGTGC	GAGAGTATCA											
1863	ACATGCTCG	TGCGCTGTTC	GATCTTGAGC	TGGGTTTGCT	CAAGCTGTGC	GAGAGTATCA											
3179	AGTCTCTGCT	GTAGATTATG	CTCGCGGAGC	TTCTTCTGAC	GCAATCTCAG	GAGGGCTGAT											
1923	AGTCTCTGCT	GTAGATTATG	CTCGCGGAGC	TTCTTCTGAC	GCAATCTCAG	GAGGGCTGAT											
3239	ACCGGGTTCT	TATCACCAAG	AGGCGCTCTT														
1983	ACCGGGTTCT	TATCACCAAG	AGGCGCTCTT														
2043	TCGAGCGCTC	CATCTGTGCT	CGTCACTGTT	GCTGTGAGAG	ACTTGATCAA	GGAATACGCC											

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 pLW100 is indicated by ↓↓. The genomic sequence from 1 to 2337 was obtained from clone pEA14; the 3' limit of this sequence is identified by double underlining. The genomic sequence from 2134 to 3269 was obtained from cosmid pW17F02 by PCR using primers CD3 and CD8. The limits of this sequence are denoted by dashed underlining. A CAAT-like box and two TATA-like boxes are italicized and underlined. The two introns are identified as gaps in the cDNA sequence.

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 λ ZAP (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) LI5 and LI13 (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 reprobated with the control gene, a fragment of the *A. nidulans* γ -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 *Bam*HI and *Sst*I 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-X₂₋₄-C/H-X₂₋₁₅-C/H-X₂₋₄-C/H (Berg, 1990).

Comparisons 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

UVS-2	MDVFGDEAPN	VPDSTDWLGT	PLACLMPVEQ	AFRCHVCKDF	YDSPMLTSCN
NUVA	1	-----MEPTFD	IPDSTDWLGT	PLTLAAFTET	ELACGVCKDF
RAD18		-----MDHQ	ITATASDFMTT	SIPSLYQSLD	LLRCHICKDF
					LKVVLVTPCG
					HTFCSLCIRR
					CLSVDSKCPL
					CRATDOESKL
					RGNWALRERV
					EAFKNSRKVL
47					HTFCSLCIRR
					CLSTEGKCP
					CLSSDQELK
					ARMVVOELV
					EGTKMAJPSI
					HTFCSLCIRT
					HLNQNCPL
					CLFEPRESLL
					RSEPLVSEII
					QSTSLRSSL
					LEFARTPP-T
					IQAILPDQAG
					PSSPSGRKAT
					EMEGPKEEDP
					ESKRPRRSTR
97					LQALMAQ-T
					OTDDSGDLAA
					HEPASTGKKI
					EPMAIVOTDG
					LFKEGIATAS
					LDALRIPKPT
					PVPNEEVPG
					PENSSWIELI
					SESEDSVNA
					ADDDLQIVAT
					STRARAELT
					AAILQEEQDT
					TPSADPDYVD
					QP-----
					-PDDGLVACP
146					QSGASGRPQ
					ATFPVVIDD
					---GSGEDTH
					-----PDGLVPCP
					SERKLAKRSM
					TDLPLSSK-
					---PSKRNPFAM
					FRSERIKKKS
					KFNEQMAQCP
					ICLTRMKEQQ
					VDR.HLDTSC
					-----PG
					SPQAASKRRP
180					VCGRNDCKEA
					VTR.HLD.SC
					-----TG
					TAEEL-KPAA
					ICQQFYPLKA
					LEKTHLD.EC
					LETLQSLGKKP
					KISTTFPTES
					NPHNK-SSSR
					IPAQTPOPT
					FPSENTRLTS
					QTNQK-----
					---PPER
					LPALAYSIVR
209					FGSLAGPRK
					---ST---LA-
					---ATGK-----
					---PPER
					LVINTSLIK
					FKVRTPEVDK
					---SS---CG-
					---ETSHVDKYL
					NSMMSAEHOR
					LPKINFSTMT
					DTALRKKLSE
					LGLSTHGSRI
					LLEKRHKEWI
					TLWNAN-CDS
					SRPKKRSSELL
241					DTVLRLKELD
					LGLTFMGPFA
					LLOAKATEWL
					MLWNAN-CDS
					ATPFAKRELL
					QSQIKQKLLS
					LGLSTNGTRQ
					NMIKRYNHYE
					MLWNSNFCDS
					LEPVDAAELK
					RDL---DEWE
					RTVGNPQTAA
					GGGGGGQGLG
					LMARAQATGA
					QIK-----
290					REL---DVWE
					RTQGGSEVTP
					TGPTN-----
					---AVM-----
					RQLLSWDVSH
					NKTPQNSNKK
					GGISK-----
					---LMIMKSNKGS
					-----DKEFPDGKAW
					ATRYGGSFGD
					LIQKAR---Q
					GIKRQTLDGN
315					-----NEDQPTKEM
					SAMTDYDFKA
					LIAAGAKKED
					AVIRSTI-PH
					SSYRKLEENF
					KNDKPNRKGW
					MVMFRKDFAR
					LIREAKMKIK
					TG---SSD-SS
					GEKADTKGGG
					GGEDVGPABL
					PTLQAREGES
					SAAPTRMDIV
					PPSSPPRPQ
353					AGQAHSDTPR
					SAQLVDOPIE
					ASL-----
					---TPQDVD
					ESTHMPQDA
					GSVGHSDNGD
					GVEKV-QSDQ
					GTE-----
					---DQMEK
					DQDTVINEDR
					VDDADTEHDG
					QAPGKDAIAE
					DTAMREQVIP
					GTPDKERQWE
					TSQQQQPPIP
403					IDMTFEPFV
					PDPPLALSGI
					DRAVSHPMEN
					VT-----
					---EADAQALPIS
					VAGERNLPIRE
					DSTADLSRE
					LMDLNEYSKD
					PP-----
					GNV 487
					GDAQLSGMKK
					PNPETC 501
453					SSA..STERT
					PH 434

Fig. 3. Comparison of the deduced amino acid sequence of *A. nidulans* NUVA to those of the *N. crassa* UVS-2 and *S. cerevisiae* RAD18. Identical residues are connected by a line, conserved residues are connected by a bold line. Evolutionarily related amino acids (as defined by Gribskov & Burgess, 1986) are indicated with dots. The conserved zinc-binding cysteine and histidine residues, present in the two zinc finger motifs, are highlighted in the UVS-2 sequence. The putative core sequence for the nucleotide binding site of RAD18 is underlined.

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), *uvs-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 RAD5 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 *nuvA11*, none of the mutants of either *uvs-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 *uvsH*, *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.

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