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MGM101, a nuclear gene involved in maintenance of the mitochondrial genome in *Saccharomyces cerevisiae*

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ABSTRACT

A nuclear mutation, *mgm101*, results in temperature sensitive loss of mitochondrial DNA (mtDNA) in the yeast *Saccharomyces cerevisiae*. The corresponding gene, *MGM101*, was isolated from a genomic DNA library by complementation. Sequence analysis shows that *MGM101* encodes a positively charged protein of 269 amino acids with a calculated molecular weight of 30 kDa. This analysis also reveals that *MGM101* is adjacent to the ribosomal protein gene *RPS7A* on chromosome X and hybridization indicates it occurs in single copy. Creation of a null mutant by targeted disruption showed that the gene has no essential cellular function, aside from its participation in mitochondrial genome maintenance. As no counterpart has been identified in databases it is a novel protein whose role has yet to be determined. Expression of *MGM101* is low on glucose medium but on galactose there is a two-fold increase in the level of the transcript.

INTRODUCTION

Most progress has been made in identifying nuclear encoded genes for mtDNA replication and maintenance in the budding yeast *Saccharomyces cerevisiae*. Mutation of these genes results in loss of mtDNA but is not lethal for the cell. Genes so far identified can be classified into the following groups. The first group includes genes which are directly involved in replication, repair or recombination of mtDNA, such as *MIP1* encoding the catalytic subunit of mtDNA polymerase (1), *RIM1* encoding a single strand DNA binding protein (2) and *PIF1* encoding a mitochondrial DNA helicase (3). The second group consists of genes related to the mitochondrial transcription machinery such as *RPO41* encoding the core enzyme of mitochondrial RNA polymerase (4), and *MTF1* encoding a mitochondrial transcription factor (5). The third group is composed of those genes required for both nuclear and mitochondrial DNA synthesis, such as *CDC21* encoding thymidylate synthetase (6), *CDC8* encoding deoxythymidylate kinase (6), and *RNR2* encoding ribonucleotide reductase (7). The fourth group of genes are ones required for mitochondrial protein synthesis (8). The final group includes those that have not been well defined for the mechanism by which they affect mtDNA maintenance, for example *ABF2* encoding a HMG1-related DNA binding protein (9), *MGM1* encoding a GTP-binding protein (10) and *PPA2* encoding a mitochondrial inorganic pyrophosphatase (11).

Recently we have initiated a study with *S.cerevisiae* to identify additional nuclear genes required for the maintenance of mtDNA. We report here the cloning and sequencing of a gene for Mitochondrial Genome Maintenance, *MGM101*. This gene can be placed in the fifth group as the predicted basic protein of 269 amino acids has no similarity to any sequence lodged in the current databases.

MATERIALS AND METHODS

Strains, media and genetic techniques

Strains of *S.cerevisiae* are listed in Table 1. AH22 and 2262 are the parental strains used for the production of mutants. The strain BJ53-6D is a kind gift from W.Fangman (University of Washington) and contains the *mgm9* mutation which is allelic to *mgm101*-1.

The complete medium for yeast cell growth consists of 0.5% yeast extract Difco (Y), 1% Bacto-Peptone Difco (P), and a carbon source as specified. GYP medium contains Y, P and 2% glucose. GlyYP contains Y, P and 2% glycerol. Minimal medium contains 0.67% Difco Yeast Nitrogen Base (YNB) without amino acids. This medium was supplemented with amino acids at 50 μg/ml or bases at 25 μg/ml to complement auxotrophic requirements.

Standard procedures were used for crossing strains, selecting diploids, inducing sporulation, and dissecting tetrads (12).

Petite colony detection

The petite frequency of a *mgm101*-1 strain at the non-permissive temperature was estimated as follows. Cells were first grown in liquid GlyYP overnight, then diluted in fresh pre-warmed GYP medium to 2×10^6 cells per ml and incubated at 37°C with shaking. At appropriate time intervals samples were taken, diluted and spread on GYP plates that were incubated at 25°C. Petites were scored as white and small colonies and respiratory competent cells as red colonies after 4 days due to the presence of an *ade2* mutation (12).

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Recombinant DNA and microbial techniques

Recombinant plasmid construction, Southern blot analysis, and *E.coli* colony hybridization were performed according to standard techniques (13). Transformation of yeast was done by the DMSO-lithium acetate method (14). The yeast/*E.coli* shuttle vector used for subcloning of *MGM101* was the centromeric plasmid pFL38 (15). The *S.cerevisiae* genomic DNA library used for cloning of *MGM101* was from Cold Spring Harbour Laboratory, constructed as a *Sau*3AI partial digest inserted into the vector YEp13M4. Genomic DNA in the genebank has been prepared from the strain SP1.

Isolation of the MGM101 gene

The *MGM101* gene was isolated by genetic complementation of the *mgm101*-1 temperature sensitive mutation by screening on medium containing the non-fermentable carbon source glycerol. The *mgm101*-1 strain M2915-8C was transformed with the yeast genomic library. Leu$^+$ transformants were selected at 25°C on minimal medium lacking leucine. The transformants were then recovered from the plate surface, pooled and replated onto GlyYP plates which were incubated at 37°C for 7 days. Colonies growing at the non-permissive temperature were subjected to further analysis. Total nucleic acid was extracted from the Mgm$^+$ Leu$^+$ transformants and used to transform *E.coli* TG1 (see ref.13) strain to ampicillin resistance. One of the complementing plasmids, pMGM101/1, which contains a truncated, but functional MGM101 gene (see RESULTS), was obtained in this way.

The *MGM101* 5′ non-coding region was cloned by colony hybridization using as a probe the *Sac*I-*Hin*dIII fragment of 0.65 kb containing part of the *MGM101* coding region and the flanking 3′ non-coding sequence. *E.coli* MC1066 strain (Casadaban, F$^-$, *lacX*74, *hsdR*, *rspL*, *galU*, *galK*, *trpC*9830, *leuB*6, *pyrF*::Tn*5*) was transformed with the yeast genomic library DNA. Approximately 8000 transformants were transferred to Nylon membrane and hybridized with the probe. Three positive clones were obtained. Isolation and characterization of the transforming plasmids indicated that they carried non-identical but overlapping genomic fragments. One of the plasmids, pMGM101-B23, was found to contain the total sequence of *MGM101* including its 5′ non-coding sequence.

DNA sequence analysis

The 1.6 kb *Sac*I-*Bgl*II fragment complementing *mgm101*-1 (carried on the plasmid pMGM101-SBB1, see Fig.1) was subcloned into the polylinker region of pTZ18U and pTZ19U (Pharmacia). Templates for sequencing were obtained either by using DNaseI to create a series of nested deletions from the ends of the yeast DNA insert or by further subcloning after cutting with appropriate restriction enzymes. The nucleotide sequence was determined by the dideoxy chain termination method (16) using a Sequenase 2.0 DNA sequencing kit (U.S.Biochemical Corp.). The deducted *MGM101* protein sequence was compared with sequences in the Swissprot databases by access through the Australian National Genomic Information Service.

Construction of an *mgm101* null allele

A disrupted allele of the *MGM101* gene was constructed by insertion of the 1.1 kb *Bgl*II fragment of pFL38 (15) that encompasses *URA3*, into the unique *Bgl*II site of the plasmid pTM-XS1. pTM-XS1 is a pTZ18U-based plasmid containing the *MGM101* sequence. *Bgl*II lies within the *MGM101* ORF at position +654 from the first ATG codon. Thus a *MGM101::URA3* construct can be isolated as a 2.2 kb *Hin*dIII DNA fragment (see Fig.1) and then introduced into the diploid strain CS4 (*MGM101/MGM101*, *ura3*-52/*ura3*-52) by selecting Ura$^+$ transformants.

RNA analysis and quantification by phosphorimaging

Total RNA from yeast was prepared according to the method described by Schmitt et al. (17). RNA was fractionated by electrophoresis in 1.2% agarose-formaldehyde gels and then transferred to Nylon membrane. After hybridization to ^{32}P-labelled DNA fragments from the *MGM101* and *ACT1* (actinomycin) genes, band intensity was measured by using the phosphorimage analyzer (Molecular Dynamics).

RESULTS

Isolation of the *mgm101*-1 mutant

An EMS (ethyl methane sulfonate) mutagenesis program has been undertaken with haploid strains AH22 and 2262 (M.X.Guan, Ph.D thesis submitted). Briefly, after EMS treatment, mutants showing temperature sensitive growth phenotype on GlyYP were retained and subsequently examined for loss of mtDNA at 37°C (but not at 25°C) by colony hybridization. Candidates exhibiting temperature sensitive loss of mtDNA were then subjected to genetic analysis. One of these mutants, *mgm101*-1, which shows a typical *mgm* phenotype at the non-permissive temperature, was selected for further examination.

The *mgm101*-1 mutation was purified from the initial isolate AH22/M29 after several rounds of meiosis by crossing to a wild type strain. One of the meiotic segregants, M2911-13D, showed the following characteristics: (1) it grows on GlyYP at 25°C but not at 37°C; (2) it grows on GYP medium at both 25°C and 37°C; (3) growth at 37°C in GYP medium leads to the complete loss of mtDNA; (4) when crossed to a wild type strain the temperature sensitive phenotype segregates 2$^+$:2$^-$ in 28 tetrads analysed and correlates with the loss of mtDNA (data not shown), indicating the presence of a single nuclear mutation. We designated this mutation as *mgm101*-1 and *MGM101* for the wild-type gene. As a *mgm101*-1/*MGM101* diploid grows on GlyYP at 37°C, *mgm101*-1 is therefore a recessive mutation.

When a *mgm101*-1 strain was crossed to BJ53-6D containing the mutation *mgm9*, which was isolated independently in W.Fangman's laboratory (University of Washington), the diploid

Table 1. Yeast strains used in this study

Strain	Genotype	Reference or source
AH22	*a, leu2*-3, *leu2*-11, *his4*	G.Fink
2262	*α, ade1, his5, leu2, lys11, ura1*	L.Hartwell
AH22/M29	*a, leu2*-3, *leu2*-11, *his4, mgm101*-1^{ts}, glu^{ts}*	This study
M2911-13D	*α, leu2, his4, mgm101*-1^{ts}	This study
M2915-8C	*α, leu2, his4, ade2, lys2, mgm101*-1^{ts}	This study
M2915-8A	*a, leu2, ade2, ade3, ura3*	This study
CS4	*α/a, leu2/leu2, his4/+, ura3/ura3, +/ade2, +/ade3*	This study
BJ53-6D	*a, ade1, ade2, ura3*-52, *leu2, tyr1, trp1, lys2, his7, mgm9*^{ts}	W.Fangman

*Aside from the *mgm101*-1 mutation, the original isolate AH22/M29 contains an additional mutation which confers temperature sensitive growth on glucose medium.

failed to grow on GlyYP at 37°C. The lack of complementation in this test indicates that *mgm101*-1 is allelic to *mgm9*.

Production of petite mutants (gly⁻ phenotype) in a *mgm101*-1 culture at the restrictive temperature is shown in Table 2. During the first two hours incubation at 37°C there is no significantly increase in petite formation. However after five hours petite mutants appeared and by nine hours the culture was totally converted. Samples of the culture transferred from 25° to 37°C were also stained with DAPI. The loss of chondriolites was observed from 4 hours and prolonged culture caused complete disappearance of chondriolites (data not shown).

Cloning of the *MGM101* gene

MGM101 was cloned by transforming M2915-8C, a strain carrying the temperature sensitive *mgm101*-1 mutation, with a yeast genomic library and selecting first for Leu⁺ transformants. The Leu⁺ transformants were then replated on GlyYP and tested for growth at 37°C. One plasmid, pMGM101/1, recovered from a Leu⁺ Gly⁺ colony, was found to complement *mgm101*-1 when it was reintroduced into M2915-8C. Also pMGM101/1 complemented the *mgm9* mutation in the strain BJ53-6D which is allelic to *mgm101*-1. pMGM101/1 contains a 4.7 kb *Sau*3AI genomic insert. As revealed by sequence analysis (see below), pMGM101/1 contains the total coding sequence of *MGM101* but lacks the 5′ non-coding region. To obtain a plasmid containing this region we have screened the *S.cerevisiae* genebank by colony hybridization using a fragment containing the *MGM101* coding sequence as probe. One such plasmid, pMGM101-B23, was identified. The physical maps of pMGM101/1 and pMGM101-B23 are illustrated in Fig.1.

Table 2. Rate of petite colony formation in the *mgm101*-1 mutant M2915-8C grown at the restrictive temperature

Hours at 37°C	0	2	5	7	9	11
Petite frequency (%)	0.66	0.92	6.4	83.3	99.4	100

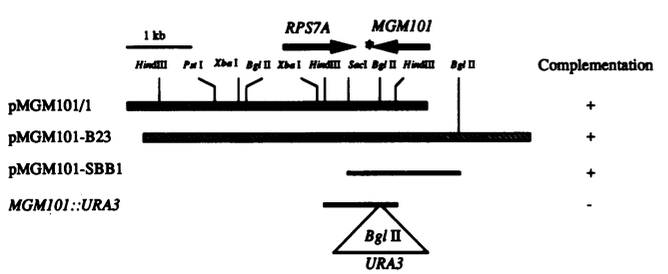

Figure 1. Maps of *MGM101* showing restriction endonuclease sites. The clone containing *MGM101* overlapped with clones containing *RPS7A* (20) and the telomere-like poly(CA) tract (in the intergenic region between *RPS7A* and *MGM101*, represented by *, 21) on chromosome X. The direction of transcription of *RPS7A* and *MGM101* are indicated as arrows on the top of the figure. pMGM101/1 and pMGM101-B23 are the original clones isolated from the gene bank (see text), and only the yeast DNA segments (hatched bars) are shown. pMGM101-SBB1 contains the complementing fragment analysed by nucleotide sequencing. *The MGM101::URA3* construct represents the DNA fragment used for creating the *MGM101* insertion-disrupted allele.

To delimit the *MGM101* gene in pMGM101/1 and pMGM101-B23, we subcloned fragments in the centromeric vector pFL38 (15) and tested their ability to complement *mgm101*-1. The results suggested that the gene lies in a 1.6 kb *Sac*I-*Bgl*II fragment (see the plasmid pMGM101-SBB1, Fig.1). The initial complementing plasmid pMGM101/1 and one of the subclones, pMGM101-P1, are truncated at the position −12 (from the first ATG codon of *MGM101*) and do not contain the *MGM101* promoter sequence (see below), but they were still able to complement *mgm101*-1. We suggest that an undefined promoter activity upstream from the truncated *MGM101* gene has allowed its expression. A promoter activity residing in the 3′ sequence of the *lacZ′* gene of pUC19-derived plasmid has been previously observed (18). This cryptic promoter was by chance correctly fused to the *MGM101* ORF in the pMGM101/1 and pMGM101-P1 plasmids and functioned in opposite orientation to *lacZ′*.

Nucleotide sequence of *MGM101*

The nucleotide sequence of the 1.6 kb *Sac*I-*Bgl*II fragment, which complements *mgm101*-1, was determined and is lodged with the EMBL-database (acquisition number X68482). Translation of the sequence in all six possible reading frames revealed a single large ORF capable of encoding a putative 269 amino acid polypeptide with a molecular weight of 30,087 daltons (Fig.2). A codon bias index of 0.18, calculated according to Bennetzen and Hall (19), suggests that *MGM101* is a weakly expressed gene. The C-terminal sequence and the 3′ non-coding region of *MGM101* overlaps with the published sequence for the 3′ non-coding region of the ribosomal protein gene *RPS7A* (20). *RPS7A* has been mapped on the right arm of chromosome X, distal to *CDC11* (20). The chromosomal organization of *MGM101* and *RPS7A* is illustrated in Fig.1. The two genes are transcribed in opposite orientation. When the total DNA from *S.cerevisiae* was digested with *Hin*dIII and hybridized to a probe containing the *MGM101* coding region, only one band of 1.1 kb was detected (data not shown), indicating that *MGM101* is a unique gene. We note that the 335 bp intergenic sequence between *MGM101* and *RPS7A* contains a poly(CA) tract. The same piece of chromosomal DNA has been identified as a telomere-like sequence by Walmsley et al. (21).

The predicted amino acid sequence of *MGM101* was compared with sequences in the Swissprot databases. No significant homology was found. The protein is rich in basic residues (17.84%), with a calculated isoelectric point of 9.5. The amino terminal amino acid sequence is rich in positively charged residues and hydroxylated amino acids. These characteristics

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MKSIFKVRGCVSHAAQFCQKRTVVSTGTSNTATAGAVRKS  40
FNSTETKPVFATKSEAGNGSHMKEYSSGINSKLGGTPLET  80
RSTADDSLNNSYKQVKGDIDWYTSWYGLGMKPFEAKVQKD 120
LIEPLDPKDIEIKPDGLIYLPEIKYRRILNKAFGAGGWGL 160
VPRSQTIVTSKLVTREYGLICHGQLISVARGEQDYFNEAG 200
IPTATEGCKSNALMRCCKDLGVGSELWDPVFIKKFKVDHC 240
TEKFVEHVTTKRKKKIWLRKDRQVEYPYK             269
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Figure 2. Deduced amino acid sequence of the *MGM101* gene. The sequence of the 1.6 kb *Bgl*II-*Sac*I fragment (Fig.1) has been transmitted to the EMBL Data Library (accession number X68482).

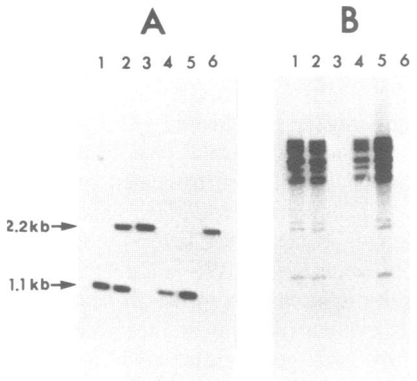

Figure 3. Southern blot analysis of total DNA from the diploid strains CS4 (*MGM101/MGM101*, lane 1), CS6 (*MGM101/MGM101::URA3*, a *MGM101*-disrupted transformant of CS4, lane 2) and the progeny of a tetratype ascus of CS6 (lanes 3–6). **(A)** Genomic DNAs were digested with *Hin*dIII and after electrophoresis, DNA fragments were transferred to Nylon membrane and hybridized to the ^{32}P-labelled DNA containing the *MGM101* sequence (0.65 kb *Sac*I-*Hin*dIII fragment). **(B)** Total DNA was digested with *Cfo*I and probed with ^{32}P-labelled total yeast mitochondrial DNA. In the two spores harbouring the disrupted *MGM101* allele (lanes 3 and 6), the mitochondrial genome was lost and no hybridization was detected (26).

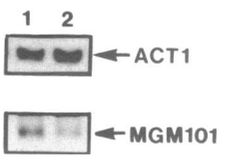

Figure 4. Northern blot analysis showing steady state levels of *MGM101* transcripts grown on different carbon sources. Total RNA was isolated from M2915-8A cells, that have been grown to exponential phase in complete medium containing 2% glucose or 3% galactose. RNA was size-fractionated on a 1.2% agarose gel, blotted onto Hybond-N and hybridized to ^{32}P-labelled probes. The probe specific for *MGM101* was a 416 bp *Hin*dIII-*Sph*I fragment corresponding to the positions from +36 to +452 of the *MGM101* coding region. The actin probe used to assess whether comparable amounts of RNA were loaded in each slot was a 0.6 kb fragment containing *S.cerevisiae ACT1* sequence (a gift from P.Stepien). Lane 1, RNA from cells grown in the presence of galactose; lane 2, RNA from cells grown in the presence of glucose. The exposure time was 1.5 hour and 24 hours for detection of *ACT1* and *MGM101* signals respectively.

together with a lack of acidic amino acid residues, suggests that the amino terminal region is a mitochondrial targeting presequence (for review, see ref.22). The C-terminal region is rich in charged residues (15 basic and 5 acidic out of 37 residues). This hydrophilic C-terminal region seems to be essential for *MGM101* function as truncation by the insertion of the *URA3* gene at the *Bgl*II site (at residue 218, nucleotide position +654 from the first ATG translation start codon) leads to loss of mtDNA (see below).

Inter-species conservation of MGM101

No cross hybridization of the *MGM101* sequence was found to DNA from *Kluyveromyces lactis, Dekkera bruxellensis* or *Schizosaccharomyces pombe* even under low-stringency (hybridizing in 3×SSC at 54°C for 18 h and washing with 2×SSC at room temperature). This result indicates either that an equivalent gene does not exist in these species or else the sequence is not conserved.

Disruption of *MGM101*

To confirm that the cloned DNA fragment complementing the *mgm101* mutation contains the authentic *MGM101* gene, we disrupted the gene in vitro by introduction of a selective marker into the coding region. The disruption, *MGM101:URA3* in which the *URA3* gene was inserted into the *Bgl*II site located at the C-terminal region of the putative *MGM101* protein (see Fig.1), was then introduced into the diploid yeast strain CS4 by one-step gene transplacement (23), selecting for uracil independence. Disruption was verified by Southern blot analysis. Total DNA from yeast transformants (Fig.3A, lane 2) was digested with *Hin*dIII and hybridized with the ^{32}P-labelled *MGM101* probe. Two copies of *MGM101* were found, one representing the intact *MGM101* gene (1.1 kb) and the other the *MGM101::URA3* construct (2.2 kb).

A *MGM101*-disrupted diploid (CS6) was allowed to undergo sporulation, and tetrad analysis was performed. Of the 8 tetrads analysed, all had four viable spores, indicating that the *MGM101* gene is not essential for mitotic viability. The *URA3* gene segregated $2^+:2^-$. All Ura^+ segregants showed slow growth on glucose medium and no growth on glycerol. Southern blot analysis of progeny from a tetrad showed that the two Gly^- clones have a disrupted copy of *MGM101* (Fig.3A, lanes 3 and 6) and have lost the mitochondrial genome (Fig.3B, lanes 3 and 6) whereas the other two Gly^+ spores have the wild-type configuration of *MGM101* and retain mtDNA (Fig.3A and 3B, lanes 4 and 5).

A *MGM101::URA3* strain was crossed to the strain M2915-8C carrying the ts *mgm101*-1 mutation. The resulting diploid strain, CS11, showed a ts phenotype for growth on glycerol. The diploid strain was sporulated at the permissive temperature and tetrad analysis was performed. From the 15 tetrads analysed, two of the spores were Ura^+ and Gly^- at both 25°C and 37°C and the other two showed the ts growth phenotype on glycerol medium. Thus we conclude that the DNA fragment complementing the *mgm101*-1 mutation contains the *MGM101* gene rather than a suppressor.

Identification of the *MGM101* transcript

Northern analysis was performed to determine the size and abundance of the yeast *MGM101* mRNA. Total RNA was prepared from exponentially growing cells in complete medium containing glucose or galactose. The ^{32}P-labelled *MGM101* probe detected a single mRNA of approximately 0.9 kb (not illustrated). The size of mRNA is in good agreement with the length of the *MGM101* open reading frame (807 nucleotides). The steady state level of *MGM101* mRNA is low in wild-type cells grown in galactose, comprising approximately 2–5% of the actin gene transcript as quantified by phosphorimaging (Fig.4). Quantification also indicated that transcription of *MGM101* is repressed by glucose. In cells grown in medium containing galactose, the level of transcription is two-fold higher than in cells grown in glucose medium (Fig.4).

DISCUSSION

By targeted disruption we have shown that the *MGM101* gene is required for maintenance of the mitochondrial genome in yeast. Furthermore *MGM101* is likely to be a specific gene for mitochondrial function as characteristic features of the N-terminal presequence predict its localization in mitochondria and no additional phenotype was observed in the *MGM101* disruptant.

MGM101 is a weakly expressed gene with the mRNA being present at only 2–5% of actin mRNA. These observations are in accord with the codon bias index of 0.18 that indicates a weakly to moderately expressed gene (19). Transcription of *MGM101* is repressed 2-fold on glucose medium. Though a CCAAT motif is present at position −386 of the putative *MGM101* promoter, we do not know whether it is involved in the activation of transcription.

The mechanism by which *MGM101* affects mtDNA maintenance is unknown. The amino acid sequence of *MGM101* does not show homology with sequences of known proteins nor does the amino acid sequence contain any motif that might suggest a function. However the basic property of the *MGM101* protein suggests that it could interact with nucleic acids.

During this study, a nuclear mutant termed *mgm9*, affected in mitochondrial genome maintenance was isolated in the laboratory of W.Fangman. This mutant was shown to be allelic to *mgm101* described in this paper.

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