The Neurospora aab-1 gene encodes a CCAAT Binding Protein Homologous to Yeast HAP5

Huaxian Chen,* 1 John W. Crabb† and John A. Kinsey*

*Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66160 and
†W. Alton Jones Cell Science Center, Inc., Lake Placid, New York 12946

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ABSTRACT

The expression of the am (glutamate dehydrogenase) gene is dependent upon two upstream activating sequences, designated URSα and URSβ. A heteromeric nuclear protein Am Alpha Binding protein (AAB) binds specifically to a CCAAT box within the URSα element. AAB appears to be composed of three components. We used polyclonal antiserum raised against the highly purified AAB1 subunit to isolate a partial aab-1 cDNA clone, which was then used to isolate a full-length cDNA and a genomic clone. The full-length cDNA has the potential to encode a 272 amino acid protein with a calculated molecular weight of 30 kD. Amino acid sequence obtained by Edman analysis of the AAB1 protein confirmed that the aab-1 gene had been cloned. AAB-1 shows similarity to the HAP5 protein of yeast and the CBF-C protein of rat. Each of these proteins is an essential subunit of their respective heteromeric CCAAT binding proteins. The aab-1 gene maps on linkage group III of Neurospora crassa near the trp-1 locus. Disruption of the aab-1 gene results in pleiotropic effects on growth and development as well as a 50% reduction in glutamate dehydrogenase levels. Transformation of the aab-1 disruption mutant strain with the cloned genomic copy of the aab-1 gene rescued all of the phenotypic alterations associated with the aab-1 mutation.

The am gene of Neurospora crassa encodes the anaerobic NADP-specific glutamate dehydrogenase (GDH). In previous reports from this laboratory we have shown that the level of expression of the am gene is dependent upon two enhancer-like elements that have been designated URSα and URSβ (Frederick and Kinsey 1990a). Using in vitro mutagenesis and targeted transformation, Frederick and Kinsey (1990a, b) demonstrated that URSα is responsible for about 50% of the normal level of am gene expression. Using gel retardation assays, Chen and Kinsey (1994) demonstrated that URSα serves as a binding site for a nuclear factor designated Am Alpha Binding protein (AAB). Purified AAB was shown to be heteromeric with probably three subunits (Chen and Kinsey 1995). In DNase I protection assays, AAB protected a portion of the URSα element that contains a motif, 5′-ACCAATAA-3′, that is identical to the consensus binding site for the yeast HAP2/3/4/5 heteromeric CCAAT binding protein (Chen and Kinsey 1995). This CCAAT motif was shown to be essential for binding of AAB (Chen and Kinsey 1995).

The CCAAT pentanucleotide motif is found in promoter and enhancer elements of a large number of eukaryotic genes and serves as a binding site for transcription factors. Although these factors share the common feature of binding to sequences that include a CCAAT motif, they can be divided into at least three families: C/EBP, HAP2/3/4/5, and HAP. Each family shares significant similarity at the amino acid sequence level, whereas there is no apparent similarity between members of different families (Dorn et al. 1987; Chodosh et al. 1988a, b; Rupp et al. 1990; Cao et al. 1991; Hooft van Huijswaal et al. 1990).

The HAP2/3/4/5 complex of Saccharomyces cerevisiae (Guarente et al. 1984; Hahn et al. 1988; Forsburg and Guarente 1989; McNabb et al. 1995) represents a family of CCAAT binding proteins that includes the CP1/NF-1 complex from humans as well as heteromeric complexes from several other vertebrate species and the fission yeast S. pombe. The HAP2/3/4/5 heteromeric complex activates transcription of several genes in the respiratory system: CYC1 (major isoform of cytochrome C), HEM1 (8-aminolevulinate synthase), COX4 (nuclear cytochrome oxidase subunit 4), and CYT1 (cytochrome C1) (Keng and Guarente 1987; Ollesen and Guarente 1990; Schneider and Guarente 1991). The HAP2, HAP3, and HAP5 subunits together form the CCAAT binding protein (McNabb et al. 1995). The HAP4 subunit is required for activation in vivo but is not required for DNA binding (Xing et al. 1993). CP1 was identified by its binding to CCAAT containing promoters of many genes, including the adenovirus major...
late promoter, the human hsp70, and the α-globin (Chodosh et al. 1988b). NF-Y was independently identified by its recognition of CCAAT-containing Y box of the major histocompatibility complex class II genes (Dorn et al. 1987). These proteins were subsequently shown to be the same. The rat homolog, CBF, activates transcription of the α1(I) and α2(I) collagen promoters (Maity et al. 1988). All of these factors share conserved blocks of sequence and in some cases genes from mammalian sources can complement yeast hap2 or hap3 mutants (Pinkham et al. 1987; Hahn et al. 1988; Forsberg and Guarante 1989; Vuorio et al. 1990; Maity et al. 1990, 1992; Hooft van Huijsduijnen et al. 1990; Becker et al. 1991). The CBF protein contains homologs of HAP2, HAP3, and HAP5 (Sinha et al. 1995). No homolog of the HAP4 subunit has been found in mammalian systems.

Here we report the cloning and sequencing of the aab-1 gene that encodes the AAB1 subunit of AAB. AAB-1 is a homolog of the recently described HAP5 (McNabb et al. 1995) and CBFC (Sinha et al. 1995) subunits of the heteromeric CCAAT binding proteins from yeast and rats, respectively. Disruption of the aab-1 gene results in a 50% decrease in expression as well as other unrelated effects on growth and differentiation.

MATERIALS AND METHODS

Strains: Escherichia coli strains DH5α, Y1090, and JM109 were used for plasmid, agt11 and λ1, and M13 propagation, respectively (Sambood et al. 1989). The Neurospora crassa strains (FGSC #4411-4430) representing parents and progeny of a cross between Mauriceville and an Oak Ridge strain described by Metzenberg et al. (1984), as well as other Oak Ridge strains, were obtained from the Fungal Genetics Stock Center, University of Kansas Medical Center. Strain TEC41, used as a recipient in targeted transformation, was from the author's collection (Cambareri and Kinsey 1994).

Amino acid sequencing: The purification of AAB protein complex has been described (Chen and Kinsey 1994). About 30 μg of the purified protein preparation was electrophoresed in a 10% SDS-polyacrylamide gel (SDS-PAGE) and transferred to Immobilon-P membrane (Millipore, Bedford, MA) as recommended by the manufacturer. After transfer, the membrane was stained for 10 min with 0.1% Cooamassie Brilliant Blue R-250 in 45% methanol, 10% acetic acid, and then destained in 45% methanol, 7% acetic acid for 10 min. The AAB1 band was excised. Cleavage of polypeptide with cyanogen bromide, trypsin digestion, peptide purification by RP-HPLC, and sequence analysis by Edman degradation were performed as previously described (Crabb et al. 1988; Stone et al. 1992).

Preparation of anti-AAB1 serum: The purified protein was separated by SDS-PAGE. The gel was stained for 30 min with 0.05% Cooamassie Blue in distilled H2O and destained with distilled H2O for 1 hr. The AAB1 polypeptide band was excised and about 30 μg was recovered by electroelution as described (Hager and Burgess 1980). A rabbit was immunized using multiple intradermal injections of the eluted protein in Freund's adjuvant and boosted twice at 2-3 mo intervals (Harlow and Lane 1988). The serum was collected 1 wk after the third injection. Prior to use for immunoscreening, the serum was preabsorbed with E. coli extract and with fractions of Neurospora proteins lacking AAB-1 activity obtained during the purification of AAB.

Western blot and immunoscreening of a λgt11 library: The purified protein was resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose. The membrane was blocked in TBST solution (10 mm Tris-HCl pH 8.0, 150 mm NaCl, 0.05% NP-40) containing 5% non-fat milk for 1 hr at room temperature. The primary antibody (rabbit antiserum) was added to the above blocking solution at a final dilution of 1:1000 and incubated for 1 hr. After washes with TBST, the membrane was incubated with a 1:3000 dilution of the second antibody, goat anti-rabbit IgG-alkaline phosphatase (Bio-Rad, Richmond, CA) for 1 hr. The membrane was washed and developed in color substrates provided by the manufacturer (Bio-Rad, Richmond, CA).

An N. crassa λgt11 cDNA library (Sachs et al. 1986) was immunoscreened by the method of Sambood et al. (1989). The plates were incubated at 42°C for 4 hr and overlaid with nitrocellulose filters, which had been soaked with 10 mm IPTG and dried. The plates were incubated for an additional 4 hr at 37°C. The filters were then blocked and screened as described for Western blots except that the incubation times with the primary and secondary antibodies was 3 and 2 hr, respectively. Phage from positive plaques were rescreened three times at lower plaque densities until a homogeneous population of immunopositive phages was obtained. Bacteriophage λDNA from positive plaques was purified using a Lambda Magic Prep Kit (Promega, Madison, WI).

DNA probes: Probes for the gel mobility shift assays were prepared by end-labeling (Sambood et al. 1989) of gel-purified DNA fragments of the isolated wild-type 90 bp HindIII-Sacl regions of the URSαm element (Chen and Kinsey 1995). Probes for hybridization assays were made by random primer labeling of appropriate fragments (Feinberg and Vogelstein 1984).

Screening libraries by hybridization: Plaques from an N. crassa λ1 genomic library constructed by Orbach (Orbach et al. 1986), or the N. crassa λgt11 cDNA were transferred to charged nylon membranes (NEN) and screened by hybridization to 32P-labelled probe under conditions recommended by the manufacturer (Dupont, Wilmington, DE).

Southern hybridization: N. crassa genomic DNA was isolated by the method of Metzenberg and Baisch (1981). DNA samples were digested with restriction enzymes, resolved on 1% agarose gel, and transferred onto Nytran membranes (Schleicher and Schuell, Keene, NH). DNA was fixed to the membrane by UV crosslinking. Hybridization and washes were performed as described (Kinsey and Helber 1989).

Northern hybridization: RNA was isolated as described (Frederick and Kinsey 1990a). Two micrograms of poly(A) + RNA from the N. crassa wild-type strain, 74-OR23-1VA (FGSC #2489) was fractionated on 1% agarose-formaldehyde gel and transferred to a membrane. Hybridization and washes were performed as described (Frederick and Kinsey 1990a). Transcript size was calculated from the migration in parallel with synthetic RNA markers (BRL) visualized by staining with ethidium bromide.

DNA sequencing: DNAs were cloned into M13mp18 and M13mp19 vectors and sequenced by the dideoxynucleotide sequencing method using Sequenase version 2 (United States Biochemical, Cleveland, OH).

Gel retardation assay: Gel retardation assays were performed as previously described (Chen and Kinsey 1994, 1995). Proteins eluted from the calf thymus DNA column were used as the AAB factor.

Transformation: Neurospora transformation was carried out as previously described (Cambareri and Kinsey 1994).
RESULTS

**AAB1 protein purification and sequence analysis:**
The AAB protein complex was purified from *N. crassa* crude cellular extracts by a combination of ion exchange and DNA affinity chromatography as described (Chen and Kinsey 1995). Purified AAB was factioned by SDS-PAGE which resolves AAB into two bands of apparent molecular mass of 40 and 30 kD (Figure 1). The separated proteins were electroblotted onto a PVDF membrane and, after staining, the AAB1 band (40 kD) was excised and incubated with cyanogen bromide. The resulting cyanogen bromide peptides were extracted from the membrane, digested with trypsin and peptide fragments purified by RPHPLC. Automated Edman analysis of isolated peptides yielded a total of 45 amino acids of sequence from three purified peptides (underlined in Figure 2).

**Cloning of AAB1 cDNAs and genomic sequences:**
A high titer rabbit polyclonal antibody against the AAB-1 was produced as described in the materials and methods. When this antiserum was used to probe Western blots of purified AAB, a band was seen only at the 40-kD position, suggesting that the 30-kD subunits were not antigenically related to AAB-1 (Figure 1A). We used this antiserum to screen a *N. crassa* cDNA expression library made in λgt11 (Sachs et al. 1986). Six strongly reactive clones were isolated among 4.5 × 10⁵ plaques. The cDNA inserts in all six clones appeared to be identical 0.8-kb fragments. One such fragment was ligated into pBS², yielding pAAB1. We then used a fragment from genomic DNA to reprobe the cDNA library. A total of 10⁶ plaques of the λgt11 library were screened with a 650-bp genomic DNA fragment. Four positive clones were identified. One of the clones contained a 1.5-kb EcoRI insert which was ligated into pBS² to give the plasmid pcAAB1-1.5. This cDNA insert contains a 3’ poly(A)⁺ tract. It is approximately the same length as the aab-1 messenger RNA (Figure 1B) and thus represents an approximately full-length cDNA.

**Detection of RNA transcripts by Northern blot analysis:** A Northern blot analysis of wild-type *N. crassa* poly(A)⁺ RNA was performed to determine the size of transcripts from the aab-1 gene, using the 0.8-kb cDNA insert as a hybridization probe. One major mRNA species of ∼1.5 kb was observed (Figure 1B). There were also two minor species of 1.3 and 3.2 kb in length. The relationship of these minor RNA species to the 1.5-kb transcript has not been determined.

**DNA sequence determination:** The complete sequence of the aab-1 gene is shown in Figure 2. Both cDNA and genomic DNA sequences were determined. There is a single long ORF with the potential to encode a 271 amino acid polypeptide. As indicated above, this ORF has the capacity to encode all of the peptide sequences obtained by peptide microsequencing. The peptide sequences are underlined in Figure 2. There are three short introns within the aab-1 coding sequences. The first interrupts codon 30, the second is between codons 34 and 35, and the last is between codons 269 and 270. Intron sequences are shown in lower case letters. The predicted molecular weight of a protein encoded by this ORF is 30 kD. The sequence surrounding the first AUG is a reasonable fit to the Neurospora consensus sequence (Bruchez et al. 1993) and codon usage is generally consistent with that found in other Neurospora proteins (Gurr et al. 1987). As with many Neurospora genes, there is no clear polyadenylation signal. The 5’ end of the longest cDNA obtained is at position 248 in Figure 2. Bruchez et al. (1993) identified a 7-bp consensus sequence (TCAT CANC) around the transcriptional start site of a number of Neurospora genes. There is a sequence identical to this at six of seven positions (TCATCATT) located between positions 214 and 221 of Figure 2. This might represent the region of the transcriptional start site(s). In common with many other Neurospora genes there is no obvious TATA box; however, interestingly there are three CCAAT boxes upstream of the likely start region.

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**Figure 1.—AAB protein and aab-1 mRNA.** (A) Western blot and silver staining analysis of AAB factor. Purified AAB factor is examined by SDS-PAGE and immunoblotting with rabbit antiserum against AAB1 (left) and silver staining (right). (B) Analysis of mRNA of the aab-1 gene. Poly(A)⁺ RNA from the wild-type strain, OR-23-1VA, was separated by electrophoresis in a formaldehyde/agarose gel and subjected to Northern blotting and hybridization with 0.8 kb cDNA insert from pcAAB1-0.8.
Comparison of the deduced amino acid sequence with the databases revealed that AAB-1 is homologous to the recently described HAP5 subunit of the yeast HAP2/3/4/5 complex and to the CBF-C subunit of the rat CCAAT binding protein CBF. Both subunits have been shown to be essential for DNA binding of their respective heteromeric complexes (McNabb et al. 1995; Sinha et al. 1995). All three polypeptides share a block of sequence ~87 residues in length that is highly similar (Figure 3). The sequence of AAB-1 between residues 81 and 167 is 75% (65/87) identical to the sequence of HAP5 between residues 127 and 212, which is 72% (63/87) identical to the sequence of CBF-C between residues 36 and 122.

**Mapping of the aab1 gene:** To locate the aab1 gene on the genetic map, we searched for restriction fragment length polymorphisms (RFLP) in the genomic region of the aab1 locus in two N. crassa strains: Mauriceville (FGSC #4416) and a multiply-marked strain in an Oak Ridge background (FGSC #4411). Genomic

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**Figure 2.—** Nucleotide sequence and open reading frame of the aab1 gene. The nucleotides of the coding strand are shown. The numbers to the right of each line refer to the nucleotide sequence. The three double underlined sequences indicate CCAAT boxes. Introns are shown in lower case letters. The three underlined amino acid sequences correspond to those obtained by microsequencing of peptides from AAB1. The asterisk indicates the location of the polyA tract in the cDNA. The 5' end of the longest cDNA sequenced was at position 248. The sequence has been deposited in Genbank. The accession number is AF026550.
Disruption of the aab-1 gene: To confirm that AAB-1 plays a role in the expression of the aab-1 gene, the aab-1 gene was disrupted by the Repeat Induced Point mutation (RIP) process (Selker et al. 1987). To accomplish this, a copy of the 1.5-kb cDNA was placed in the targeting vector pAL1 (Cambareri and Kinsey 1994) and targeted to the aab-1 locus in the recipient strain, TEC41. Using this system, the aab-1 cDNA was introduced as a single copy at the aab-1 locus with a selectable marker (hygromycin resistance). Transformants were screened by Southern blots to confirm the correct integration and linkage to the aab-1 locus. Consequently, genomic DNA from the progeny of a cross between Mauriceville and an Oak Ridge derived strain that have been extensively used for RFLP mapping in N. crassa (Metzenberg et al. 1984), was digested with XbaI and probed with the 0.8 kb cDNA. Segregation of the XbaI RFLP in the progeny gave a pattern identical to that of con-7 or trp-1. This indicates that the aab1 gene is located on the right arm of linkage group III.

DNA was prepared from the two strains and digested with more than a dozen restriction enzymes. Southern blots of the digested DNAs were hybridized with 32P-labeled 0.8 kb cDNA from pAAB1-0.8. The data from all Southern blots indicated that there is a single genomic sequence homologous to the cDNA probe. RFLPs were detected with only two restriction enzymes: SalI and XbaI. Consequently, genomic DNA from the progeny of a cross between Mauriceville and an Oak Ridge background strain. When strains with two copies of a gene go through the sexual cycle both copies of the gene can be mutated by the RIP process (Selker et al. 1987). Thus, we expected copies of the aab-1 gene to be mutated in some fraction of progeny resulting from the cross. We were particularly interested in mutated copies of the native gene (the copy integrated at the aab-1 locus is a cDNA copy lacking a promoter and is thus inherently nonfunctional). The native aab-1 locus is linked to the trp-1 gene; therefore, the native copy of the aab-1 gene that might have been subjected to RIP should be found among tryptophan requiring progeny from this cross. DNA from 19 morphologically normal progeny digested with several restriction enzymes showed no evidence of RIP of the native aab-1 locus. However, among the tryptophan requiring progeny there were three slow-growing morphologically altered strains. Two of these showed clear indication of RIP mutation at the native locus.

One of the RIP mutants, RM 94-62-93, had the am::aab-1 allele that has the aab-1 cDNA sequence replacing the am gene and thus could not be assayed for GDH; however, the other mutant, RM 94-62-100, had a wild-type am allele at the am locus and thus could be assayed for GDH. RM 94-62-100 showed in repeated assays a reduction in GDH activity to a level that was ~50% of that of wild-type activity. This is equivalent to values obtained when the URSamα element was deleted. This would be expected if aab-1 encodes a protein that is an essential component of the complex that binds and activates URSamα in vivo. The morphological mutant strain RM 94-62-100 was crossed to the wild-type ORSa strain, and eight tetrads were isolated. The morphological phenotype segregated 2:2 and showed linkage to trp-1. A typical tetrad is shown in Figure 4. GDH assays on the eight spore cultures from one tetrad showed that the specific activity of GDH in all of the morphologically altered strains was ~50% of that of wild-type strains. Analysis of random spores from crosses involving RM 94-62-100 and its descendants routinely gave map values of about 1 cM for the interval between aab-1 (scored as a morphological mutant) and trp-1. Furthermore, the morphological phenotype has segregated with RFLPs at aab-1 and reduced expression of the am gene through four generations of backcrosses.

To determine if the aab-1 mutations affected CCAAT binding activity, extracts were made from RM 94-62-93 and RM 94-62-100 and used in gel mobility shift experiments (Figure 5). The major CCAAT binding activity seen in normal strains (lane B) was absent in both mu-
tant strains; however, a faint band with altered mobility is visible with extracts from RM 94-62-93 (lane C), but not in extracts of RM 94-62-100 (lane D). From these results it would appear that RM 94-62-93 has some residual binding activity; whereas, RM 94-62-100 appears to be a complete null for binding to the CCAAT motif found in the URSαα element. There is no obvious difference in the phenotype of the two mutant strains.

Not unexpectedly, the aab-1 mutation has pleiotropic effects on Neurospora growth and development. As indicated above, all isolated aab-1 mutants have highly altered morphology. When grown on solid media only very short aerial hyphae are produced, giving the culture a patchy appearance (Figure 4). Supplementation with glutamate does not relieve this morphological phenotype. Conidiation of aab-1 strains is greatly reduced with only sporadic single chains appearing very late in development. In addition to the reduced aerial hyphae, growth is much slower than wild-type whether the strains are grown in liquid or on solid medium. On race tubes (Ryan et al. 1943), aab-1 strains showed erratic growth with extension rates of 0.02–0.15 mm/hr at 25°. On some race tubes, aab-1 strains showed a start-stop growth pattern in which linear extension might halt for up to 48 hr before resuming at a slow extension rate. This should be compared to the steady extension rates of ~3.4 mm/hr for the wild-type strain and 2.5 mm/hr for a strain from which the URSαα element had been deleted, grown under the same conditions. Because of poor conidiation, it was difficult to compare the growth of aab-1 with wild-type in liquid cultures; however, using sheared hyphal fragments as an inoculum, aab-1 strains appeared to have a doubling time in excess of seven hours as compared to a 2-hr doubling time for wild-type strains grown under the same conditions. In addition to effects on growth and conidiation, aab-1 strains are female sterile; however, they are fertile as males. This complex of characteristics has segregated with aab-1 through four rounds of backcrosses.

**Rescue of the aab-1 phenotype:** To demonstrate conclusively that the pleiotropic effects were because of aab-1 disruption, an aab-1 strain was rescued by transformation with a plasmid containing the cloned aab-1 genomic sequences. Since aab-1 strains grow so poorly, the heterokaryon-assisted transformation system as described by Yamashiro et al. (1996) was used. An mtr;aab-1 double mutant strain was constructed and placed in a forced heterokaryon with a lys-1 strain. This heterokaryon conidiated and grew normally, allowing normal spheroplasting and transformation. Spheroplasts from the heterokaryon were cotransformed with an aab-1 plasmid and a plasmid, pCNS43, (Staben et al. 1988) conferring hygromycin resistance. Transformants were selected on hygromycin and p-fluorophenylalanine (the latter compound selects for homokaryotic strains that have the mtr mutant gene) and approximately half of the selected transformants had normal morphology and conidiation. After repeated single condial isolation to insure homokaryosis, the conidiating transformants were screened by Southern blot analysis. Each of five tested transformants had the resident disrupted copy of aab-1 and at least one ectopic wild-type copy. GDH assays on the same transformants indicated that all had regained wild-type levels of GDH.

![Figure 4](image)

**Figure 4.**—Segregation of the aab-1 phenotype among the products of a single ascus from a cross of aab-1 mutant strain 94-62-100 to the standard wild-type strain, ORS a (FGSC 2490). The cultures shown had grown for seven days at 25°.

![Figure 5](image)

**Figure 5.**—Gel mobility shift analysis with the URSαα DNA fragment and extracts from wild-type and aab-1 mutant strains. All lanes contain the same DNA probe. Lane A contains no protein extract. Lane B contains wild-type extract. Lane C contains extract from the 94-62-93 mutant strain. Lane D contains extract from the 94-62-100 mutant strain.
DISCUSSION

The most difficult step of isolating clones of the aab-1 gene from N. crassa has been obtaining sufficient pure AAB1 protein from which to produce antisera and to obtain protein sequence information. Previously, we purified the AAB transcription factor complex to near homogeneity and demonstrated that it consists of at least two components (Chen and Kinsey 1994). Here we have further purified the AAB1 component to apparent homogeneity by electrophoresis. A high titer polyclonal antisera was prepared against the purified AAB1 subunit and used to isolate a partial cDNA clone from an N. crassa cDNA expression library. Sequence analysis of the cDNA clone confirmed an open reading frame containing the deduced amino acid sequence of three peptides determined by direct Edman sequence analysis of purified AAB1. The cDNA clone was used to isolate the corresponding genomic clone, a fragment of which was used to isolate a cDNA that contained the entire coding region (Figure 2). The deduced AAB1 protein has a calculated molecular mass of 29.6 kD but migrates in SDS-PAGE as a 40-kD protein (Figure 1). Such discrepancies are not uncommon and notably have been observed for transcription factors GCN4 from yeast (Hope and Struhl 1985) and NF-Y from mouse (Hooft van Huysduijnen et al. 1990). Either AAB1 migrates anomalously in SDS-PAGE, or it is post-translationally modified.

Comparison of the sequence of the deduced protein with sequences present in protein data bases indicated that AAB-1 is homologous to HAP5 and CBF-C. The region of clear homology between these proteins covers a sequence of 87 residues (Figure 3). Interestingly, this region includes all parts of CBF-C that are known to be required for interaction with the other CBF subunits as well as sequences required for DNA binding (Kim et al. 1996). Kim et al. (1996) have also suggested that this region has homology to the histone-fold motif of histone H2A, suggesting that the protein/DNA interaction may resemble that of histones. The sequence homology between AAB-1, HAP5, and CBF-C make it clear that AAB is a Neurospora CCAAT binding protein. Therefore AAB-1 is almost certainly one of three essential subunits. This suggests that the other two subunits are represented by the doublet of bands of an apparent molecular mass of ~30 kD observed by Chen and Kinsey (1995) on denaturing PAGE gels of purified AAB protein.

When RIP was used to mutate the aab-1 gene no alteration of aab-1 sequences was seen in morphologically normal progeny. However, 3–10% of the progeny produced in RIP crosses were morphologically altered. When these morphologically altered progeny were examined by Southern blot analysis, alterations in restriction sites within the aab-1 gene were clearly present in many cases. GDH assays indicated that the strains with alterations at the aab-1 locus produced only about 50% of the GDH produced by normal strains. This is equivalent to the reduction in am expression seen in strains that have deletions of the CCAAT site upstream of the am locus (Frederick and Kinsey 1990a). Since the aab-1 strains also have altered morphology and slow growth, it is reasonable to ask whether the reduction in GDH level is responsible for this phenotype. Clearly this is not the case since mutants lacking either the URSamα or the URSamβ, each of which reduce am expression by ~50%, have no effect on morphology and result in only a minor reduction in growth rate. The fact that all of the aberrant phenotypes associated with the aab-1 disrupted mutant strains can be rescued by the cloned aab-1 gene indicates that the aberrant phenotypes must be because of reduced expression of other genes whose expression is driven by the AAB transcription factor.

Given that expression of a number of genes must be affected by the aab-1 mutation, it could be argued that the effect on the expression of an am gene might be indirect. Although this is a formal possibility it seems unlikely. Mutation of the CCAAT box in URSamα to CCGGT results in a reduction in am expression that is equivalent to deletion of the entire 90-bp element (J. A. Kinsey, unpublished results). This argues that the crucial sequence in URSamα is the CCAAT box. Further, the CCAAT box found in URSamα is a perfect fit to the consensus sequence recognized by both the yeast and mammalian homologs of AAB (Chodosh et al. 1988a,b) but is different from the consensus sequences bound by other mammalian CCAAT binding proteins (Chodosh et al. 1988a). Also, during the experiments that resulted in the purification of AAB-1, we made an extensive search for other proteins that bound to URSamα. None were found. Yeast appears to have only a single CCAAT binding protein, whether this will prove true for other fungi such as Neurospora is unknown.

Although little is known regarding the regulation of the genes that encode CCAAT binding proteins, it is interesting to note that there are three CCAAT boxes in the 5′ noncoding sequences of the aab-1 gene (double underlined sequences in Figure 2). The first box is identical to the HAP2/HAP3/HAP4/HAP5 consensus binding site. The other two boxes are identical in six of the seven residues in this consensus sequence. This raises the possibility that the expression of aab-1 may be autoregulated.

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LITERATURE CITED


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