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Characterization of the Formate (*for*) Locus, Which Encodes the Cytosolic Serine Hydroxymethyltransferase of *Neurospora crassa*†

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Serine hydroxymethyltransferase (SHMT) occupies a central position in one-carbon (C_1) metabolism, catalyzing the reaction of serine and tetrahydrofolate to yield glycine and 5,10-methylenetetrahydrofolate. Methylenetetrahydrofolate serves as a donor of C_1 units for the synthesis of numerous compounds, including purines, thymidylate, lipids, and methionine. We provide evidence that the formate (*for*) locus of *Neurospora crassa* encodes cytosolic SHMT. The *for*$^+$ gene was localized to a 2.8-kb *Bgl*II fragment by complementation (restoration to formate-independent growth) of a strain carrying a recessive *for* allele, which confers a growth requirement for formate. The *for*$^+$ gene encodes a polypeptide of 479 amino acids which shows significant similarity to amino acid sequences of SHMT from bacterial and mammalian sources (47 and 60% amino acid identity, respectively). The *for*$^+$ mRNA has several different start and stop sites. The abundance of *for*$^+$ mRNA increased in response to amino acid imbalance induced by glycine supplementation, suggesting regulation by the *N. crassa* cross-pathway control system, which is analogous to general amino acid control in *Saccharomyces cerevisiae*. This was confirmed by documenting that *for*$^+$ expression increased in response to histidine limitation (induced by 3-amino-1,2,4-triazole) and that this response was dependent on the presence of a functional cross-pathway control-1 (*cpc-1*) gene, which encodes CPC1, a positively acting transcription factor. There are at least five potential CPC1 binding sites upstream of the *for*$^+$ transcriptional start, as well as one that exactly matches the consensus CPC1 binding site in the first intron of the *for*$^+$ gene.

Reactions involving one-carbon (C_1) units are essential for the synthesis of numerous compounds, including purines, thymidylate, lipids, and methionine. In the majority of these syntheses, the C_1 units are donated from C_1-substituted tetrahydrofolate derivatives. The principal enzyme involved in the generation of C_1-substituted tetrahydrofolates is serine hydroxymethyltransferase (SHMT; EC 2.1.2.1), which catalyzes the reaction of serine and tetrahydrofolate to yield glycine and 5,10-methylenetetrahydrofolate. Methylenetetrahydrofolate can then be converted to methyl-, methenyl- and formyltetrahydrofolate, which serve as the primary sources of C_1 groups for biosynthetic reactions in biological systems (11, 12). SHMT is central to one-carbon (C_1) metabolism in both prokaryotes and eukaryotes. In the absence of SHMT activity, C_1-substituted tetrahydrofolates can be synthesized by the conversion of formate and tetrahydrofolate to formyltetrahydrofolate, which is then converted to methylene-, methyl-, and methenyl-substituted tetrahydrofolates. Abnormalities in C_1 metabolism and specifically in SHMT activity have been detected in human colon carcinoma (46) and in schizophrenic and psychotic patients (54, 55).

Genes (*glyA*) encoding prokaryotic forms of SHMT have been isolated from *Escherichia coli* (47), *Salmonella typhimurium* (50), and *Bradyrhizobium japonicum* (42). Complete nucleotide sequences have been determined for the *E. coli* and *B. japonicum* genes, allowing the deduction of the amino acid sequences (41, 42). Although genes encoding SHMT have not yet been isolated from eukaryotic sources, both cytosolic and mitochondrial isozymes of SHMT have been purified from rabbit liver, and the complete amino acid sequences of the proteins have been determined by amino acid sequencing (31, 32). There is significant amino acid sequence similarity among all forms of SHMT characterized to date, suggesting extensive conservation of catalytically or conformationally important residues (31, 32, 41, 42).

The isolation and characterization of the gene encoding SHMT from a eukaryote with a well-established system of genetic manipulation would facilitate the in vivo analysis of the structure and function of SHMT. Strains of *Neurospora crassa* mutated at the formate (*for*) locus have a growth requirement for formate (20) and lack cytosolic, but retain mitochondrial, SHMT activity (7, 14, 15). The *for* locus maps to the right arm of linkage group VII, ~4.5 map units distal to the oligomycin resistance gene (*oli* [40]). The *oli* locus has been cloned and shown to encode the proteolipid subunit of the mitochondrial ATP synthase (51). We have recently cloned the *for*$^+$ gene of *N. crassa* by complementation of a *for* mutant strain with a cosmid clone of *N. crassa* genomic DNA identified by chromosomal walking from *oli* (33). We now report the physical characterization of the *for*$^+$ gene and show that it encodes the cytosolic isozyme of SHMT. We further demonstrate that the regulation of SHMT activity results, at least in part, from changes in the mRNA abundance of the *for*$^+$ gene. This regulation involves the cross-pathway control system (3, 8, 9), analogous to general amino acid control in *Saccharomyces cerevisiae* (24), and requires a functional copy of the cross-pathway control-1 (*cpc-1*) gene (3).

MATERIALS AND METHODS

***N. crassa* strains and clones.** Strains FGSC 4264 *cpc-1* (the CD15 allele) and FGSC 878 acriflavin resistance-2 (*acr-2* [the KH2 allele]) were obtained from the Fungal Genetic Stock Center (University of Kansas Medical Center, Lawrence). Strains carrying the *for* mutation (the C24 allele) were

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obtained from J. Feldman and J. Loros. Plasmids carrying the *N. crassa* arginine-2 (*arg-2* [35]) and *cpc-1* (37) genes were obtained from D. Ebbole and C. Yanofsky. *for*$^+$ cDNA clones were isolated from a library of *N. crassa* mycelial cDNA in the vector λgt10 (30).

Media and culture conditions. Liquid or plate cultures of an *N. crassa* wild-type (*for*$^+$) strain or of the *for* strain were grown in Horowitz complete medium or Vogel's minimal medium (16) plus 2% sucrose supplemented with sodium formate (10 mM). The effect of various supplements on mRNA abundance was determined in mycelia harvested from log-phase cultures grown, with shaking, at 30°C in Vogel's minimal medium plus 2% sucrose. Where indicated, the medium was supplemented with sodium formate (1 or 10 mM), glycine (1 or 10 mM), L-serine (1 or 10 mM), or L-methionine (1 or 10 mM). Cultures were harvested after 24 h of growth except for those supplemented with glycine, which grew slowly and were harvested after 36 h of growth. In some experiments, 3-amino-1,2,4-triazole (3-AT; 10 mM) was added 1 h prior to harvest to induce histidine starvation by competitive inhibition of the histidine biosynthetic enzyme imidazoleglycerolphosphate dehydratase (EC 4.2.1.1 [23]).

Transformation of *N. crassa*. Preparation of *for* spheroplasts for transformation with cosmid DNA was done as described by Vollmer and Yanofsky (52). For transformation with individual DNA restriction fragments or with plasmid subclones, DNA prepared by the boiling method (25) was further purified by electrophoresis through low-melting-point agarose (SeaPlaque; FMC, Rockland, Maine) and transformed as described by Liu and Dunlap (29). Individual DNA restriction fragments were cotransformed with pSV50, which encodes benomyl resistance (52). Primary benomyl-resistant (Bm^r) transformants of *for* strains were identified in regeneration agar on FIGS minimal medium plates (52) containing benomyl (500 μg/liter) and supplemented with sodium formate (10 mM). Individual Bm^r transformants were maintained on Horowitz complete medium containing benomyl (500 μg/liter) and supplemented with sodium formate (10 mM) and were screened for formate-independent growth in liquid cultures of Vogel's minimal medium with or without sodium formate (10 mM).

Nucleic acid manipulations. RNA purification and Northern (RNA) blot analyses were done as previously described (30). RNA slot blots were prepared by using a Minifold II apparatus (Schleicher & Schuell, Keene, N.H.) according to the manufacturer's instructions. RNA was quantitated by densitometric analysis with a MasterScan densitometer (Scanalytics, Billerica, Mass.). Plasmid subclones prepared in the Bluescript vectors (Stratagene, La Jolla, Calif.) were subjected to exonuclease III deletion (22), and DNA sequence was determined by the chain termination method (44), using Sequenase (U.S. Biochemicals, Cleveland, Ohio) according to the manufacturer's instructions. 3′ and 5′ ends of *for*$^+$ mRNAs were determined by S1 protection (5), using the modifications described by Ausubel et al. (2). Briefly, a DNA primer (5′-GCCTTGTGAGTCTCGGA-3′, complementary to nucleotides [nt] 602 to 587 according to the numbering scheme of Fig. 2) was allowed to anneal to a single-stranded DNA template and elongated with the Klenow fragment of DNA polymerase I in the presence of radiolabeled dCTP to generate a single-stranded, radiolabeled probe complementary to the *for* mRNA. The elongation products were digested to completion with *Eco*RI (which cuts at nt 368) and resolved on a denaturing 50% urea–4% polyacrylamide gel. The 234-nt fragment extending from nt 602 to 368 was eluted from the gel and annealed with 25 μg of poly(A)$^+$ RNA from *N. crassa*, digested with S1 nuclease, and analyzed on an 8% denaturing acrylamide gel. 5′ end analysis was confirmed by primer extension (34), using the modifications described by Ausubel et al. (2), and by direct sequencing of *for*$^+$ RNA (19). The GCG programs were used for computer analysis of nucleic acid sequences (17).

Nucleotide sequence accession number. The nucleotide sequence of the *N. crassa for*$^+$ gene has been registered with GenBank under accession number M81918.

RESULTS

Localization of the *for*$^+$ gene. The loss of SHMT activity resulting from the *for* mutation blocks the major pathway of biosynthesis of C_1-substituted tetrahydrofolates and thus disrupts C_1 metabolism. The resulting growth limitation can be alleviated by supplying formate (20), because the reaction of formate and tetrahydrofolate yields formyltetrahydrofolate, which is then converted to other C_1-substituted tetrahydrofolate derivatives (12). We had previously shown that DNA sequences capable of complementing (restoring to formate independence) the *for* strain were carried on cosmid 31:5E from the Vollmer-Yanofsky library (33). By transformation with *Bgl*II-digested cosmid 31:5E, we determined that sequences sufficient to restore formate-independent growth to a *for* strain lay on a single *Bgl*II restriction fragment (33). Systematic transformation of *for* spheroplasts with each of the *Bgl*II fragments of cosmid 31:5E indicated that the *for*$^+$ locus was entirely contained on a 2.8-kb *Bgl*II fragment (Fig. 1a). The *Bgl*II restriction fragment capable of complementing the *for* strain (Fig. 1b) was subcloned in both orientations into the Bluescript vector SK$^-$ as clones pSAD1-2 and pSAD4. The physical map of the *oli-frq-for* region (33) allowed the orientation of the restriction map of cosmid 31:5E, with the centromere to the left and the telomere to the right, as drawn in Fig. 1. The minimal sequences necessary for function of the *for*$^+$ locus were determined by cotransformation of the *for* strain with subclones of pSAD1-2 together with the plasmid pSV50, which confers benomyl resistance (52). Bm^r transformants were selected and scored for formate-independent growth (Fig. 1c). With intact pSAD1-2 (nt 1 to 2856) and pSV50, all of the Bm^r transformants were complemented (restored to formate independence). Transformation with pSV50 alone failed to restore formate-independent growth to the Bm^r transformants (data not shown). Deletions of up to 438 nt from the 5′ end of pSAD1-2 had no effect on the frequency of complementation. However, further deletions to nt 518 and 578 resulted in reduced frequency of complementation. Deletion from the 3′ end of pSAD1-2 to nt 2230 had no effect on complementation frequency, but further deletion to nt 2019 eliminated complementation among Bm^r transformants (Fig. 1c).

Physical characterization of the *for*$^+$ transcription unit. The direction of transcription was determined to be toward the telomere by probing Northern blots with single-stranded hybridization probes prepared from pSAD1-2 and pSAD4 (data not shown). cDNA clones of the *for*$^+$ mRNA were identified by hybridization with the 2.8-kb *Bgl*II fragment. No full-length cDNA clone was recovered. Therefore, the complete nucleotide sequences of both strands of several of these cDNAs which collectively spanned the entire length of the *for*$^+$ mRNA, as well as the complete sequence of both strands of the entire 2.8-kb *Bgl*II genomic fragment (Fig. 2),

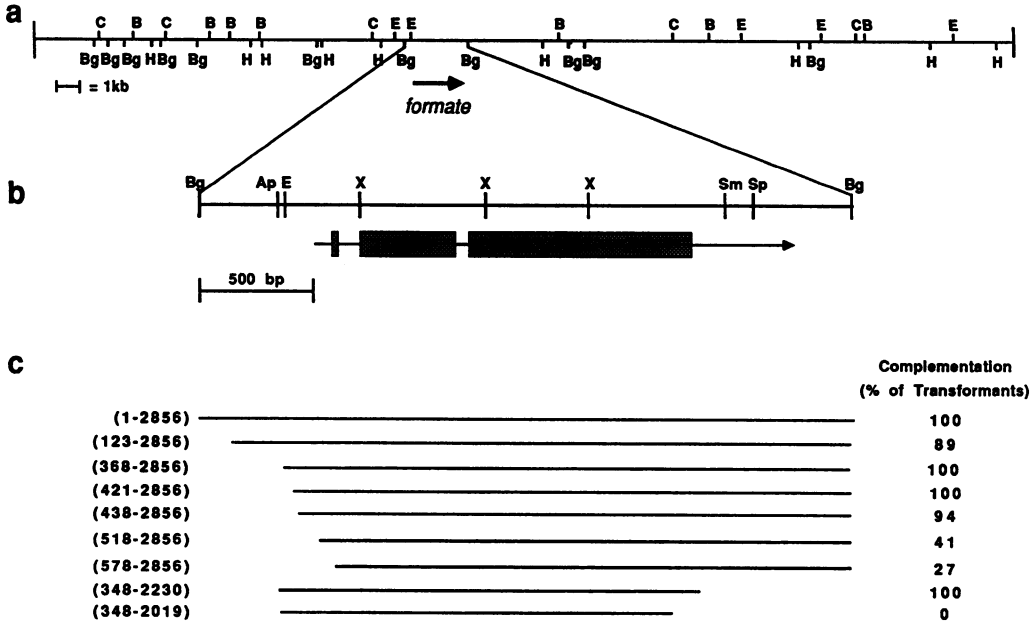

FIG. 1. (a) Restriction map of the insert of cosmid 31:5E (in the vector pSV50 [52]), with the position and direction of transcription of the *for*$^+$ gene indicated by the arrow. The centromere of linkage group VII is to the left, and the telomere is to the right. (b) Enlargement of the 2.8-kb *Bgl*II fragment containing the *for*$^+$ gene, which was subcloned into the Bluescript vector SK$^-$ as pSAD1-2 and pSAD4 (which differ in the orientation of the insert relative to the vector). The *for*$^+$ transcription unit is indicated by the arrow under the restriction map, with exons indicated by the hatched boxes. Restriction sites: *Apa*I (Ap), *Bam*HI (B), *Bgl*II (Bg), *Cla*I (C), *Eco*RI (E), *Hin*dIII (H), *Sma*I (Sm), *Spe*I (Sp), and *Xho*I (X). (c) Determination of minimal sequences of the *for*$^+$ gene sufficient to complement (restore formate-independent growth) when transformed (along with pSV50) into the *for* recipient. Inserts of plasmid subclones used to transform *for* spheroplasts are indicated by horizontal lines, with the endpoint nucleotides of the inserts indicated in parentheses to the left of each line. The percentage of Bm^r transformants restored to formate-independent growth is indicated at the right.

were determined. The nucleotide sequence indicates an open reading frame interrupted by two introns. The open reading frame begins at an ATG at nt 569 and ends at a TAA at nt 2157. The ATG at nt 569 is preceded by a sequence (gTCACC) which corresponds at five of six positions (indicated by capital letters) to the consensus sequence for *N. crassa* translational initiation sites (37). A second, in-frame ATG at codon 13 (immediately 3′ to the first intron) is not in a consensus translational initiation context. The first intron, 88 nt in length, lies between codons 12 and 13, and the second intron, 58 nt in length, interrupts codon 151 between the second and third positions. Both introns have the conserved *N. crassa* boundary sequences 5′ exon R/GTR(C/A)GT . . . CAG/exon 3′ (R = purine). Furthermore, both introns have sequences, ACTCATA and ACTAACC, respectively, beginning at positions 26 nt upstream of the 3′ exon, which closely resemble the *N. crassa* branch point consensus ACTRACA (43). By Southern analysis, we determined that the *for* mutant allele had not undergone any gross rearrangement, insertions, or deletions relative to the wild type (data not shown). The nature of the mutation resulting in the *for* phenotype remains unknown.

The *for*$^+$ mRNA has multiple 5′ and 3′ ends. Northern analysis of either total or poly(A)$^+$ RNA hybridized with coding sequence probes indicated the existence of at least four mRNA species of ~2,600, ~2,450, ~2,300, and ~2,050 nt for the *for*$^+$ gene (Fig. 3c, lanes B to E). No hybridization to mRNA was observed with probes extending from nt 1 to 372 (Fig. 3c, lane A) or from nt 2851 downstream for 2.4 kb (Fig. 3c, lane F), indicating that the entire transcription unit of the *for*$^+$ gene lies between nt 372 and 2851. Two 5′ ends of the *for*$^+$ mRNAs were identified by S1 nuclease protection (Fig. 4) and were confirmed by primer extension and RNA sequencing (data not shown). These 5′ ends were at nt 506 and 509 (indicated by arrows in Fig. 2) and are 65 and 62 nt, respectively, upstream of the translation initiation site at nt 569. Clustering of multiple 5′ ends has been frequently observed for *N. crassa* transcripts (for examples, see references 10 and 43 and references therein). Conserved TATA (TATATCT at nt 438) and CCAAT (nt 290) sequences are present upstream of the transcriptional initiation sites. The transcriptional start sites are preceded by a pyrimidine-rich sequence, characteristic of genes of filamentous fungi, particularly of highly expressed genes (6).

The 3′ ends of the *for*$^+$ mRNA transcripts were determined by comparison of cDNA and genomic sequences. At least three 3′ ends, indicated by asterisks in Fig. 2, were confirmed by S1 nuclease protection (data not shown), with the major end (double asterisk) at position 2591 (432 nt downstream of the termination codon) and minor ends (single asterisk) at positions 2531 and 2740 (372 and 581 nt, respectively, downstream of the termination codon). A fourth 3′ end is inferred to lie between nt 2293 and 2364, because a probe extending from nt 2293 to 2856 hybridizes to at least four mRNA species on a Northern blot (Fig. 3c, lane C) and a probe extending from nt 2364 to 2856 hybridizes to only three species and does not hybridize to the shortest mRNA (Fig. 3c, lane D). Two potential polyadenylation signals (Fig. 2, underscored with hatched boxes) are located in the 3′ untranslated sequences.

Deduced amino acid sequence of the *for*$^+$ gene. From open reading frame analysis of the combined cDNA and genomic sequences, the *for*$^+$ gene is deduced to encode a protein of 479 amino acids in length. Codon utilization within this open

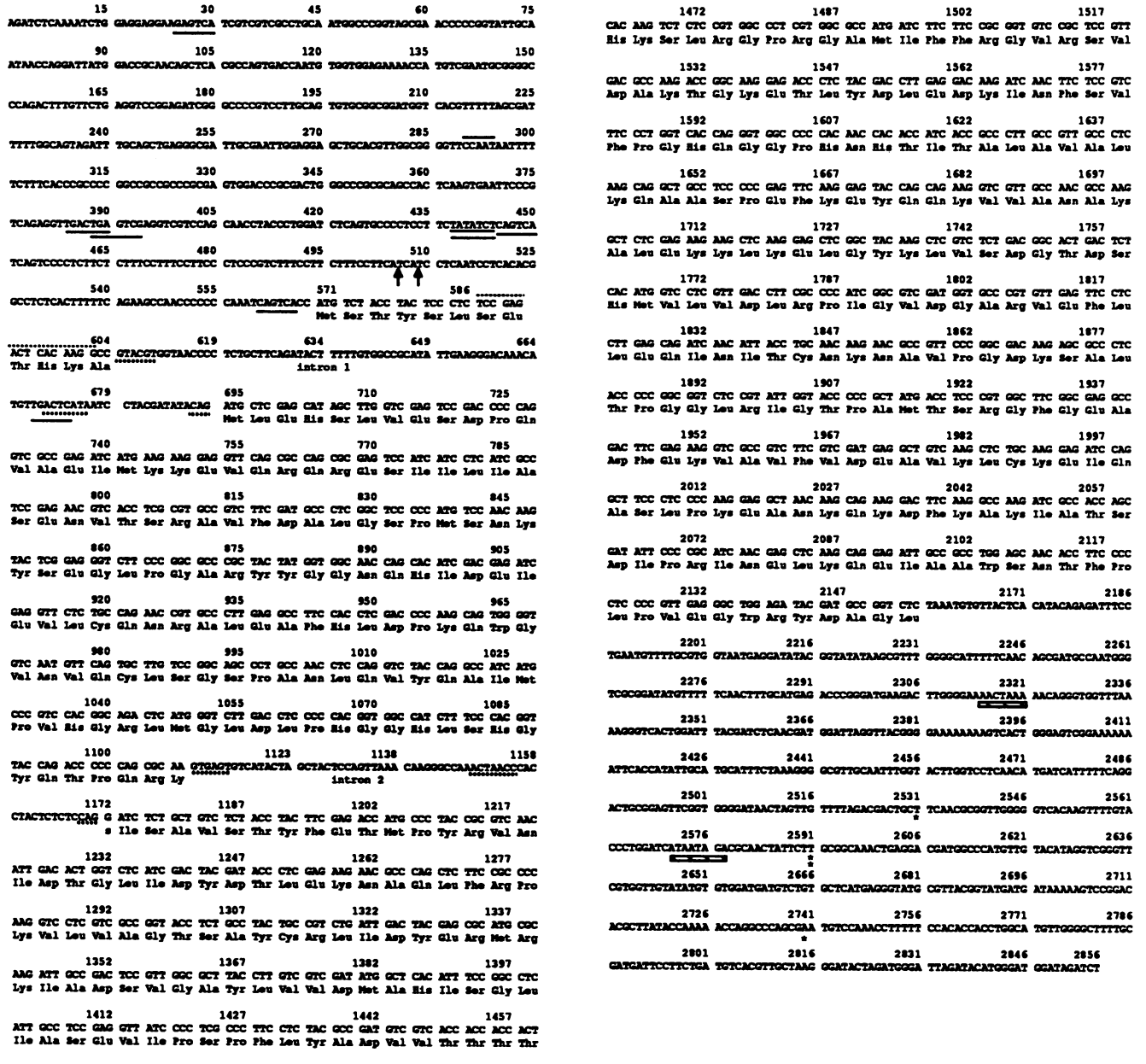

FIG. 2. Nucleotide sequence of the *N. crassa for*$^+$ gene region (noncoding strand) and deduced amino acid sequence of the SHMT protein. The nucleotide sequence is numbered relative to the upstream *Bgl*II site. Consensus intron boundary and internal splice sequences are indicated by dotted underlines. A putative CAAT sequence is overlined with a solid line. A potential TATA sequence is indicated with a double underline. Transcription start sites are indicated by the arrows. The sequence complementary to the primer (5′-GCCTTGTGAGTC TCGGA-3′) used for the determination of transcription start sites by nuclease protection is indicated by the dotted overline (nt 587 to 602). 3′ ends of the mRNAs are indicated by asterisks, with the major 3′ end indicated by a double asterisk. Sequences similar to the canonical polyadenylation signal, AATAAA, are underlined with hatched boxes. Sequences similar to the consensus CPC1 binding site (TGACTCA or its complement [18, 37]) are underlined.

reading frame shows strong bias against purines, especially adenines, and also against uracils in the third position, as is seen in highly expressed *N. crassa* nuclear genes (10). This amino acid sequence, which would encode a protein of 53 kDa, was used to search GenBank and NBRF data bases and showed significant similarity to amino acid sequences deduced from the *E. coli* and *B. japonicum glyA* genes (41, 42), which encode SHMT, and to the protein sequences determined for cytosolic and mitochondrial isozymes of rabbit liver SHMT (31, 32). These five SHMT amino acid sequences are aligned in Fig. 5, with residues conserved in at least three of the five sequences boxed. The *N. crassa* sequence shows 47% identity to the *E. coli* and *B. japonicum* SHMT sequences, 60% identity to the rabbit liver cytosolic SHMT sequence, and 56% identity to the rabbit liver mitochondrial SHMT sequence. Allowing for conservative substitutions, similarity values of the *N. crassa* sequence to the SHMT sequences for *E. coli*, *B. japonicum*, rabbit liver

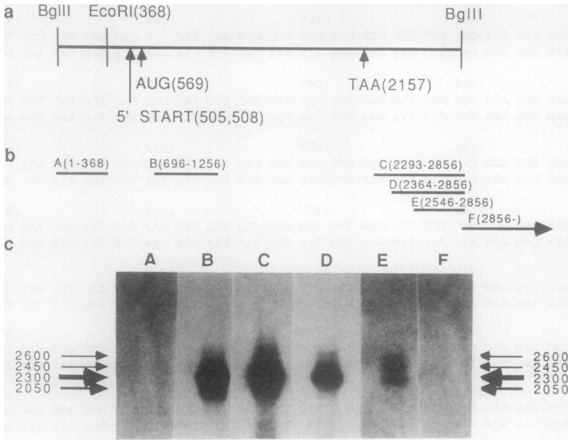

FIG. 3. Northern blot analysis of *for*$^+$ gene expression. (a) Restriction map of the 2.8-kb *Bgl*II fragment which contains the complete *for*$^+$ gene. Major transcriptional start sites and translational start and stop sites are indicated below the restriction map (nucleotides according to numbering scheme of Fig. 2 are indicated in parentheses). (b) Restriction fragments used as hybridization probes (first and last nucleotides of the probes, according to the numbering scheme of Fig. 2, are indicated by the numbers in parentheses). (c) Samples (2 μg) of poly(A)$^+$ RNA purified from wild-type mycelia grown in Vogel's minimal medium supplemented with sodium formate (10 mM) were separated on a 1.2% agarose–6% formaldehyde gel, transferred to nylon (ICN Biodyne), and hybridized to the probes (see above) indicated by the letters above the lanes. Transcript sizes, in nucleotides, are indicated at the left and right.

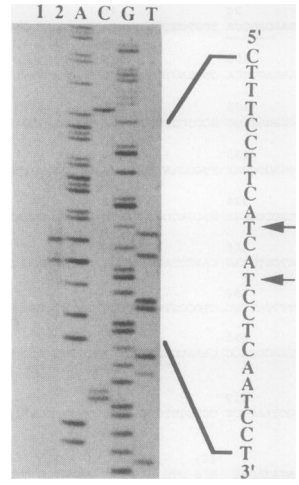

FIG. 4. Mapping the 5′ ends of the *for* mRNA by S1 nuclease protection. A primer (5′-GCCTTGTGAGTCTCGGA-3′) was used to generate a single-stranded, radiolabeled probe from nt 602 to 368 which would be complementary to the mRNA (see Materials and Methods). This strand was annealed with 25 μg of poly(A)$^+$ RNA from *N. crassa*, digested with S1 nuclease, and analyzed on denaturing 8% acrylamide gel in lane 2. Lane 1 shows the same probe incubated without RNA followed by S1 nuclease digestion. Lanes A, C, G, and T show the sequencing ladders generated from single-stranded DNA of the noncoding strand, using the same primer used to generate the radiolabeled probe used in the S1 nuclease treatments. The noncoding strand sequence (that is, the inverse of the sequence generated by the sequencing ladder shown) is shown at the right, with the two nucleotides corresponding to the 5′ ends of the mRNA indicated by arrows.

cytosol, and mitochondria are 65, 66, 74, and 73%, respectively. Using the rdf2 program (28, 39), we calculated *z* values (ktup = 1,500 shuffles) of 81, 81, 161, and 141 for comparisons of the *N. crassa* SHMT with SHMTs of *E. coli*, *B. japonicum*, rabbit cytosol, and rabbit mitochondria, respectively. A *z* value of >10 is consistent with common evolutionary origin (28). Thus, we conclude that the *for* locus encodes the *N. crassa* structural gene for SHMT.

Regulation of *for* gene expression. The effects of supplementation of the growth medium with methionine, serine, glycine, or sodium formate on *for* expression in a wild-type (*for*$^+$) strain are shown in Fig. 6a and 7. Supplementation with methionine or serine produced little effect on *for*$^+$ mRNA abundance. However, supplementation with glycine (at 10 mM but not at 1 mM) resulted in a ~2.8-fold increase in *for*$^+$ mRNA abundance. Similarly, supplementation with formate (at both 1 and 10 mM) resulted in increased (~2-fold) abundance of *for*$^+$ mRNA.

The increased level of *for*$^+$ mRNA observed in response to amino acid imbalance induced by supplementation with glycine suggested regulation by the cross-pathway control system (4). Similarly, the increase in *for*$^+$ mRNA in response to formate supplementation might result in amino acid imbalance and thus induce the cross-pathway control system. To determine whether the increased *for*$^+$ expression in response to glycine or formate supplementation (Fig. 6a and 7) represented regulation by the cross-pathway control system, we examined whether that induction was dependent on the presence of a functional *cpc-1* gene, which is required for cross-pathway control (3). In Fig. 8a we show that the increased abundance of *for*$^+$ mRNA in response to glycine or formate supplementation was dependent on the presence of a functional *cpc-1* gene product. We also examined the effect of histidine starvation induced by addition of 3-AT and found that it resulted in a four- to fivefold increase in *for*$^+$ mRNA (Fig. 6b and 9). This increase was not seen in a strain resistant to 3-AT because of mutation at the *acr-2* locus (27). Furthermore, *for*$^+$ mRNA did not accumulate in response to 3-AT addition in a strain lacking a functional *cpc-1* gene (Fig. 6b and 9) and hence lacking cross-pathway control (3). As a control, we also show that in our experiments, the expression of *arg-2*, which is known to be regulated by *cpc-1* (36), is induced by 3-AT addition in a *cpc-1*-dependent fashion (Fig. 9b). As a second control, we also show that the expression of *cpc-1* itself is induced by 3-AT addition in a *cpc-1*-independent manner (Fig. 9b), as was previously demonstrated (37). Consistent with this finding is our observation that glycine supplementation induces *cpc-1* expression in a *cpc-1*-independent fashion (Fig. 8b). Interestingly, formate supplementation also results in increased *cpc-1* mRNA accumulation, but this induction is abolished in a *cpc-1* strain (Fig. 8b).

In the *for* mutant strain, grown in Vogel's minimal medium supplemented with 10 mM formate, *for* mRNA of apparently unaltered size relative to the wild type (*for*$^+$) was present in somewhat higher (~1.5-fold) abundance than in a *for*$^+$ strain grown in the same medium. The *for* strain is an auxotroph and requires supplementation with formate for growth in minimal medium. We therefore investigated the effects of glycine, serine, or methionine supplementation on *for* expression in a *for* strain grown in minimal medium plus formate or in a rich (Horowitz complete) medium. In the wild type, *for*$^+$ expression in rich medium is 0.8-fold that in minimal medium without supplementation (data not shown). There was no difference in *cpc-1*$^+$ expression in wild-type

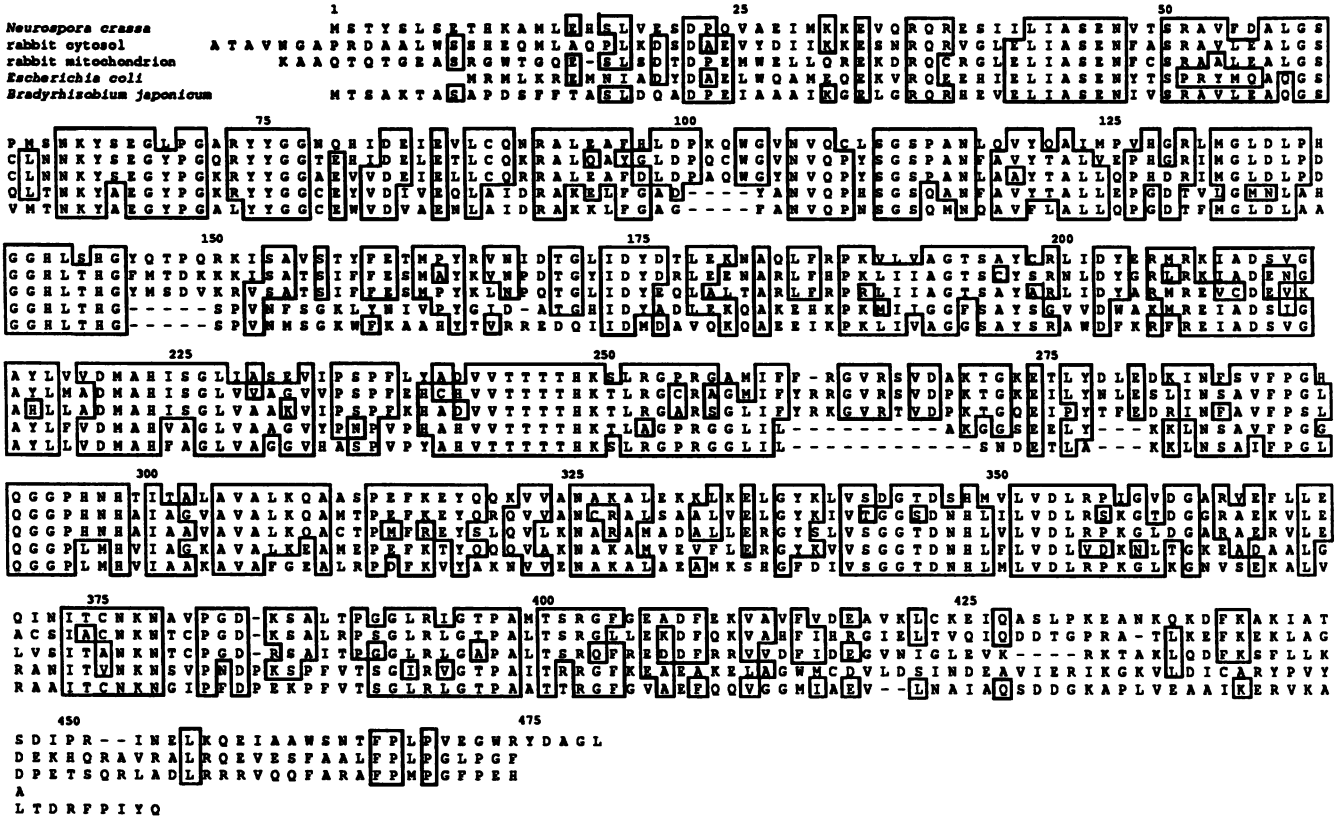

FIG. 5. Comparison of deduced amino acid sequence of *N. crassa* SHMT encoded by *for*$^+$ with amino acid sequences determined for rabbit cytosolic and mitochondrial SHMT isozymes (31, 32) and with SHMT amino acid sequences deduced from nucleotide sequences for *glyA* genes from *E. coli* (41) and *B. japonicum* (42). Boxes indicate residues identical in at least three of the five sequences. Numbering refers to the *N. crassa* SHMT deduced amino acid sequence.

cultures grown in rich or in unsupplemented minimal medium (data not shown). In the *for* mutant, *for* expression was increased ~2.7-fold in minimal (plus formate) medium relative to rich medium (Fig. 10). Additional supplementation with glycine, serine, or methionine did not result in further

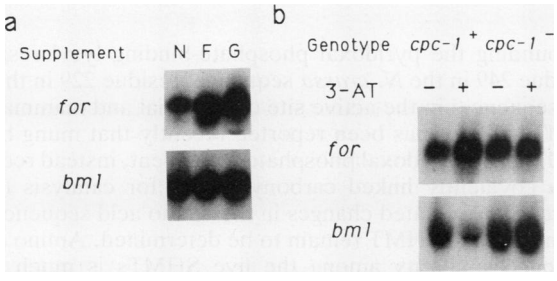

FIG. 6. Northern analysis of expression of the *for* gene in *for*$^+$ mycelia grown in Vogel's minimal medium with indicated supplements. (a) Effect on *for* expression of supplementation with 10 mM formate (F) or with 10 mM glycine (G) compared with no supplement (N) in a *cpc-1*$^+$ strain. A Northern blot loaded with 10 μg of total RNA per lane was hybridized to a *for*-specific probe, autoradiographed, stripped, rehybridized to a *bml* (β-tubulin)-specific probe, and reautoradiographed. (b) Effect on expression of *for* and *bml* genes of histidine starvation induced by 3-AT addition in *cpc-1*$^+$ and *cpc-1* mutant strains. Duplicate Northern panels each loaded with 10 μg of total RNA per lane were probed with either a *for*-specific probe or a *bml*-specific probe.

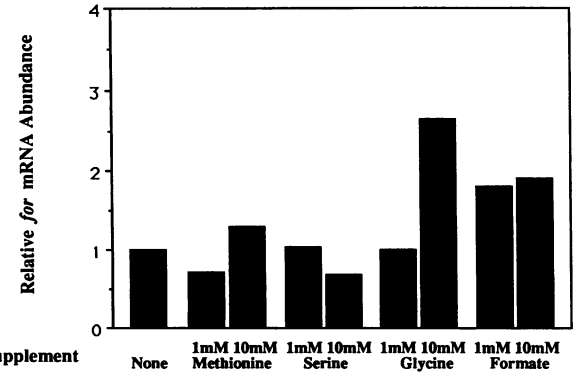

FIG. 7. Expression of the *for* gene in wild-type (*for*$^+$) mycelia grown in Vogel's minimal medium with indicated supplements. Slot blots loaded with 3 μg of total RNA were hybridized to a *for*-specific probe, autoradiographed, stripped, rehybridized to a *bml* (β-tubulin)-specific probe, and reautoradiographed. mRNA abundance was determined by densitometry using a Masterscan densitometer (Scanalytics). Abundance of *for* mRNA is expressed relative to *bml* mRNA abundance. mRNA abundance in wild-type cultures grown in minimal medium without supplementation was arbitrarily defined as 1.0, and mRNA abundance in other treatments was expressed relative to that value. The data presented represent means from at least two independent experiments. Northern analysis (not shown) indicated that the *for*-specific probe (the insert of pSAD1-2) hybridized only to the *for*$^+$ mRNA bands seen in Fig. 3 and that the *bml* probe hybridized to a single *bml* mRNA species (35).

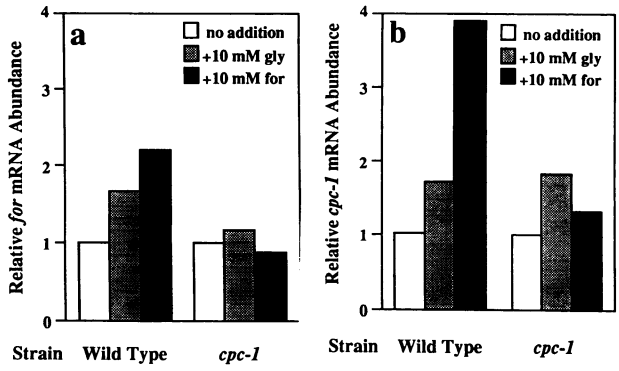

FIG. 8. Expression of the *for*$^+$ gene (a) and the *cpc-1* gene (b) in response to glycine (gly) or formate (for) supplementation in wild-type and *cpc-1* strains. Methods are as described for Fig. 7. The data represent means from at least two independent experiments. In each case, mRNA abundance is normalized to *bml* mRNA abundance. mRNA abundance in wild-type cultures grown in minimal medium without supplementation was arbitrarily defined as 1.0, and mRNA abundance in other treatments was expressed relative to that value.

increases in *for* expression. In the *for* mutant strain, *cpc-1*$^+$ expression paralleled *for* expression (Fig. 10).

DISCUSSION

The high degree of similarity observed between the amino acid sequence deduced from the nucleotide sequence of the *N. crassa for*$^+$ locus and the amino acid sequences of known SHMTs confirms that the *for*$^+$ locus encodes SHMT. Strains carrying a *for* mutation lack cytosolic but retain mitochondrial SHMT activity (7, 14, 15), indicating that the *for*$^+$ locus encodes the cytosolic isozyme of SHMT. Consistent with this conclusion, there is no evidence of a mitochondrial transit peptide in the deduced amino acid sequence. Mitochondrial transit peptides, although lacking in characteristic sequence homology blocks, typically are rich in serine, leucine, and basic residues (especially arginine) and are lacking in acidic residues (21, 53). In particular, arginine residues have been noted at positions −2 and −10 relative to the bond cleaved by the matrix processing protease (21, 53). The first arginine residue in the *N. crassa* SHMT is at amino acid 35 (nt 759) and corresponds to a residue conserved in all five SHMT sequences, including the rabbit mature mitochondrial SHMT. Cleavage downstream from this arginine would remove a number of residues which are conserved in all five SHMTs, including the mature mitochondrial SHMT. Thus, it seems unlikely that the *for*$^+$ gene encodes a mitochondrial SHMT.

Inspection of Fig. 5 shows clearly that there is considerable sequence conservation among SHMTs from diverse taxa, presumably reflecting catalytic and/or structural constraints. The *N. crassa* SHMT amino acid sequence is most similar to the sequence of the rabbit liver cytosolic isozyme, showing 60% identical residues. The *N. crassa* SHMT shows 56% sequence identity with the rabbit liver mitochondrial isozyme and also shows considerable sequence conservation with prokaryotic SHMTs (47% identity to the *E. coli* and *B. japonicum* sequences). For comparison, the rabbit sequences share 62% identical residues with one another (32), and the prokaryotic sequences share 58% identical residues (41, 42). Sequence conservation among the five SHMTs extends throughout most of the length of the proteins, with the greatest degree of sequence conservation

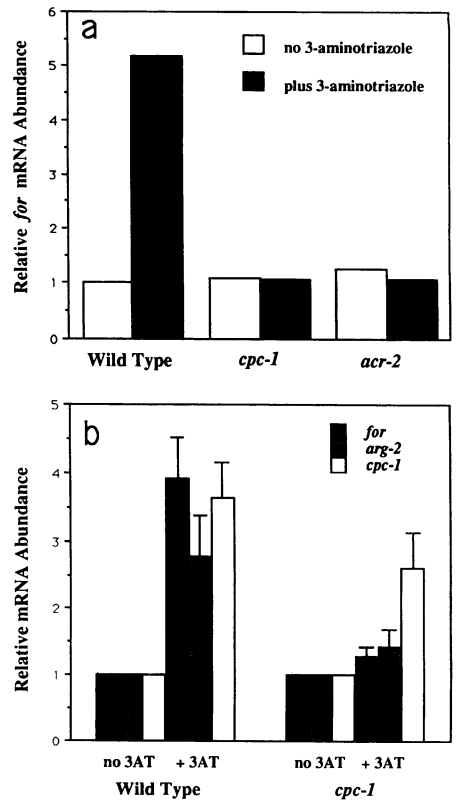

FIG. 9. (a) Expression of the *for*$^+$ gene in response to amino acid limitation in wild-type, *cpc-1*, and *acr-2* strains. Open bars represent cultures grown in Vogel's minimal medium without supplementation; solid bars represent cultures in which histidine limitation was induced by supplementation with 3-AT (10 mM) for 1 h prior to harvest. mRNA was quantified as described for Fig. 7. *for*$^+$ mRNA abundance was normalized to *bml* mRNA abundance. *for*$^+$ mRNA abundance in unsupplemented wild-type cultures was arbitrarily defined as 1.0, and *for*$^+$ mRNA abundance in other treatments was expressed relative to that value. (b) Abundance of *for*$^+$, *arg-2*, and *cpc-1* mRNA in response to 3-AT supplementation in wild-type and *cpc-1* strains. mRNA levels were quantified as described for Fig. 7 and normalized to *bml* mRNA levels. Data represent the means ± standard errors of four (three for *cpc-1* mRNA) replicate experiments.

surrounding the pyridoxal phosphate-binding lysyl residue (residue 249 in the *N. crassa* sequence, residue 229 in the *E. coli* sequence) in the active site of bacterial and mammalian SHMTs (12). It has been reported recently that mung bean SHMT is not pyridoxal phosphate dependent, instead requiring a covalently linked carbonyl group for catalysis (49). However, associated changes in the amino acid sequence of the mung bean SHMT remain to be determined. Amino acid sequence similarity among the five SHMTs is much less conserved at the amino and carboxy termini. The three eukaryotic sequences can be distinguished from the prokaryotic sequences by a series of short insertions, at residues 100, 145, 261, and 278 of the *N. crassa* sequence, as well as by a carboxy-terminal extension. These inserted sequences are moderately conserved among the three eukaryotic forms, but the functional significance of the inserted residues is not known.

The sequences necessary for function of the *for*$^+$ gene were determined by complementation of *for* strains, using subclones of pSAD1-2 (Fig. 1c). Deletion of all sequences

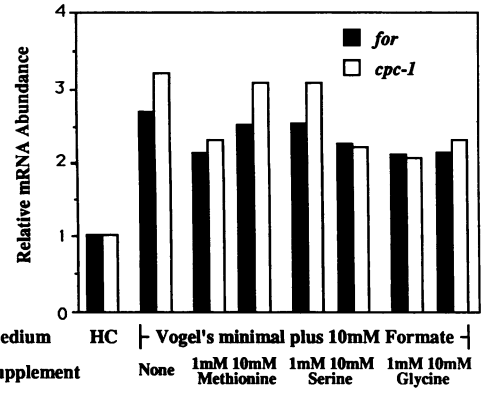

FIG. 10. Expression of the *for*$^+$ gene and the *cpc-1* gene in a *for* strain grown in Horowitz complete (HC) medium or in Vogel's minimal medium plus 10 mM formate with additional supplements as indicated. Methods are as described in the legend to Fig. 7. The data represent means from at least two independent experiments. In each case, mRNA abundance is normalized to *bml* mRNA abundance. mRNA abundance in *for* cultures grown in a rich medium (Horowitz complete medium) was arbitrarily defined as 1.0, and mRNA abundance in other treatments was expressed relative to that value.

upstream of the putative TATA sequence (nt 438) did not affect the ability to restore formate-independent growth. Furthermore, transformation with subclones of pSAD1-2 deleted to nt 518 (pSAD518-2856) or to nt 578 (pSAD578-2856) resulted in formate-independent growth in 41 and 27% of transformants, respectively. Thus, deletion of the transcriptional start sites and even of the translation initiation site and the three amino-terminal amino acid residues reduced the frequency of complementation to *for*$^+$ but did not eliminate complementation entirely. However, we scored only for formate-independent growth and did not determine the level of *for*$^+$ expression necessary for formate-independent growth. The presence of *for* mRNA of apparently unaltered size in unaltered abundance (or perhaps slightly increased abundance [data not shown]) in the *for* strain renders such analysis problematic. Whether complementation of *for* strains resulting from transformation with constructs apparently lacking transcriptional and translational signals resulted from expression utilizing transcription and translation signals present in the Bluescript vector or acquired during integration into the *N. crassa* genome either at the homologous *for* locus or at nonhomologous sites is unclear. Note, however, that there is an in-frame methionine residue (amino acid 13) at nt 694, and it is possible that translation beginning at that methionine results in a functional protein. The lack of sequence conservation at the amino terminus of SHMT may indicate that the amino-terminal residues are not catalytically or structurally critical. Indeed, removal (by proteolytic cleavage) of the amino-terminal 14 amino acids from the rabbit cytosolic SHMT does not affect in vitro catalytic activity (45).

In a similar analysis of 3′ signals, transformation of the *for* strain with *for*$^+$ subclones deleted of all sequences downstream of nt 2230 (pSAD345-2230) resulted in complementation of 100% of the Bm^r transformants to formate-independent growth. Thus, the coding sequence plus 71 nt of untranslated trailer sequences which do not include putative polyadenylation signals is sufficient for complementation of the *for* mutant. Transformation with a subclone deleted of all sequences downstream of nt 2019 (pSAD345-2019) and lacking the carboxy-terminal 46 amino acid residues did not yield transformants capable of formate-independent growth. Thus, despite the lack of amino acid sequence conservation at the carboxy terminus among the five known SHMTs, nucleotide sequences encoding the carboxy-terminal 46 residues are required for complementation of the *for* strain.

The central function of SHMT in C_1 metabolism makes it likely that the regulation of the *for*$^+$ gene is complex and responsive to a variety of regulatory signals. Previous studies in *N. crassa* indicated that SHMT activity increased in response to supplementation of the growth medium with serine or glycine and decreased in response to supplementation with formate (7, 13). We wished to determine whether changes in SHMT activity reflected changes in steady-state mRNA levels. Supplementation with glycine resulted in an ~2.8-fold increase in the steady-state level of *for*$^+$ mRNA (Fig. 6 and 7), which is consistent with the enzyme activity increase previously seen (7, 13). Although serine supplementation resulted in increased SHMT activity (7), we observed a slight decrease in *for*$^+$ mRNA level in cultures supplemented with serine. In contrast to the decrease in SHMT activity observed by Burton and Metzenberg (7) in response to formate addition, we observed that supplementation with formate resulted in ~2-fold increases of *for*$^+$ mRNA in wild-type strains. Thus, although we conclude that the regulation of SHMT activity includes regulation of *for*$^+$ mRNA abundance, it seems likely that there are additional mechanisms of regulation of SHMT.

The role of SHMT in amino acid (methionine) biosynthesis suggests that *for*$^+$ expression might be regulated according to the amino acid status of the mycelium. In *N. crassa*, amino acid biosynthetic genes are regulated by the cross-pathway control system (3, 8, 9). Cross-pathway control is analogous to the system of general control of amino acid biosynthesis in *S. cerevisiae*, in which starvation for any of several amino acids results in induction of genes for biosynthesis of all amino acids (24). In *N. crassa*, amino acid imbalance produced by supplementation with amino acids such as glycine results in global induction of amino acid biosynthetic enzymes which are regulated by the cross-pathway control system (4). Supplementation with formate may also result in a situation of amino acid imbalance, leading to the observed increase in *for*$^+$ expression. The increased *for*$^+$ expression seen in response to glycine or formate supplementation (Fig. 6a and 7) was dependent on the presence of a functional *cpc-1* gene (Fig. 8), which is required for cross-pathway control (3). Furthermore, the *cpc-1*-dependent induction of *for*$^+$ expression in response to histidine limitation (due to 3-AT addition) was similar to that of *arg-2* (Fig. 9), a gene known to be regulated by the cross-pathway control system (36). In contrast, the induction of *cpc-1* mRNA in response to glycine or 3-AT addition does not require the presence of a functional *cpc-1* gene, consistent with the results of Paluh et al. (37). Thus, we conclude that the *for* gene is regulated, at least in part, by the cross-pathway control system.

CPC1, the product of the *cpc-1* gene, is a DNA-binding protein which serves as a transcriptional activator of amino acid biosynthetic genes in *N. crassa* (37, 38). CPC1 shows similarity to GCN4, particularly in domains required for DNA binding and transcriptional activation (26, 37), and both proteins recognize the DNA element 5′-TGACTCA-3′ (1, 18). Inspection of the nucleotide sequence of the *for* gene reveals a number of sequences showing conservation of six of seven nucleotides in this recognition sequence (underlined in Fig. 2) as well as a single perfect (seven of seven conserved nucleotides) element at nt 668 to 674; it is not yet

known whether CPC1 binds to any of these DNA elements. The single completely conserved CPC1 recognition element is in the first intron of the *for*$^+$ gene, consistent with the ability of truncated *for*$^+$ genes lacking all sequences 5′ to the putative TATA box and even lacking the first several codons of the coding sequence to complement *for* mutant strains. A similar situation has been observed for the *arg-2* locus, which is subject to cross-pathway control (36). Transformation of mutants with presumed promoterless *N. crassa arg-2* subclones resulted in a high frequency of restoration of arginine-independent growth (36). Nucleotide sequence analysis of the *arg-2* locus has shown putative CPC1 binding sequences within both introns of the upstream open reading frame.

We have shown that mRNA abundance of the *N. crassa for*$^+$ gene is regulated by the cross-pathway control system in a *cpc-1*-dependent manner. However, the basal expression of *for* mRNA does not differ between a *cpc-1*$^+$ or a *cpc-1* mutant strain (Fig. 6b), indicating that CPC1 is not required for basal *for* expression. The effects of amino acid supplementation on *for*$^+$ mRNA accumulation do not always parallel the effects on SHMT activity, indicating other levels of regulation of *for*$^+$ expression. Given the role of SHMT in purine synthesis, it seems likely that *for*$^+$ expression may also be subject to regulation according to the purine status of the cell, as has been reported for the *E. coli glyA* gene (48). *for*$^+$ expression may also be responsive to regulation by other products of C_1 metabolism, including lipids and thymidylate. The *N. crassa for*$^+$ gene thus offers a useful system for the investigation of complex gene regulation in a genetically manipulable eukaryote.

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REFERENCES

1. **Arndt, K., and G. R. Fink.** 1986. GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5′ TGACTC 3′ sequences. Proc. Natl. Acad. Sci. USA **83**:8516–8520.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology. Wiley-Interscience, New York.
3. **Barthelmess, I. B.** 1982. Mutants affecting amino acid cross-pathway control in *Neurospora crassa*. Genet. Res. **39**:169–185.
4. **Barthelmess, I. B.** 1986. Regulation of amino acid synthetic enzymes in *Neurospora crassa* in the presence of high concentrations of amino acids. Mol. Gen. Genet. **203**:533–537.
5. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
6. **Burns, D. M., and C. Yanofsky.** 1989. Nucleotide sequence of the *Neurospora crassa trp-3* gene encoding tryptophan synthetase and comparison of the *trp-3* polypeptide with its homologs in *Saccharomyces cerevisiae* and *Escherichia coli*. J. Biol. Chem. **264**:3840–3848.
7. **Burton, E. G., and R. L. Metzenberg.** 1975. Regulation of methionine biosynthesis in *Neurospora crassa*. Arch. Biochem. Biophys. **168**:219–229.
8. **Carsiotis, M., and R. F. Jones.** 1974. Cross-pathway regulation: tryptophan-mediated control of histidine and arginine biosynthetic enzymes in *Neurospora crassa*. J. Bacteriol. **119**:889–892.
9. **Carsiotis, M., R. F. Jones, and A. C. Wesseling.** 1974. Cross-pathway regulation: histidine-mediated control of histidine, tryptophan, and arginine biosynthetic enzymes in *Neurospora crassa*. J. Bacteriol. **119**:883–898.
10. **Chow, C. M., and U. L. Rajbhandary.** 1989. Regulation of the nuclear genes encoding the cytoplasmic and mitochondrial leucyl-tRNA synthetases of *Neurospora crassa*. Mol. Cell. Biol. **9**:4645–4652.
11. **Cossins, E. A.** 1980. One-carbon metabolism, p. 365–418. *In* D. D. Davies (ed.), The biochemistry of plants, vol. 2. Academic Press, New York.
12. **Cossins, E. A.** 1987. Folate biochemistry and the metabolism of one-carbon units, p. 317–353. *In* D. D. Davies (ed.), The biochemistry of plants, vol. 11. Academic Press, New York.
13. **Cossins, E. A., P. Y. Chan, and G. Combepine.** 1975. Stimulation of folate metabolism by exogenous glycine in *Neurospora crassa* wild type. FEBS Lett. **54**:286–290.
14. **Cossins, E. A., P. Y. Chan, and G. Combepine.** 1976. One-carbon metabolism in *Neurospora crassa* wild-type and in mutants partially deficient in serine hydroxymethyltransferase. Biochem. J. **160**:305–314.
15. **Cossins, E. A., and S. H. Y. Pang.** 1980. Loss of cytosolic serine hydroxymethyltransferase in a formate mutant of *Neurospora crassa*. Experientia **36**:289–290.
16. **Davis, R. H., and F. J. deSerres.** 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17A**:79–143.
17. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
18. **Ebbole, D. J., J. L. Paluh, M. Plamann, M. S. Sachs, and C. Yanofsky.** 1991. *cpc-1*, the general regulatory gene for genes of amino acid biosynthesis in *Neurospora crassa*, is differentially expressed during the life cycle. Mol. Cell. Biol. **11**:928–934.
19. **Geliebter, J., R. A. Zeff, R. W. Melvold, and S. G. Nathenson.** 1986. Mitotic recombination in germ cells generated two major histocompatibility complex mutant genes shown to be identical by RNA sequence analysis: K^{bm9} and K^{bm6}. Proc. Natl. Acad. Sci. USA **83**:3371–3375.
20. **Harrold, C. E., and M. Fling.** 1952. Two mutants of *Neurospora crassa* which utilize formate or formaldehyde for growth. J. Biol. Chem. **194**:399–406.
21. **Hendrick, J. P., P. E. Hodges, and L. E. Rosenberg.** 1989. Survey of amino-terminal proteolytic cleavage sites in mitochondrial precursor proteins: leader peptides cleaved by two matrix proteases share a three-amino acid motif. Proc. Natl. Acad. Sci. USA **86**:4056–4060.
22. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155**:156–165.
23. **Hilton, J. L., P. C. Kearney, and B. N. Ames.** 1965. Mode of action of the herbicide, 3-amino-1,2,4-triazole (amitrole): inhibition of an enzyme of histidine biosynthesis. Arch. Biochem. Biophys. **112**:544–547.
24. **Hinnebusch, A. G.** 1988. Mechanisms of gene regulation in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:248–273.
25. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
26. **Hope, A. I., and K. Struhl.** 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell **46**:885–894.
27. **Hsu, K. S.** 1965. Acriflavin resistance controlled by chromosomal genes in *Neurospora*. Neurospora Newsl. **8**:4–6.
28. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
29. **Liu, Q., and J. C. Dunlap.** 1988. A rapid and efficient approach for *Neurospora crassa* transformation using low melting point agarose purified DNA. Fungal Genet. Newsl. **35**:24–25.
30. **Loros, J. J., S. A. Denome, and J. C. Dunlap.** 1989. Molecular cloning of genes under the control of the circadian clock in

Neurospora. Science **243**:385–388.

31. **Martini, F., S. Angelaccio, S. Pascarella, D. Barra, F. Bossa, and V. Schirch.** 1987. The primary structure of rabbit liver cytosolic serine hydroxymethyltransferase. J. Biol. Chem. **262**:5499–5509.
32. **Martini, F., B. Maras, P. Tanci, S. Angelaccio, S. Pascarella, D. Barra, F. Bossa, and V. Schirch.** 1989. The primary structure of rabbit liver mitochondrial serine hydroxymethyltransferase. J. Biol. Chem. **264**:8509–8519.
33. **McClung, C. R., B. A. Fox, and J. C. Dunlap.** 1989. The *Neurospora* clock gene *frequency* shares a sequence element with the *Drosophila* clock gene *period*. Nature (London) **339**: 558–562.
34. **McKnight, S. L., and R. Kingsbury.** 1982. Transcription control signals of a eukaryotic protein-coding gene. Science **217**:316–324.
35. **Orbach, M. J., E. B. Porro, and C. Yanofsky.** 1986. Cloning and characterization of the gene for β-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol. Cell. Biol. **6**:2452–2461.
36. **Orbach, M. J., M. S. Sachs, and C. Yanofsky.** 1990. The *Neurospora crassa arg-2* locus. J. Biol. Chem. **265**:10981–10987.
37. **Paluh, J. L., M. J. Orbach, T. L. Legerton, and C. Yanofsky.** 1988. The cross-pathway control gene of *Neurospora crassa*, *cpc-1*, encodes a protein similar to GCN4 of yeast and the DNA-binding domain of the oncogene v-*jun*-encoded protein. Proc. Natl. Acad. Sci. USA **85**:3728–3732.
38. **Paluh, J. L., and C. Yanofsky.** 1991. Characterization of *Neurospora* CPC1, a bZIP DNA-binding protein that does not require aligned heptad leucines for dimerization. Mol. Cell. Biol. **11**:935–944.
39. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
40. **Perkins, D. D., A. Radford, D. Newmeyer, and M. Bjorkman.** 1982. Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. **46**:426–570.
41. **Plamann, M. D., L. T. Stauffer, M. L. Urbanowski, and G. V. Stauffer.** 1983. Complete nucleotide sequence of the *E. coli glyA* gene. Nucleic Acids Res. **11**:2065–2075.
42. **Rossbach, S., and H. Hennecke.** 1991. Identification of *glyA* as a symbiotically essential gene in *Bradyrhizobium japonicum*. Mol. Microbiol. **5**:39–47.
43. **Sachs, M. S., H. Bertrand, R. L. Metzenberg, and U. L. RajBhandary.** 1989. Cytochrome oxidase subunit V gene of *Neurospora crassa*: DNA sequences, chromosomal mapping, and evidence that the *cya-4* locus specifies the structural gene for subunit V. Mol. Cell. Biol. **9**:566–577.
44. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
45. **Schirch, V., D. Schirch, F. Martini, and F. Bossa.** 1986. Serine hydroxymethyltransferase: effect of proteases on the activity and structure of the cytosolic enzyme. Eur. J. Biochem. **161**: 45–49.
46. **Snell, K., Y. Natsumeda, J. N. Eble, J. L. Glover, and G. Weber.** 1988. Enzymic imbalance in serine metabolism in human colon carcinoma and rat sarcoma. Br. J. Cancer **57**:87–90.
47. **Stauffer, G. V., M. D. Plamann, and L. T. Stauffer.** 1981. Construction and expression of hybrid plasmids containing the *Escherichia coli glyA* gene. Gene **14**:63–72.
48. **Steiert, J. G., R. J. Rolfes, H. Zalkin, and G. V. Stauffer.** 1990. Regulation of the *Escherichia coli glyA* gene by the *purR* gene product. J. Bacteriol. **172**:3799–3803.
49. **Sukanya, N., M. Vijaya, H. S. Savithri, A. N. Radhakrishnan, and N. A. Rao.** 1991. Serine hydroxymethyltransferase from mung bean (*Vigna radiata*) is not a pyridoxal-5′-phosphate-dependent enzyme. Plant Physiol. **95**:351–357.
50. **Urbanowski, M. L., L. T. Stauffer, M. D. Plamann, and G. V. Stauffer.** 1984. Cloning and characterization of the gene for *Salmonella typhimurium* serine hydroxymethyltransferase. Gene **27**:47–54.
51. **Viebrock, A., A. Perz, and W. A. Sebald.** 1982. The imported preprotein of the proteolipid subunit of the mitochondrial ATP synthase from *Neurospora crassa*. Molecular cloning and sequencing of the mRNA. EMBO J. **1**:565–571.
52. **Vollmer, S. J., and C. Yanofsky.** 1986. Efficient cloning of genes of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **83**:4869–4873.
53. **von Heijne, G., J. Steppuhn, and R. G. Herrmann.** 1989. Domain structure of mitochondrial and chloroplast targeting peptides. Eur. J. Biochem. **180**:535–545.
54. **Waziri, R., S. Baruah, T. S. Hegwood, and A. D. Sherman.** 1990. Abnormal serine hydroxymethyl transferase activity in the temporal lobes of schizophrenics. Neurosci. Lett. **120**:237–240.
55. **Waziri, R., J. Wilcox, A. D. Sherman, and J. Mott.** 1984. Serine metabolism and psychosis. Psychiatry Res. **12**:121–136.