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Cloning of *GLN4*: an Essential Gene That Encodes Glutaminyl-tRNASynthetase in *Saccharomyces cerevisiae*

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The structural gene for glutaminyl-tRNA synthetase has been isolated from a gene bank of *Saccharomyces cerevisiae* chromosomal DNA. Cloning was achieved by complementation of a recently described yeast strain that is auxotrophic for glutamine. A multicopy recombinant plasmid with a 5-kilobase-pair genomic insert conferred sixfold elevation in glutaminyl-tRNA synthetase activity and restored a Gln^+ phenotype to strains that were Gln^- by virtue of a mutant *gln4* allele. Subfragments of the 5-kilobase insert directed integration of *URA3* to *GLN4*. Further experiments established that *GLN4* is an essential gene that is located on chromosome XV. RNA blots with a *GLN4*-specific probe detected a single transcript of approximately 2,900 nucleotides.

The availability of cloned genes coding for aminoacyl-tRNA synthetases has contributed to our understanding of their enzymatic and regulatory properties. Partial deletions of *alaS* cloned from *Escherichia coli* have led to a definition of the various functional domains of alanyl-tRNA synthetase (10). Availability of a cloned aminoacyl-tRNA synthetase gene also allows functional manipulation of the level of aminoacylation of the cognate tRNA species. These manipulations can be useful for determining the role of aminoacylated tRNA in the regulation of specific bacterial and eucaryotic genes (19).

Structural genes for two eucaryotic aminoacyl-tRNA synthetases have been cloned. *ILS1* and *MES1*, encoding isoleucyl- and methionyl-tRNA synthetases from the yeast *Saccharomyces cerevisiae*, were both isolated by complementation of strains bearing temperature-sensitive defects at the respective alleles (7, 12). Isolation of other aminoacyl-tRNA synthetase genes in *S. cerevisiae* has been hindered by a lack of functionally altered alleles whose activity can be complemented by transformation.

We have recently reported the isolation and characterization of a yeast strain which has reduced levels of glutaminyl-tRNA synthetase (Gln-tRNA synthetase) activity (13). This reduction is due to an altered Gln-tRNA synthetase which has a high K_m for glutamine. When the allele responsible for this alteration is coupled with a variant allele of *GLN1* which produces an altered form of glutamine synthetase with reduced activity, an absolute requirement of glutamine for growth results; in the same genetic background, neither allele alone produces such an effect.

We report the isolation of *GLN4*, the structural gene for Gln-tRNA synthetase, by complementation with the auxotrophy of a strain which bears mutations at both *GLN1* and *GLN4*. The cloned gene is shown to be *GLN4* by its ability to overproduce Gln-tRNA synthetase activity in strains which harbor this gene on an autonomously replicating plasmid and by its ability to direct integration of *URA3* to *GLN4*. We have also used the cloned gene to map *GLN4* to chromosome XV and to identify its transcript.

MATERIALS AND METHODS

Strains. A complete list of the yeast strains used in these studies is given in Table 1. Strains bearing the *gln1-105* or *gln4-1* mutations are derivatives of AM483-5B, previously described by Mitchell and Ludmerer (13). Such strains used in these studies are the result of standard genetic manipulations with transformable strains bearing either the *ura3-52* or the *leu2-3 leu2-112* mutation. Genotypes cited throughout the remainder of this work are those relevant to the given experiment. Yeast growth media were prepared and tetrad analysis was done as described in the Cold Spring Harbor yeast manual (17). Where appropriate, medium was supplemented with 2 g of glutamine per liter.

E. coli HB101, which was used for transformation and plasmid purification, was grown in LB medium, which contains 0.5% yeast extract (Difco Laboratories), 0.5% NaCl, and 1% tryptone (Difco); where appropriate, ampicillin (100 μg/ml) was added. Transformation was done by the method of Dagert and Ehrlich (5).

Yeast transformation and DNA purification. Yeast strains were transformed by the lithium acetate procedure of Ito et al. (9). Plasmid and chromosomal DNA purifications were done as described in the Cold Spring Harbor yeast manual (17).

The following vectors were used: YEp13 (3), YEp24 (2), YIp5 (20), and pBR322 (1). The yeast genomic library constructed in YEp13 was obtained from W. Courchesne, Massachusetts Institute of Technology, Cambridge, Mass. (15).

RNA isolation. Total RNA from AM644-2B(pSWL203) was isolated from yeast cells grown in minimal medium supplemented only with methionine to a concentration of 20 μg/ml. Cells were grown to a Klett reading of 100 and broken with glass beads, and RNA was isolated by the method of Carlson and Botstein (4). Total RNA from S288C was a gift from P. Drain, Massachusetts Institute of Technology.

Gel transfer hybridization. DNA fragments were separated on 0.8% agarose gels. RNA transcripts were separated on 1.5% agarose–3% formaldehyde gels. Nucleic acids were transferred onto nitrocellulose (BA85; 0.45 μm; Schleicher & Schuell, Inc.) as described by Maniatis et al. (11).

Hybridization buffer for DNA contained 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 20 mM sodium phosphate (pH 7.0), 100 μg of denatured salmon sperm DNA per ml, and 0.1% each bovine serum albumin, Ficoll, polyvinylpyrrolidone, and sodium dodecyl sulfate. Hybridizations were performed at 65°C for 16 h.

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TABLE 1. Strains used in these studies

Strain[a]	Genotype	Source
AM644-2B	a *met13-25 ura3-52 gln1-105 gln4-1*	A. P. Mitchell
AM644-2B(pSWL203)	AM644-2B bearing pSWL203	Present work
AM644-11D	a *met13-25 ura3-52 gln1-105*	A. P. Mitchell
JT1399	α *ade2 ura3-52 lys2-801*	J. Thomas
K417	α *ade2-101 ura3-52 trp1-1 his3-200*	K. Struhl
KY419	a/α *ade2-101 ura3-52 trp1-1 lys2-801 his3-200* / *ade2-101 ura3-52 trp1-1 lys2-801 his3-200*	K. Struhl
SWL206-1	a *leu2-1,112 gln1-105 gln4-1*	S. W. Ludmerer
SWL207-1	α *lys2(oc) ura3-52 gln1-105*	S. W. Ludmerer
SWL280-1	a *met13-25 ura3-52 gln1-105 gln4-1*::pSWL204	Present work
SWL281-1	a *met13-25 ura3-52 gln1-105 gln4-1*::pSWL205	Present work
SWL285	a *met13-25 ura3-52 gln1-105 gln4-1*::pSWL203	Present work
SWL301	a *GLN4*::pSWL210 *ura3-52 ade2-101 trp1-1 lys2-801 his3-200* / α *GLN4 ura3-52 ade2-101 trp1-1 lys2-801 his3-200*	Present work
SWL301-1A	a *ura3-52 ade2-101 trp1-1 lys2-801 his3-200*	Present work
SWL301-1B	α *ura3-52 ade2-101 trp1-1 lys2-801 his3-200*	Present work
SWL340	a *met13-25 ura3-52* + *gln1-105 gln4-1*::pSWL203 / α + *ura3-52 ade2* + +	SWL285 × JT1399
SWL341	a *met13-25 ura3-52* + *gln1-105 gln4-1*::pSWL203 + + + / α + *ura3-52 his3-200* + + *trp1-1 ade2-101 lys2-801*	SWL285 × KY417

[a] Additional strains used: SWL280-1 and SWL280-2 are independently isolated integrants of pSWL204 in AM644-2B; SWL281-1 and SWL281-2 are independently isolated integrants of pSWL205 in AM644-2B; SWL301, SWL302, SWL303, and SWL304 are four independent isolates of pSWL210 integrants of KY419; SWL302-1A and SWL302-1B are two spores from the same tetrad whose genotypes are identical to that of SWL301-1A.

Hybridization buffer for RNA contained 50% formamide and 5× SSC; all other components were the same as those used in the DNA hybridizations. Hybridizations were done at 37°C for 2 days.

All filters were washed in various concentrations of SSC and visualized with Kodak AR X-ray film with a Dupont Cronex screen at −80°C for 1 to 2 days.

DNA probes. Probes were made from mp8-containing inserts as described by Hsu and Schimmel (8). Reaction mixtures were passed through a Sephadex G-75 column to separate the high-molecular-weight material from the unincorporated nucleotides. The high-molecular-weight fractions were pooled for use as probes.

Cell extract preparation and aminoacyl-tRNA synthetase assays. Cell growth, extract preparation, and aminoacyl-tRNA synthetase assays were done as described by Mitchell and Ludmerer (13). A single batch of L-[^{3}H]glutamine (Amersham Corp.) was used for the determinations shown in any one table to avoid variability due to differences between batches of glutamine. Values were normalized to the wild-type control used in the given set of assays. A normalized value of 1 corresponds to a rate of approximately 20 pmol of glutamine converted to trichloroacetic acid-precipitable material by 1 mg of crude extract protein per min in the assay system described earlier (13).

Miscellaneous. Restriction endonucleases, *E. coli* DNA polymerase I, and T4 DNA ligase were purchaed from New England Biolabs and used as recommended by the supplier. *E. coli* DNA polymerase I large fragment (Klenow enzyme) was obtained from Boehringer Mannheim Chemicals. [α-^{32}P]dATP was obtained from Amersham Corp. at a specific activity of >400 Ci/mmol. L-[^{3}H]glutamine was purchased from Amersham Corp. Specific activity varied from batch to batch with a range from 22 to 42 Ci/mmol.

RESULTS

Isolation of *GLN4*. Strain SWL206-1 (*leu2-1 gln1-105 gln4-1*) was transformed to leucine prototrophy with a plasmid gene bank which consists of haploid wild-type strain S288C chromosomal DNA partially digested with *Sau*3A and ligated into the *LEU2* vector YEp13 (16). From 5,000 Leu$^+$ transformants screened for a Gln$^+$ phenotype, one transformant, harboring plasmid pSWL201, was judged to contain the *GLN4* gene by the following criteria. (i) Plasmid loss can be induced by growth of the transformant in nonselective media. Loss of the plasmid was detected by a reversion to a Leu$^-$ phenotype. Screening of such Leu$^-$ revertants showed that they had also acquired a glutamine deficiency. No Gln$^-$ revertant was identified which was not also Leu$^-$. This result shows that the abilities to complement the leucine and glutamine deficiencies are linked and plasmid borne. (ii) Back transformation of SWL206-1 with pSWL201 gave Leu$^+$ Gln$^+$ transformants at a frequency of 200 trans-

TABLE 2. Effect of pSWL203 on Gln-tRNA synthetase activity

Strain	Relevant genotype	Gln-tRNA synthetase activity[a]
AM644-11D	*GLN4*	1.0
AM644-2B	*gln4-1*	ND[b]
AM644-2B(pSWL203)	*gln4-1*::pSWL203	6.0

[a] Exponential cultures growing on minimal media supplemented with glutamine, methionine, and uracil [no uracil in the case of AM644-2B(pSWL203)] were harvested, extracted, and assayed for Gln-tRNA synthetase as described previously (12). Activities are normalized to the level of a wild-type control (AM644-11D) as described in the text.

[b] ND, Not detectable.

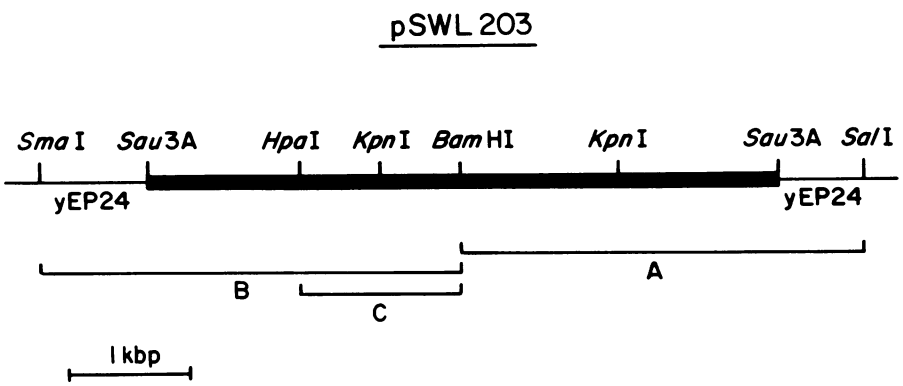

FIG. 1. Recombinant plasmid pSWL203. The plasmid was constructed by partial *Sau*3A digestion of pSWL201 (isolated from a yeast gene bank) and religation into the *Bam*HI site of YEp24 (2). No *Bam*HI site was created on either end of the insertion. Major restriction sites are shown. The bracketed letters indicate regions used in further subcloning.

formants per μg of plasmid DNA. The frequency of Leu^+ transformants was the same as that observed in a control transformation where a plasmid pool of a YEp13 genomic library (15) was used in place of pSWL201. (iii) Though a plasmid harboring *GLN1* would also give a Gln^+ phenotype, it should not overproduce the Gln-tRNA synthetase activity. When harboring pSWL201, haploid strain SWL206-1 has six times the Gln-tRNA synthetase activity of a wild-type strain; in contrast, untransformed strain SWL206-1 has no detectable activity under the conditions of the assay.

Plasmid pSWL201 contains a 10-kilobase-pair (kbp) insert of yeast chromosomal DNA. The plasmid was partially digested with *Sau*3A, and fragments of 4 to 6 kbp were isolated by gel electrophoresis and religated into the *Bam*HI site of the *URA3*-containing plasmid YEp24 (2). Transformation of AM644-2B (*gln1-105 gln4-1 ura3-52*) to a Ura^+ Gln^+ phenotype with the YEp24 recombinant plasmids led to isolation of plasmid pSWL203. This plasmid contains a 5-kbp insert and confers a sixfold elevation of Gln-tRNA synthetase activity relative to a wild-type strain. Table 2 shows typical activities for a set of such experiments.

***GLN4* is a single-copy gene which is essential for cell viability.** Shown in Fig. 1 is a restriction map of the insert of pSWL203. Table 3 lists the regions of *GLN4*, shown in Fig. 1, which were subcloned into various vectors for further studies.

We previously observed that *GLN4* and *URA3* are unlinked. Plasmids pSWL204 and pSWL205, which contain fragments A and B, respectively, of pSWL203 (Fig. 1), both direct integration of *URA3* to *GLN4*. Only pSWL205, which contains the *Sma*I-*Bam*HI fragment of pSWL203, is able to complement the Gln^- phenotype of haploid strain AM644-2B (Tables 4 and 5). This indicates that the *gln4-1* mutation lies in the region encoded by fragment B. Because the *gln4-1* mutation causes a K_m defect for glutamine in Gln-tRNA synthetases (13), fragment B is likely to span much of the coding region for this enzyme. Furthermore, Gln^+ strains resulting from such an integration event have a wild-type level of Gln-tRNA synthetase activity (data not shown). We decided, therefore, to focus on this region in constructing a suitable plasmid for a gene disruption.

Integrating plasmid pSWL210, which contains the *Hpa*I-*Bam*HI subfragment of *GLN4*, was used to transform diploid strain KY419 (*ura3-52/ura3-52 GLN4/GLN4*) to uracil prototrophy. Integration was directed to the *GLN4* locus by digestion of pSWL210 with *Kpn*I before transformation. Four independent Ura^+ transformants were randomly selected for further analysis.

Each Ura^+ phenotype was judged to be stably inherited because no Ura^- derivatives were observed among 100 clonal derivatives screened after more than 20 generations of growth. The four diploid strains were sporulated, and the results of tetrad analysis are given in Table 6. No diploid gave more than two viable spores per tetrad; every viable spore was Ura^- in phenotype. This result is expected if integration of *URA3* causes a gene disruption at *GLN4* and an intact allele is necessary for cell viability. Integration was confirmed by Southern analysis of chromosomal DNA prepared from parent diploid strain KY419, integrant diploid strain SWL302, and the two viable haploid spores of a single tetrad of SWL302 (Fig. 2).

A single band was detected when a *Bam*HI digest of chromosomal DNA from a *GLN4/GLN4* diploid strain was

TABLE 3. Plasmids constructed during the course of this work[a]

Plasmid	Fragment	Vector	Site
pSWL204	A	YIp5	*Bam*HI-*Sal*I
pSWL205	B	YIp5	*Bam*HI-*Nru*I
pSWL208	C	pBR322	*Pvu*II
pSWL210	C	YIp5	*Bam*HI-*Nru*I
pSWL211	C	mp8	*Bam*HI-*Sma*I
pSWL212	C	mp8	*Bam*HI-*Hin*cII

[a] Recombinant plasmid pSWL203 served as a parent vector for subcloning. A restriction map of pSWL203, showing the areas used for subcloning, is given in Fig. 1. Details of subcloning are described in the text.

TABLE 4. pSWL204 directs *URA3* to *GLN4*

Diploid strain	Relevant genotype			No. of the following tetrads[a]		
				PD	NPD	T
SWL280-1 × SWL207-1	*ura3-52* / *ura3-52*	*gln1-105* / *gln1-105*	*gln4-1*::pSWL204 / *GLN4*	10	0	0
SWL280-2 × SWL207-1	*ura3-52* / *ura3-52*	*gln1-105* / *gln1-105*	*gln4-1*::pSWL204 / *GLN4*	4	0	0

[a] A parental ditype (PD) is defined as 2 Ura^+ Gln^-:2 Ura^- Gln^+ segregation. A nonparental ditype (NPD) is defined as 2 Ura^+ Gln^+:2 Ura^- Gln^- segregation. Haploid strains SWL280-1 and SWL280-2 are Gln^- in phenotype. Haploid strain SWL207-1 is Gln^+ in phenotype. T, Tetratype.

TABLE 5. pSWL205 restores a stable Gln$^+$ phenotype segregating with the *gln4-1* allele

Diploid	Relevant genotype	No. of tetrads with the following phenotypic ratio among spores[a]				
		0:4	1:3	2:2	3:1	4:0
SWL281-1×SWL207-1	*ura3-52* *gln1-105* *gln4-1*::pSWL205 / *ura3-52* *gln1-105* *GLN4*	0	0	0	0	10
SWL281-2×SWL207-1	*ura3-52* *gln1-105* *gln4-1*::pSWL205 / *ura3-52* *gln1-105* *GLN4*	0	0	0	0	10

[a] Phenotypic ratio, Gln$^+$ to Gln$^-$. Meiotic tetrads from sporulated diploids were dissected by micromanipulation, and the spore phenotypes were analyzed by replica plating. Haploid strains SWL281-1, SWL281-2, and SWLI207-1 are all Gln$^+$ in phenotype. Ten of 10 tetrads were 2 Ura$^+$:2 Ura$^-$.

probed with pSWL211 (Table 3). Because *Bam*HI is an internal restriction site of *GLN4*, this indicates that in a wild-type strain, *GLN4* is not duplicated and there are no other chromosomal regions of high homology to *GLN4*. In contrast, *Bam*HI-digested chromosomal DNA of the Ura$^+$ diploid SWL302 displayed an additional band migrating at 7.0 kbp. This is the expected position of the pSWL210 plasmid liberated by *Bam*HI digestion.

We have previously showed that haploid and diploid strains have equal specific activities of Gln-tRNA synthetase in crude extract preparations (12). Diploid strains of heterozygous genetic composition *GLN4/gln4-1* have a specific activity which is intermediate between those of *GLN4/GLN4* and *gln4-1/gln4-1* diploid strains. Ura$^+$ diploid strains SWL301 and SWL302 (*ura3-52/ura3-52 GLN4/GLN4*::pSWL210) exhibited half the specific activity of parent diploid strain KY419 (*ura3-52/ura3-52 GLN4/GLN4*) (Table 7). Furthermore, haploid segregants showed a level of activity equal to that of the parent diploid strain KY419.

We conclude that the integration of pSWL210 at *GLN4* creates a disrupted allele which is incapable of supporting growth, suggesting that there is a single *GLN4* allele in a haploid strain.

***GLN4* encodes a single transcript.** Identical amounts of total RNA from strain AM644-2B/pSWL203 were electrophoresed in two separate wells of an agarose-formaldehyde gel and transferred onto a nitrocellulose filter. The filter was cut in half and hybridized to probes isolated from pSWL211 and pSWL212. These probes correspond to opposite strands of the *Hpa*I-*Bam*HI fragment of pSWL203. Only probe pSWL211 showed strong hybridization to a single position on the filter (data not shown). This indicates that the direction of transcription is from *Hpa*I to *Bam*HI (Fig. 1). Hybridization of pSWL211 to total RNA isolated from wild-type strain S288C also produced a single band (Fig. 3). The migration of the *GLN4* mRNA, relative to the 35S and 18S ribosomal RNAs, suggests that *GLN4* mRNA is approximately 2,900 nucleotides in length.

Chromosomal mapping of *GLN4*. *GLN4* was mapped to chromosome XV by the 2μm mapping technique of Falco and Botstein (6). This map position was confirmed and

TABLE 6. Integration of pSWL210 at *GLN4*: Ura$^+$ segregates with a lethal phenotype

Diploid strain[a]	No. of tetrads with the following viability ratio[b]:				
	0:4	1:3	2:2	3:1	4:0
SWL301	0	2	11	0	0
SWL302	0	1	9	0	0
SWL303	0	2	10	0	0
SWL304	0	1	10	0	0

[a] Diploids (all Ura$^+$ in this table) have the relevant genotype *ura3-52/ura3-52 GLN4*::pSWL210/*GLN4*. No haploid Ura$^+$ segregants were produced by any of these strains.

[b] Meiotic tetrads from sporulated diploids were dissected by micromanipulation, and the spore phenotypes were analyzed by replica plating. Viability ratio is the ratio of viable spores to nonviable spores.

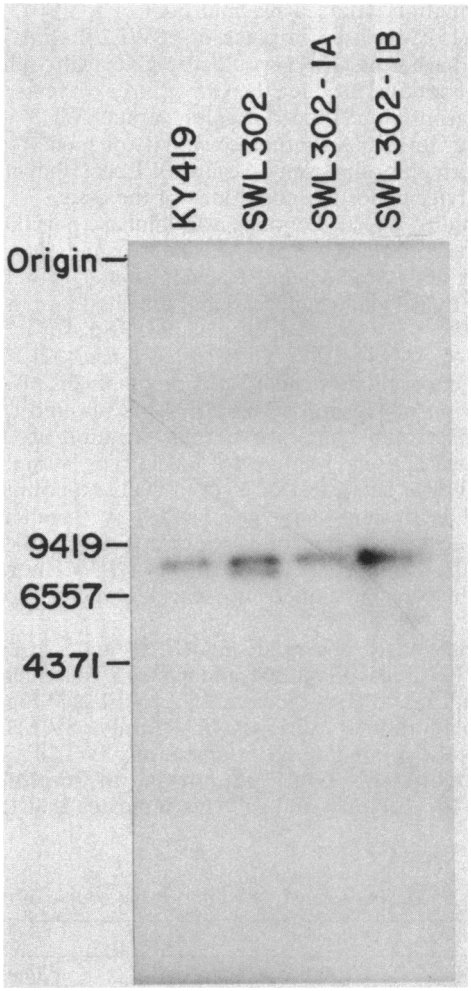

FIG. 2. A rearrangement at *GLN4* is not transmitted to viable progeny. Chromosomal DNA was isolated from parent diploid strain KY419, Ura$^+$ integrant SWL302, and the two viable haploid spores of a single tetrad from SWL302. DNA was digested with *Bam*HI and probed with pSWL211. The additional band seen with SWL302 corresponds to the expected size of pSWL210 which was liberated upon *Bam*HI digestion. This chromosomal rearrangement is not transmitted to viable progeny.

TABLE 7. Gln-tRNA synthetase activity reduction caused by plasmid pSWL210

Strain	Relevant genotype	Gln-tRNA synthetase activity[a]
KY419	*GLN4* / *GLN4*	1.0
SWL301	*GLN4*::pSWL210 / *GLN4*	0.6
SWL301-1A	*GLN4*	1.1
SWL301-1B	*GLN4*	1.0
SWL302	*GLN4*::pSWL210 / *GLN4*	0.5
SWL302-1A	*GLN4*	1.1
SWL302-1B	*GLN4*	1.0

[a] Exponential cultures growing on minimal media supplemented with adenine, histidine, lysine, tryptophan, and uracil were harvested, extracted, and assayed for Gln-tRNA synthetase activity as previously described (12). Activity is normalized to that of KY419, which is assigned the value of 1.0 (see the text).

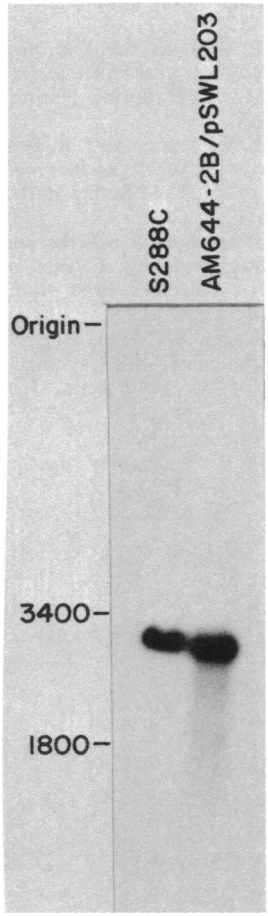

FIG. 3. A subclone of GLN4 hybridizes to a single RNA transcript. Total RNA from strains S288C and AM644-2B(pSWL203) was purified and separated on a 0.8% agarose–1.5% formaldehyde gel. RNA was tansferred to nitrocellulose and incubated with an α-^{32}P-labeled probe of pSWL211, which contains an internal coding fragment of *GLN4* (Table 3). A single band was detected in both cases. The slight difference in molecular weight of the *GLN4* RNA in the two strains may reflect differences in their genetic backgrounds.

TABLE 8. Meiotic mapping of *GLN4*

Diploid strain	Relevant genotype			No. of the following tetrads[a]		
				PD	NPD	T
SWL340	*ADE2* / *ade2*	*ura3-52* / *ura3-52*	*gln4-1*::pSWL203 / *GLN4*	14	0	14
SWL341	*HIS3* / *his3*	*ura3-52* / *ura3-52*	*gln4-1*::pSWL203 / *GLN4*	11	0	13

[a] Meiotic tetrads from sporulated diploids were dissected by micromanipulation, and the spore phenotypes were analyzed by replica plating. We observed anomalous segregation of the *ade-2* allele in SWL341 and, therefore, did not include it in these results. For SWL340, a parental ditype (PD) is defined as 2 Ura$^+$ Ade$^+$:2 Ura$^-$ Ade$^-$, and a nonparental ditype (NPD) is defined as 2 Ura$^+$ Ade$^-$:2 Ura$^-$ Ade$^+$. For SWL341, a PD is defined as 2 Ura$^+$ His$^+$:2 Ura$^-$ His$^+$, and NPD is defined as 2 Ura$^+$ His$^-$:2 Ura$^-$ His$^+$. T, Tetratype.

localized by standard meiotic mapping. Haploid strain SWL285, which was used in the mapping, has pSWL203 integrated at *GLN4*. Hence segregation of *GLN4* can be followed from the segregation of *URA3*. Results of the mapping are given in Table 8. *GLN4* maps to approximately 20 centimorgans from both *ADE2* and *HIS3*, and the order most consistent with these results is centromere-*ADE2*-*GLN4*-*HIS3*.

DISCUSSION

We provide evidence for the isolation of the structural gene for Gln-tRNA synthetase. The gene is in single copy in a haploid genome, and its function is essential for cell viability. We used the cloned gene to determine the size of the mRNA transcript and its position relative to restriction sites on the cloned gene.

By using the 2μm mapping method of Falco and Botstein, we mapped *GLN4* to chromosome XV. Standard meiotic mapping has confirmed the assignment and localized its position to approximately 20 centimorgans from both *ADE2* and *HIS3*. Thus, there appears to be no clustering of genes coding for aminoacyl-tRNA synthetases because *ILS1* and *MES1*, which encode isoleucyl and methionyl-tRNA synthetases, are located on chromosomes II and VII, respectively (18).

In some cases in which a synthetase has been studied in both *E. coli* and *S. cerevisiae*, the subunit composition is conserved (16). Because *E. coli* Gln-tRNA synthetase consists of a single subunit, we anticipate that this is likely for the *S. cerevisiae* enzyme. Furthermore, the cloned *GLN4* gene overproduces the synthetase activity sixfold, a level of activity which we feel would be unlikely if Gln-tRNA synthetase required more than one type of subunit, encoded at separate loci, for activity. Possession of AM644-2B(pSWL203), a strain that overproduces Gln-tRNA synthetase (Table 2), should facilitate purification of this enzyme and provide critical protein data.

The genes which encode the cytoplasmic forms of glutaminyl-, isoleucyl-, and methionyl-tRNA synthetases are all in single copy in a haploid genome (18). To date, no gene encoding the mitochondrial form for any of these synthetases has been identified. Note that if a mitochondrial form of Gln-tRNA synthetase is encoded at a separate locus, then this gene lacks the capacity to rescue a strain that has a disruption at *GLN4*. Sequencing of *GLN4* will allow us to establish whether or not there is homology to the *E. coli glnS* gene (21). A region of high homology will be useful as a

probe to detect and isolate a putative mitochondrial *glnS* gene (14).

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