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Abstract

We report the isolation and identification of a gene encoding GMP synthetase from a *Neurospora crassa* cDNA library. Phage infection of the purine-requiring *Escherichia coli* strain SØ3834 using the NO_3-induced cDNA phage library from the Fungal Genetics Stock Center resulted in colonies able to grow on minimal media with no added purine source. A plasmid, termed pGMPS1, was isolated from one of these colonies and shown to reproducibly support growth of strain SØ3834 in the absence of purines in the media. Identification of this gene as one encoding GMP synthetase is confirmed by DNA sequencing and comparison to the known *guaA* gene from yeast.

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Identification of a gene encoding GMP synthetase from a *Neurospora crassa* cDNA library by bacterial complementation

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We report the isolation and identification of a gene encoding GMP synthetase from a *Neurospora crassa* cDNA library. Phage infection of the purine-requiring *Escherichia coli* strain SØ3834 using the NO_3^- induced cDNA phage library from the Fungal Genetics Stock Center resulted in colonies able to grow on minimal media with no added purine source. A plasmid, termed pGMPS1, was isolated from one of these colonies and shown to reproducibly support growth of strain SØ3834 in the absence of purines in the media. Identification of this gene as one encoding GMP synthetase is confirmed by DNA sequencing and comparison to the known *guaA* gene from yeast.

Nucleotide biosynthetic enzymes are common targets for anti-nucleic acid drug therapy, and deficiencies of the corresponding genes in humans are implicated in a number of inherited metabolic disorders. GMP synthetase (EC 6.3.5.2) has implications in anticancer and immunosuppressive therapies; additionally, this enzyme is used industrially in the semi-enzymatic synthesis of GMP (Fujio *et al.* 1997, *Bioscience, Biotechnology, and Biochemistry* **61**: 840-845). The enzyme is a member of the amidotransferase family, which utilizes glutamine hydrolysis as a source of ammonia for a subsequent enzymatic step, and catalyzes the replacement of O2 in XMP with an amino group to yield GMP.

To extend the collection of GMP synthetase genes from different organisms, we attempted isolation of the gene from *Neurospora crassa* using bacterial complementation. The auxotrophic *Escherichia coli* strain, SØ3834 (Chang *et al.* 1991, *Biochemistry* **30**: 2273-2280), lacks both genes for GMP synthetase (*guaA*) and adenosine deaminase (*add*), and requires a purine source that can be metabolized to GMP for growth. In *guaA*$^-$ *add*$^+$ strains, the purine source can be 2,6-diaminopurine, since this compound can be enzymatically ribosylated and subsequently deaminated by adenosine deaminase to guanosine, but in SØ3834, 2,6-diaminopurine is incapable of providing the purine requirement. Thus, colonies in the complementation screen that arise on media containing 2,6-diaminopurine could contain either the *guaA* or *add* genes, but those that arise on purine-free media could only contain *guaA*.

From colonies arising in a complementation screen of about 10^5 phage [NO_3^- induced cDNA phage library, Fungal Genetics Stock Center; *Fungal Genetics Newsletter*, **45** (Supplement), 8], one colony yielded plasmid DNA from an alkaline lysis preparation that, when reinserted into the host strain, provided the ability to grow on purine-free media. This plasmid was presumed to contain the GMP synthetase gene and was designated pGMPS1. Sequencing of the inserted region within the vector was carried out at the Automated DNA Sequencing Facility, University of North Carolina, Chapel Hill, using the M13 (-20) primer and primers designed from newly acquired sequence information. An open reading frame of 1629 nucleotides was identified; when translated into the amino acid sequence, a high degree of similarity was found with the yeast GMP synthetase gene (duJardin *et al.* 1994, *Gene* **139**:127-132). Of the 543 amino acids from the deduced sequence, 354 (65%) have identical counterparts in the yeast sequence (Figure 1). The high degree of sequence similarity with the yeast GMP synthetase gene and the ability of the gene to support growth of *E. coli* strain SØ3834 in the absence of purines confirm the identification of a GMP synthetase gene from an *N. crassa* library.

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Figure 1. Amino acid sequence comparison of GMP synthetases. Yeast sequence is shown in light type, sequence from *N. crassa* is bold. Vertical dashes between sequences indicate identical amino acids.

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MAA   GEQV SNMFDTILVL DFGSQYSHLI TRRLREFNIY AEMLPCTQKI
MAAATLGEVP TKAFDTILTL DFGSQYTHLI TRRMRELNIL SEMLPCTTKI

SELGWTPKGV ILSGGPYSVY AEDAPHVDHA IFDLNVPILG ICYGMQELAW  97
ADLDYKPKGV ILSGGPYSVY EDGAPHVDPA VFELGVPILG ICYGMQEIAW 100

 INGKQVGRG DKREYGPATL KVI   DDSNS   LFKGMNDS  TVWMSHGDK
RASPENVIAG VHREYGHSNL KALKGDDAHV DRLFAGLEDS MRVWMSHGDK

LHGLPTGYKT IATSDNSPYC GIVHETKPIY GIQFHPEVTH STQGKTLLKN 191
LGALPEGFHT VAVSDNSEYA AIAHKTKPIY GLQFHPEVTH SQNGTQLLKN 200

FAVDLCHAKQ NWTMENFIDT EINRIRKLVG PTAEVIGAVS GGVDSTVASK
FAVDICGCAQ NWTMARFLDQ EIARIRDLVG PEGQVLGAVS GGVDSTVAAK

LMTEAIGDRF HAILVDNGVL RLNEAANVKK TLVEGLGINL MVVDASEEFL 291
LMKEAIGDRF WAVLVNNGVM RLDECEQVER DLKQHLGINL TVIDASKDFL 300

SKLKGVTDPE KKRKIIGNTF IHVFEREAEK IKP    KDGK   EIQFLLQG
EGLKGLHDPE QKRKFIGGKF IDVFEAEAQK IEEAAAKSGK GTKIGFFLQG

TLYPDVIESI SFKGPSQTIK THHNVGGLLE NMK     LKL IEPLRELFKD 382
TLYPDVIESL SFKGPSATIK THHNVGGLPE RMTNGQGLQL IEPLRSLYKD 400

EVRHLGELLG IPHDLVWRHP FPGPGIAIRV LGEVTKEQVE IARKADNIYI
EVRELGRTLG IHEELVMRHP FPGPGIAVRI LGEVTEEKVR IARQADHIFI

EEIKKAGLYN QISQAFACLL PVKSVGVMGD QRTYDQVIAL RAIETTDFMT 482
SEIRKAGLYD QISQAYAAVD PSRAVGVMGD KRVYGYIIIL RAVTTTDFMT 500

ADWFPFEHSF LKKVASRIVN EVDGVARVTY DITSKPPATV EWE        525
AEAFNFPWDF LQRVMNRIVN EVNGVCRVTY DITSKPPGTI ELE        543
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