The Saccharomyces cerevisiae mRNA cap methylating enzyme is a 436-amino acid protein encoded by the essential ABD1 gene. To identify structural features of ABD1 required for enzyme function, we introduced alanine mutations at 19 positions within a 205-amino acid region of similarity to the methyltransferase domain of the vaccinia virus capping enzyme. Three new recessive lethal mutations, E170A, D194A, and R206A, were identified. Structure-function relationships were clarified by introducing conservative substitutions at Glu-170, Asp-194, and Arg-206, and at Tyr-254 (an essential residue identified previously). Alleles E170D and D194E were viable, whereas E170Q and D194N were lethal; hence, acidic side chains were critical at both positions. R206K was viable, suggesting that a basic residue sufficed. Y254S was lethal, whereas Y254F was viable, albeit slow growing; thus, an aromatic side chain was important. The ABD1 mutations that were deleterious in vivo elicited catalytic defects in vitro. By studying the effects of amino- and carboxy-terminal deletions, we defined a fully active catalytic domain of ABD1 from residues 130 to 426. Residues 110–129 were dispensable for methyltransferase activity in vitro, but essential for function in vivo. This analysis allowed us to delineate a subfamily of ABD1-like proteins within the superfamily of AdoMet-dependent methyltransferases. In addition, we identify a candidate Caenorhabditis elegans gene encoding a putative cap methyltransferase. All residues essential for ABD1 activity are conserved in the C. elegans homologue.

Eukaryotic mRNAs contain a 5'-terminal cap structure, m7GpppN, which is synthesized by the following series of three enzymatic reactions (1):

$$\text{pppN(pN)}_n \rightarrow \text{ppN(pN)}_n + P$$  \hspace{1cm} (Eq. 1)

$$\text{ppN(pN)}_n + \text{ppG} \rightarrow \text{G}^{\delta}\text{pppN(pN)}_n + \text{PP}_i$$  \hspace{1cm} (Eq. 2)

$$\text{G}^{\delta}\text{pppN(pN)}_n + \text{AdoMet} \rightarrow \text{m}^{\delta}\text{G}^{\delta}\text{pppN(pN)}_n + \text{AdoHcy}$$  \hspace{1cm} (Eq. 3)

The enzyme RNA (guanine-N7-) methyltransferase (referred to hereafter as cap methyltransferase) catalyzes the third step in this pathway: the transfer of a methyl group from AdoMet to the GpppN terminus of RNA to produce the m7GpppN-termi- nated RNA and AdoHcy. The Saccharomyces cerevisiae cap methyltransferase is the product of the ABD1 gene (2). ABD1 encodes a 436-amino acid polypeptide that is homologous in part to the methyltransferase catalytic domain of the vaccinia virus capping enzyme. We have shown that ABD1 is an essential gene and that the cap methyltransferase activity of ABD1 is critical for yeast cell growth (2, 3). The latter assertion was based upon the concordance of in vitro and in vivo mutational effects discerned from a small collection of ABD1 mutants, to wit: (i) deletion and missense mutations in ABD1 that preserved methyltransferase activity in vitro also supported cell growth, (ii) mutations that severely affected methyltransferase activity in vitro were either lethal or severely defective for cell growth, and (iii) no mutations were identified that abolished methyltransferase activity yet permitted cell growth. These results provided the first genetic evidence that the cap methyl group is essential for cellular function.

ABD1 is presently the sole example of a cap methylating enzyme encoded by a cellular gene. Detailed study of this protein is clearly warranted, given that we know little about the mechanism of cap methylation and its potential regulation. In the present study, we use targeted mutagenesis to identify structural elements of the 50-kDa ABD1 protein that are essential for enzyme function in vivo and in vitro. We establish structure-function relationships for yeast cap methyltransferase that may extend to homologous members of a distinct class of proteins within the AdoMet-dependent methyltransferase superfamily. In addition, we identify, on phylogenetic grounds, a putative cap methyltransferase from Caenorhabditis elegans.

**MATERIALS AND METHODS**

**Site-directed Mutagenesis—Missense mutations in the ABD1 gene were programmed by synthetic oligonucleotides using the two-stage PCR-based overlap extension strategy as described (3).** NH2-terminal deletion mutations were generated by one-stage PCR using sense-strand oligonucleotide primers that introduced an NdeI restriction site (CATATG) with an in-frame methionine codon. A carboxy-terminal deletion mutation was made via PCR using an antisense oligonucleotide that introduced a stop-codon at amino acid 427 and a downstream BamHI restriction site. An NdeI-BamHI restriction fragment of each PCR-amplified ABD1 gene was inserted into pET16b. The presence of the desired mutation was confirmed in every case by sequencing the entire ABD1 insert; the occurrence of PCR-generated mutations outside the targeted region was thereby excluded. Mutated ABD1 genes were transferred from the pET vectors to the yeast plasmid p358-5’ (CEN TRP1). Expression of ABD1 in this context is driven by its natural promoter.

**Test of ABD1 Function by Plasmid Shuffle—**Strain YBS6 (MATa her3 trp1 lys2 his2 leu2 abi1::hisG), which is deleted at the chromosomal ABD1 locus, is viable when it maintains an extrachromosomal copy of ABD1 on a CEN URA3 plasmid (p360-ABD) (2). p358-ABD plasmids bearing missense mutations of ABD1 were introduced into YBS6. Trp+ transformants were selected at 30 °C on medium lacking tryptophan. Individual colonies were patched on medium lacking tryptophan. Cells from each patch were then streaked at 30 °C on...

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*The abbreviations used are: PCR, polymerase chain reaction; 5-FOA, 5-fluoroorotic acid; PAGE, polyacrylamide gel electrophoresis; ITPG, isopropyl-1-thio-β-D-galactopyranoside.*
medium containing 0.75 mg/ml 5-FOA.

**Purification of Recombinant ABD1—**PET-His-ABD1 plasmid derivatives were transformed into *Escherichia coli* BL21(DE3). Expression of the plasmid-encoded protein was induced by addition of 0.4 mM IPTG to an exponentially growing 200-ml bacterial culture. Cells were harvested by centrifugation at 4 °C postinduction. Soluble cell lysates were prepared as described (2). The His-ABD1 proteins were purified by Ni-agarose and phosphocellulose column chromatography steps as described (2, 3). The polypeptide composition of the column fractions was monitored by SDS-PAGE. Protein concentrations were determined using the Bio-Rad dye reagent with bovine serum albumin as a standard.

**Methyltransferase Assay—**RNA (guanine-7)-methyltransferase was assayed by conversion of [32P]-cap-labeled poly(A) to methylated capped poly(A) in the presence of unlabeled AdoMet (2). Standard assay mixtures (10 μl) contained 50 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 40 mM NaCl, 50 μM AdoMet, [32P]-cap-labeled acceptor RNA, and enzyme as specified. After incubation at 37 °C for 5 min, the reaction mixtures were heated at 95 °C for 3 min, then adjusted to 50 mM sodium acetate (pH 5.5). Samples were incubated with 5 μg of nuclease P1 for 30–60 min at 37 °C. The digests were then spotted on polyethyleneimine cellulose TLC plates that were developed with 0.2 M (NH4)2SO4. The extent of methylation of the cap (as [m7GpppA]/[m7GpppA + GpppA]) was determined by scanning the TLC plate using a FUJIX BAS1000 PhosphorImager.

**ABD1 Antiserum—**Polyclonal rabbit antiserum was prepared against full-length His-ABD1 protein that had been expressed in bacteria and purified to homogeneity under native conditions (2). Immunization was performed at Pocono Hill Rabbit Farm, Canadensis, PA. The reactivity and specificity of the immune serum was verified by Western blotting against recombinant ABD1.

**Western Blot Analysis—**Yeast cells were grown in liquid culture at 30 °C until A600 reached 0.6–0.8. Cells were harvested by centrifugation of 6-ml aliquots of the culture; the pellets were washed once with distilled water, and the cells were resuspended in 0.1 ml of SDS-sample buffer containing β-mercaptoethanol and an equivalent volume of acid-washed glass beads. The samples were vortexed vigorously for 2 min, then heated for 5 min at 100 °C. After removing the glass beads by centrifugation, the soluble lysates were electrophoresed through a 10% polyacrylamide gel containing 0.1% SDS. Proteins were transferred electrophoretically to a nitrocellulose membrane, which was then blocked with TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20) containing 5% bovine serum albumin and 1% dried milk. The membranes were incubated for 1 h at 37 °C with anti-ABD1 polyclonal antiserum diluted 1:2000 in TBST and then washed with TBS containing 0.4% Tween-20. The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin, and the reactive polypeptides were visualized using an enhanced chemiluminescence system (ECL; Amersham Corp.).

**RESULTS**

**Deletion Mutants—**It was shown previously that the deletion of 52 or 109 amino acids from the NH2 terminus of ABD1 did not affect the ability of the ABD1(53–436) or ABD1(110–436) genes to support cell growth. However, a more extensive deletion of 155 amino acids from the NH2 terminus was lethal, as was a deletion of 55 amino acids from the COOH terminus (3). To define the minimal functional ABD1 protein, three new NH2-terminal deletions, ABD1(121–436), ABD1(130–436), and ABD1(143–436), were tested for function in vivo using the plasmid shuffle procedure. Wild-type and truncated ABD1 alleles on CEN plasmids marked with Trp1 were introduced into YBS6 cells. YBS6 is deleted at the chromosomal ABD1 locus and is therefore dependent for growth on maintenance of a copy of ABD1 on a CEN plasmid marked with URA3. The Trp+ transformants were then plated on medium containing 5-FOA to select against retention of the wild type ABD1 gene on a URA3 plasmid. Cells bearing ABD1 and ABD1(110–436) plasmids grew similarly, whereas the three more extensively truncated NH2-terminal mutant alleles could not support colony formation on 5-FOA (not shown). However, a 10-amino acid COOH-terminal truncation, ABD1(1–426), was viable (not shown).

The effects of the amino- and carboxyl-terminal truncations on cap methyltransferase activity were examined after expressing the wild type and truncated ABD1 proteins in bacteria. The ABD1 genes were inserted into an inducible T7 RNA polymerase-based PET vector such that a histidine-rich NH2-termini leader (His-tag) was fused to each ABD1 polypeptide. The expression plasmids were introduced into *E. coli* BL21(DE3), a strain that contains the T7 RNA polymerase gene under the control of a lacUV5 promoter. Polypeptides corresponding to His-tagged ABD1, ABD1(121–436), ABD1(130–436), ABD1(143–436), and ABD1(1–426) were recovered in soluble extracts of IPTG-induced bacteria and enriched by adsorption to Ni-agarose and elution with 20 mM imidazole. Each protein was purified further by adsorption to phosphocellulose and step-elution with 0.5 M NaCl. SDS-PAGE analysis of the phosphocellulose preparations indicated that the ABD1, ABD1(121–436), ABD1(130–436), ABD1(143–436), and ABD1(1–426) proteins were highly enriched (Fig. 1). The truncated proteins migrated more rapidly during SDS-PAGE than the wild type ABD1, as expected.

**RNA (guanine-7)-methyltransferase activity was assayed by the conversion of [32P]-cap-labeled poly(A) to methylated capped poly(A) in the presence of unlabeled AdoMet (2). The reaction products were digested to cap dinucleotides with nuclease P1 and then analyzed by polyethyleneimine-cellulose thin layer chromatography, which resolved GpppA from m7GpppA. The extent of methylation was proportional to the amount of ABD1 added (Fig. 1). In the mid-range of the titration curve, ABD1 formed about 2–3 fmol of methylated capped ends per fmol of protein. Note that the assay measured yield of product rather than reaction rate (2). At saturating enzyme, the capped RNA substrate was methylated quantitatively. The specific activities of the ABD1(121–436), ABD1(130–436), and ABD1(1–426) preparations were comparable to that of the full-length ABD1, whereas the activity of ABD1(143–436) was reduced to ~5% that of wild type ABD1 (Fig. 1).

Any number of factors might account for the finding that ABD1(121–436) and ABD1(130–436), which were catalytically active in vitro, were incapable of sustaining cell growth. In particular, we wished to evaluate whether the mutant proteins were actually produced in vivo. To do this, we prepared extracts of yeast cells bearing wild type and mutant ABD1 alleles, alone or in combination. Polypeptides were resolved by SDS-PAGE, transferred to membranes, and probed with antiserum raised against recombinant His-ABD1 purified from bacteria under
native conditions. The antisera recognize the recombinant 52-kDa His-ABD1 polypeptide in the Western blot assay (Fig. 2, lane 1). An immunoreactive 50-kDa polypeptide was detected in whole-cell extracts of yeast cells bearing the wild type ABD1 gene on a CEN TRP1 plasmid and on a CEN URA3 plasmid (Fig. 2, lane 2). Note that 50 kDa is the predicted size of the full-length ABD1 polypeptide; the His-tagged version is 2 kDa larger than the native protein. (Several minor bands of lower molecular weight were also detected by Western blotting. These may be proteolytic derivatives of ABD1; alternatively they may be yeast polypeptides distinct from ABD1 that cross-react with the antibody probes used for immunoblotting.)

The 50-kDa full-length ABD1 protein was absent in cells bearing ABD1(110–436). Rather, extracts of ABD1(110–436) cells contained an immunoreactive polypeptide of 37 kDa, the size expected for the truncated ABD1(110–436) protein (Fig. 2, lane 3). A protein of 49 kDa was detected in extracts of cells carrying the COOH-terminal deletion mutant ABD1(1–426) (lane 8). The ABD1(1–426) protein migrated faster during SDS-PAGE than did the wild type ABD1 protein analyzed in parallel (compare lanes 8 and 9). The full-length and truncated ABD1 polypeptides were present in seemingly equal amounts in extracts of yeast cells carrying ABD1 on a CEN URA3 plasmid and ABD1(1–426) on a CEN TRP1 plasmid (note the doublet in lane 7).

A novel immunoreactive species of 34 kDa was detected along with full-length ABD1 protein in cells carrying wild type ABD1 and the NH2-terminal deletion mutant ABD1(130–436) (Fig. 2, lane 5). Hence, the inability of the catalytically active ABD1(130–436) enzyme to support cell growth was not attributable to lack of protein production. A different scenario applied to ABD1(121–436), for which we did not detect any immunoreactive mutant protein in whole cell extracts (lane 4). The more extensively truncated derivative, ABD1(143–436), was produced in vivo, as evinced by the presence of a 32-kDa immunoreactive species in whole cell extracts (lane 6). We infer that cell viability is contingent on a threshold level of methyltransferase specific activity and that debilitated activity of ABD1(143–436) does not meet that threshold.

Alanine-substitution Mutations—Individual amino acid residues essential for ABD1 function were identified by alanine-scanning mutagenesis. We targeted the mutations to a 205-amino acid segment of the ABD1 protein (residues 168–372) which displays sequence similarity to the cap methyltransferases encoded by the poxviruses (vaccinia virus, Shope fibroma virus, molluscum contagiosum virus) and by African swine fever virus (Fig. 3). These mutations were introduced at 19 new positions within the conserved region. The residues mutated are denoted by asterisks and filled arrowheads in Fig. 3.

The ABD1-Ala mutants were tested for function in vivo by plasmid shuffle. Sixteen of the mutants formed wild-type size colonies under 5-FOA selection at 30 °C for 3 days (Table I). Three mutants, E170A, D194A, and R206A, did not grow under 5-FOA selection at 30 °C (Table I). Even after prolonged incubation (up to 10 days) E170A, D194A, and R206A showed no evidence of growth. We discerned no dominant negative effect on cell growth when the E170A, D194A, or R206A mutant alleles were introduced into a wild type ABD1 strain.

Structure-Function Relationships at Essential Residues—Alanine substitution eliminates the side chain beyond the β-carboxyl group of essential serines and threonines. It was of interest to establish whether alanine substitutions would affect catalysis by the ABD1 enzyme. As illustrated in Fig. 4, alanine substitution of serine 174, threonine 178, or threonine 362 resulted in enzyme with little or no activity in vitro. The substitutions at conserved residues Gly-174, Asp-178, and Tyr-362, had no effect on cell growth (3). Five other alanine-substitution mutations, at His-253, Thr-282, Glu-287, Glu-361, and Tyr-362, had no effect on cell growth (3). (These nonessential residues are indicated by + in Fig. 3.) Now, we introduced mutations at 19 new positions within the conserved region. The residues mutated are denoted by asterisks and filled arrowheads in Fig. 3.
Effect of alanine-substitution mutations on ABD1 function in vivo

YBS6 was transformed with p358-ABD-Ala plasmids containing the indicated mutant alleles. Trp+ transformants were selected and then streaked on medium containing 5-FOA (0.75 mg/ml). The plates were incubated at 30 °C. Alleles that supported the formation of wild-type sized colonies after 3 days are indicated by + + + growth. Lethal mutations (growth −) were those that formed no colonies after 7 days.

| Mutation | Growth on 5-FOA |
|----------|-----------------|
| V168A    | + + +           |
| E170A    | + + +           |
| G172A    | + + +           |
| G176A    | + + +           |
| K181A    | + + +           |
| Y183A    | + + +           |
| D194A    | + + +           |
| R206A    | + + +           |
| G276A    | + + +           |
| G277A    | + + +           |
| E347A    | + + +           |
| Y348A    | + + +           |
| V349A    | + + +           |
| G363A    | + + +           |
| V350A    | + + +           |
| G366A    | + + +           |
| L366A    | + + +           |
| V367A    | + + +           |
| F372A    | + + +           |

FIG. 4. Purification of mutant ABD1 proteins. Aliquots (2 μg) of the phosphocellulose preparations of wild-type and mutant ABD1 proteins were analyzed by SDS-PAGE. Polypeptides were visualized by staining with Coomassie Blue dye. The identity of the protein is specified above the lanes. The positions and sizes (kDa) of marker polypeptides are indicated at the left.

Methyltransferase Activity of ABD1 Missense Mutants—Histagged versions of the wild type and 10 mutant ABD1 proteins were expressed in bacteria and purified by Ni-agarose and phosphocellulose chromatography. The polypeptide compositions of the phosphocellulose preparations revealed similar extents of purification (Fig. 4). The methyltransferase activity of the recombinant proteins was assayed as a function of protein concentration (Fig. 5). There was a clear correlation between mutational effects on yeast cell growth and effects on the catalytic activity of the recombinant enzymes. At position Glu-170, the lethal E170A and E170Q changes reduced the methyltransferase activities to −8% and <1% of the wild type enzyme, respectively (Fig. 5A). The viable E170D mutation apparently increased methyltransferase specific activity compared with the wild type enzyme (Fig. 5A). At Asp-194, the lethal D194A and D194N substitutions abrogated methyltransferase activity in vitro, while the viable D194E substitution had little effect (Fig. 5B). The lethal R206A mutation reduced activity to −6% of wild type, whereas the lysine substitution was well tolerated (Fig. 5C). The Y254F mutant displayed near-wild-type specific activity, while the activity of the lethal Y254S mutant was −10% of wild type (Fig. 5D). These results further support the idea that viability is contingent on a threshold level of ABD1 methyltransferase activity.

Effect of conservative substitutions on ABD1 function in vivo

YBS6 was transformed with p358-ABD-Ala plasmids containing the indicated mutant alleles. Trp+ transformants were selected and then streaked on medium containing 5-FOA (0.75 mg/ml). The plates were incubated at 30 °C. Alleles that supported the formation of wild type sized colonies after 3 days are indicated by + + + growth. Formation of small colonies after 5 days is denoted by + growth. Lethal mutations (growth −) were those that formed no colonies after 7 days.

| Mutation | Growth on 5-FOA |
|----------|-----------------|
| E170D    | + + +           |
| E170Q    | −               |
| D194E    | + + +           |
| D194N    | −               |
| R206K    | + +             |
| Y254F    | +               |
| Y254S    | −               |

DISCUSSION

Structure-function relationships for the yeast cap methylating enzyme were illuminated by testing the effects of deletion and point mutations on cell growth and on methyltransferase activity in vitro. From the results of the deletion analysis, we conclude: (i) the NH2-terminal 109 residues and COOH-terminal 10 residues of ABD1 are dispensable for cell growth and for cap methyltransferase activity; (ii) residues 110–129 are not required for catalysis in vitro, but are important for ABD1 function in vivo; (iii) elimination of residues 130–142 attenuates, but does not abolish, catalytic activity. These experiments define an autonomous catalytic domain of the cap methyltransferase that extends from residues 130 to 426.

The role of the segment from 110 to 129 in ABD1 function in vivo remains unclear; the catalytically active ABD1(130–436) polypeptide accumulates in vivo, but this derivative is not sufficient to support growth in the absence of wild type ABD1. Several explanations come to mind: (i) the truncated protein may not fold correctly in yeast, although it does when expressed in bacteria; (ii) the missing residues are required for correct subcellular localization; or (iii) the deleted segment is required for interaction of ABD1 with other cellular proteins, e.g. with other enzymes of the mRNA capping pathway or with the RNA polymerase II transcription complex. Such protein-protein interactions, if they do occur, are likely to be either transient or weak, insofar as the bulk of the cap methyltransferase activity isolated from whole cell yeast extracts sediments as a monomer (2). The catalytic domain defined by our deletion analysis includes the entire 205-amino acid region of homology between ABD1 (from residues 168 to 372) and the catalytic domain of

TABLE I

| Mutation | Growth on 5-FOA |
|----------|-----------------|
| Y168A    | + + +           |
| E170A    | + + +           |
| G172A    | + + +           |
| G176A    | + + +           |
| K181A    | + + +           |
| Y183A    | + + +           |
| D194A    | + + +           |
| R206A    | + + +           |
| G276A    | + + +           |
| G277A    | + + +           |
| E347A    | + + +           |
| Y348A    | + + +           |
| V349A    | + + +           |
| G363A    | + + +           |
| V350A    | + + +           |
| G366A    | + + +           |
| L366A    | + + +           |
| V367A    | + + +           |
| F372A    | + + +           |
the vaccinia virus cap methyltransferase. We have now characterized the effects of mutations at 27 amino acids within this region (Fig. 3). Alanine substitutions at 21 positions had no effect on ABD1 function \textit{in vivo}, despite the fact that most of the 21 nonessential residues are conserved among the cap methyltransferases (Fig. 3).

So far we have shown that six residues within this region are important for ABD1 function \textit{in vivo} and \textit{in vitro}. Glu-170, Gly-174, and Asp-178 are located near the NH$_2$-terminal border of the conserved region of ABD1, within or immediately flanking a putative AdoMet binding motif VL(D/E)X$_3$G$_X$X$_3$, that is conserved among AdoMet-requiring methyltransferases (4, 5). This sequence element has been named motif I. We have replaced all three glycine residues by alanine and found that only the second glycine of motif I (Gly-174 in ABD1) is essential. Ala substitution of the corresponding Gly in the vaccinia virus cap methyltransferase (Gly-600 of the D1 subunit) reduced the specific activity of the viral methyltransferase to 4% that of wild type enzyme (6). The glycine residue corresponding to Gly-174 of ABD1 is strictly conserved in all known cap methylating enzymes (Fig. 3). The third glycine residue of motif I is also conserved, but ABD1 function is not compromised when this residue (Gly-176) is replaced by alanine. Ala substitution at Gly-606 of the vaccinia methyltransferase (the equivalent of Gly-176 in ABD1) was similarly without effect on enzyme activity (6). We found that the first glycine in motif I of ABD1 (Gly-172) is also nonessential. This position is Gly in the African swine fever virus, but not in the vaccinia and other poxviruses, where it is instead an aspartic acid. Replacement of the vaccinia aspartic acid residue by alanine had little effect on enzyme activity (the D598A mutant retained 50% the activity of wild type enzyme) (6). Hence, the identity of this position is not critical in either cellular or viral cap methylating enzymes.

We found that Glu-170 in motif I of ABD1 can be replaced by aspartate, but not glutamine or alanine; hence, an acidic residue is essential for ABD1 function \textit{in vivo}. The African swine fever virus homologue contains an aspartic acid at this position (Fig. 3). Most other AdoMet-requiring methyltransferases that contain motif I also have an acidic residue at this position.

![Fig. 5. Methyltransferase activity of ABD1 mutants. Reaction mixtures contained 20 fmol of cap-labeled poly(A), 50 \textmuM AdoMet, and either wild-type ABD1 or mutant ABD1 proteins as indicated. The extent of cap methylation (average of two experiments) is plotted as a function of the molar amount of input ABD1 protein.](http://www.jbc.org/)

![Fig. 6. A subfamily of ABD1-like methyltransferases and putative methyltransferases. The amino acid sequence of ABD1 from residues 168 to 206 is aligned with those of nine known AdoMet-dependent methyltransferases (top group) and 22 other putative methyltransferases of unknown function (bottom group). The organism from which each protein derives, the gene name, and the predicted size of the polypeptide (number of amino acid residues) are indicated to the left of each sequence. Amino acids essential for ABD1 function \textit{in vivo} are denoted by asterisks. Conserved motifs I and Ia are demarcated by horizontal bars below the aligned sequences.](http://www.jbc.org/)
Yeast mRNA Cap Methyltransferase

However, there are exceptions (5, 7), even among the cap methylating enzymes, e.g., the three poxvirus proteins contain alanine (Fig. 3). It is noteworthy that alanine substitution of Glu-170 reduces, but does not eliminate catalytic activity. Thus, Glu-170 is not strictly essential for catalysis, but an acidic side chain is apparently required to exceed the threshold of activity required for yeast cell growth. The vaccinia cap methyltransferase, which has a higher specific activity to begin with than does ABD1 (2, 6), may be less dependent on the contributions of this side chain than is ABD1. Conceivably, replacement of the alanine in the vaccinia enzyme by an acidic side chain might even increase its specific activity (analogous to the increase in ABD1 specific activity seen upon substitution of Glu by Asp). Although alanine replacement in ABD1 reduced activity in vitro, replacement by glutamate virtually abolished activity. Introducing an amide moiety is evidently more deleterious than is side chain removal.

Essential residue Asp-194 is located 18 amino acids downstream of motif I. An aspartate is present at this position in all cap methyltransferases (Fig. 3). An acidic side chain at this position is critical for ABD1 function in vivo and for catalytic activity in vitro. Methyltransferase activity was abrogated by replacing Asp-194 with either asparagine or alanine. Downstream residue Arg-206 is also essential for cell growth. Lysine suffices at this position, but alanine does not. The R206A mutant, like E170A, has significantly reduced activity in vitro, replacement by glutamine virtually abolished activity. Introducing an amide moiety is evidently more deleterious than is side chain removal.

It was shown previously that Y254A substitution in ABD1 was lethal and that this mutation reduced methyltransferase activity in vitro \( \sim 5\% \) of the wild type level (3). We now found that phenylalanine could substitute for tyrosine in both in vivo and in vitro (although cell growth was slowed). In contrast, a serine at position 254 was lethal and the Y254S protein displayed reduced methyltransferase activity in vitro, and for catalytic activity in vivo. Methyltransferase activity was abrogated by replacing Y254 with either asparagine or alanine. Downstream residue Arg-206 is also essential for cell growth. Lysine suffices at this position, but alanine does not. The R206A mutant, like E170A, has significantly reduced activity in vitro, replacement by glutamine virtually abolished activity. Introducing an amide moiety is evidently more deleterious than is side chain removal.

The correlation of in vitro and in vivo missense mutational effects on ABD1 function can be summarized as follows: (i) all mutations that severely affected methyltransferase activity were lethal, and (ii) no mutations were encountered that abolished methyltransferase activity yet permitted cell growth. These results confirm that cap methyltransferase activity (and, by inference, the cap methyl group) is essential for cellular function.

Essential Residues in ABD1 Define a Subfamily of Methyltransferases and Putative Methyltransferases—Structure-function relationships revealed by mutating the conserved domain of ABD1 are likely to be relevant to other methyltransferases. We performed a search of the NCBI data base with the segment of ABD1 from residues 168 to 194, which includes four of the essential residues. This illuminated a distinct subfamily of ABD1-like methyltransferase enzymes that display conservation at essential ABD1 residues 170, 174, and 194. The aligned sequences are shown in Fig. 6. Members of this subfamily include: EcaI adenine-N6 DNA methyltransferase; magnesium-protoporphyrin O-methyltransferase (bchM); ribosomal protein L11 methyltransferase (prmA); cyclosporin synthetase (simA); enniatin synthetase (esyn1), alkanic acid methyltransferase (dauC), ribosomal RNA adenine-N6 methyltransferase (ermA), 3-demethylubiquinone-9 3-methyltransferase (COQ3), and hnrNP methyltransferase (HMT1). Two of the methyltransferases (EcaI and bchM) also contained an Arg residue at the position corresponding to the essential amino acid Arg-206 of ABD1. We identified a number of methyltransferase-like proteins of unknown function that fall into this subfamily. The aligned sequences of 22 of these proteins are shown in Fig. 6. Nine of them contain arginine at the position corresponding to Arg-206 of ABD1, whereas three others contain a lysine at this position.

The alignment highlights several other conserved residues in addition to the three used as criteria for assembling this subfamily. A cysteine-containing version of motif I, (V/L/I)-L-[D/E]-V/L/I)-G-C-G-X-G, was especially highly conserved. A second conserved element, (P[N]-I/V)-G-[I]-I/V)-D-[I/L/V)-S, is located 13 amino acids downstream in the ABD1 protein. We will refer to this sequence element as methyltransferase motif Ia. In other subfamily members, motifs I and Ia are separated by a nonconserved spacer of 12–15 amino acids (Fig. 6).

A striking aspect of the alignment is that essential ABD1 residue Asp-178 is not conserved in other subfamily members; a mere three out of 31 ABD1-like proteins in Fig. 6 contain an aspartate at this position. Furthermore, none of the 25 different E. coli methyltransferases aligned by Koonin et al. (7) contains an acidic residue at this position. Yet Asp-178 is...
strictly conserved among the five cap methyltransferases (Fig. 3). This suggests that this acidic residue contributes uniquely to cap methylation, perhaps by interacting with the GppN cap structure of the RNA methyl acceptor.

A Candidate Cap Methyltransferase from C. elegans—By imposing complete conservation of all six residues essential for ABD1 function, we have identified a putative cap methyltransferase from C. elegans. The predicted C. elegans C25A1.f gene product is a 402-amino acid polypeptide identified by the Nematode Sequencing Project (GenBank™ accession no. Z81038). Alignment of the sequence of the nematode protein with ABD1 reveals identity or conservation at 149/402 positions (Fig. 7). The nematode protein includes a 30-amino acid carboxyl-terminal extension not found in ABD1. The worm protein does not contain a counterpart of the NH2-terminal 96 amino acids of ABD1; our deletion analysis showed these nonconserved residues are dispensable for ABD1 function. It is worth underscoring that the similarity between yeast ABD1 and the C. elegans gene product is greater than the similarity of ABD1 to the vaccinia virus cap methyltransferase. The C. elegans polypeptide is first candidate cap methylating enzyme identified from a metazoan species. It will be of interest to obtain a cDNA clone encoding this C. elegans polypeptide and then test whether the nematode gene complements an abd1 mutant of S. cerevisiae.

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