Homocitrate Synthase Is Located in the Nucleus in the Yeast Saccharomyces cerevisiae*

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We have generated monoclonal antibodies against nuclear proteins from the yeast Saccharomyces cerevisiae. The monoclonal antibodies react with proteins of 47 and 49 kDa on immunoblots and with partially overlapping sets of proteins on two-dimensional nonequilibrium pH gradient electrophoresis-SDS blots. Immunofluorescence localization shows a nuclear staining pattern. Immunoscreening a yeast expression library yielded five independent full-length clones of two open reading frames from chromosome IV, corresponding to YDL182w in the Saccharomyces genome data base. These two open reading frames are predicted to encode homocitrate synthase isoforms of 47 and 49 kDa, respectively. A clone carrying YDL182w was sequenced in its entirety and directs the expression of a 47-kDa protein in Escherichia coli. A clone carrying YDL131w expresses a 48-kDa protein in E. coli. Yeast grown in minimal medium plus lysine show significant reductions in nuclear immunofluorescence staining. Cell fractionation studies localize the 47- and 49-kDa proteins to the nucleus. Nuclear fractionation studies reveal that a portion of the 47- and 49-kDa proteins can only be extracted with DNase digestion and high salt. The localization of homocitrate synthase to the nucleus is unexpected given previous reports that homocitrate synthase is present in mitochondria and the cytoplasm in S. cerevisiae.

In yeast, higher fungi, and euglenids, lysine is synthesized via the α-aminoadipate pathway, which is only found in these organisms (1). Homocitrate synthase catalyzes the first committed reaction in this pathway and is thought to be an important site of control of metabolic flow. In Saccharomyces cerevisiae, two isoforms have been identified by isoelectric focusing of purified enzyme preparations (2). Both isoforms are feedback inhibited by lysine, but only one is transcriptionally repressed by lysine (2).

Genes for the homocitrate synthase isoforms have not been identified until recently, despite extensive genetic analyses of lysine auxotrophs, which have revealed most of the enzyme-encoding (LYS) genes required in this pathway. During the sequencing of chromosome IV of S. cerevisiae, an open reading frame (ORF)† was identified that encoded a protein with significant homology to homocitrate synthase from other yeasts (3). This ORF is designated YDL182w in the Saccharomyces genome data base (GenBank™ accession number X83276, ORF D1298). Ramos et al. (4) have disrupted this gene, examined the effects on lysine production and levels of homocitrate synthase enzymatic activity, and named this gene LYS20.

The subcellular localization of enzymes of the α-aminoacidopate pathway has been investigated in S. cerevisiae, and the enzymes for the first half of the pathway have been reported to be located in the mitochondrion (reviewed in Ref. 5). Two reports place homocitrate synthase from S. cerevisiae in mitochondria (6, 7). Jaklitsch and Kubicek (8) reported that homocitrate synthase from Penicillium chrysogenum is present in the mitochondrion and cytosol.

We have generated four monoclonal antibodies against homocitrate synthase isoforms and present evidence that the majority of homocitrate synthase is localized to the nucleus in S. cerevisiae. As discussed below, this unexpected finding may be reconciled with previous results by consideration of the cell fractionation techniques used in previous studies. Thus, our findings extend previous cell fractionation studies and do not necessarily contradict them. The localization of homocitrate synthase to the nuclear compartment is likely to provide new insights into the α-aminoacidopate pathway for lysine biosynthesis, and more generally, into aspects of nuclear function in S. cerevisiae.

EXPERIMENTAL PROCEDURES

Monoclonal Antibodies—mAb 31F5 was generated during the preparation of monoclonal antibodies to a nucleolus-enriched fraction derived from yeast nuclei.§ Balb/c mice were immunized with nucleoli suspended in RIBI adjuvant (Immunochrom Research, Inc., Hamilton, MT), boosted on a regular schedule, and bled for analysis of the immune response by Western blotting and immunofluorescence. Spleen cells were harvested from the mouse with the most robust and complex immune response, and fused with NS myeloma cells, and plated in four media: (i) HCM (hybridoma complete medium, Ref. 10) + 10% conditioned HCM; (ii) HCM + 10% conditioned HCM + origin supplement (Igen Inc., Gaithersburg, MD); (iii) HCM + 10% conditioned HCM + spleenocyte feeder + origin supplement; (iv) HCM + 10% conditioned HCM + spleenocyte feeder. Hybridomas in each medium were plated in 10, 96-well dishes to attain an average one colony per well. The yields of colonies from each medium were as follows: 17 colonies in medium i; 297 colonies in medium ii; 136 in medium iii; and 287 in medium iv. Wells were scored for the presence of one or more colonies at more than one time after fusion. Priority was given to single colonies. Supernatants (737) from 96-well plates were screened by immunofluorescence using a hexaploid yeast strain (Yeast Genetic Stock Center) and multiwell slides that were prepared in advance and stored dry at 4 °C until use. Clones (199) giving a nucleolar or nuclear staining pattern were expanded to 48-well plates, from which supernatants were screened by trophoresis; PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactopyranoside; DAPI; NLS, nuclear localization sequence.

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† The abbreviations used are: ORF, open reading frame; mAb, monoclonal antibody; HCM, hybridoma complete medium; PAGE, polyacrylamide gel electrophoresis; NEPHGE, nonequilibrium pH gradient electrophoresis; PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactopyranoside; DAPI; NLS, nuclear localization sequence.

§ Dove, J. E., Brockenbrough, J. S., and Aris, J. P. (1997) Methods Cell Biol., in press.
Western blotting. Of 199 supernatants that produced a nuclear staining pattern, 8 reacted with proteins of 45–50 kDa on Western blots. One of these, 31F5, was selected for further study. mAbs C65, D61, and D62 were generated in a screen for antinuclear antibodies that was described previously (11). mAb B47 recognizes Nop1p, the nuclear protein encoded by YSR7 (12), based on results from screening a λgt11 library. All of the mAbs are IgG, isotype 1-κ. Cell fusion, hybridoma culture, and ascites fluid production were done using standard methods by the Hybridoma Laboratory of the Interdisciplinary Center for Biotechnology Research at the University of Florida.

**Gel Electrophoresis and Immunoblotting—SDS-PAGE in one dimension and 30% gels, transfer to nitrocellulose membrane, and incubations with antibodies were done as described previously (13, 14).** For two-dimensional gels, separation in the first dimension was done using nonequilibrium pH gradient electrophoresis (NEPHGE), followed by SDS-PAGE on 10.5% gels in the second dimension, as described previously (11). The resolution of nuclear proteins (100 μg of total protein) was improved by digesting nucleic acids with 2 units of micrococcal nuclease for 10 min at 25 °C prior to NEPHGE.

**Immunofluorescence Localization—The protease-deficient haploid strain BJ2168 (15) and a W303 diploid were grown at 30 °C in YPD medium (1% yeast extract, 2% peptone, 2% dextrose) and were used for indirect immunofluorescence localization as described (13). Growth in synthetic dextrose or synthetic glycerol-lactate medium (synthetic base plus 1% glycerol, 1% lactate), with or without 100 μg/ml l-lysine was done at 30 °C (16). Ascites fluids or mAb supernatants of 31F5, or C65, D61, and D62, were used at dilutions of 1/250 or 1/5, respectively. Secondary antibody anti-mouse-Cy5 conjugate (Jackson Immunoresearch Laboratories) was diluted 1/200.

**Library Immunoselection, PCR Analysis, and DNA Sequencing—**A yeast genomic expression library in λgt11 (Clontech) was screened using standard techniques (17), using mAb 31F5 ascites fluid at a dilution of 1/1000. Positive plaques were purified, and the insert DNA analyzed by direct PCR amplification of A phage suspensions using primers flanking the EcoRI site. Of 25 positives analyzed by PCR, 8 had unique sized inserts. Five positives (#9, #15, #20, #25, and #39) were selected for further analysis on the basis of efficient amplification of a single PCR product band. DNA sequence from both ends of each of the six PCR products was obtained through the DNA Sequencing Laboratory of the Interdisciplinary Center for Biotechnology Research at the University of Florida. A DNA was purified from #9 and the 2.6-kilobase pair insert was excised with EcoRI and cloned into pBluescript II KS+ to give plasmid pJP67. The 2.6-kilobase pair PCR product from #20 was ligated into the TA site of the pT7-Blue vector to give plasmid pJPA72. E. coli DH5α was used for transformations and plasmid preparations (17). Amino acid alignments were generated using the method of Lipman and Pearson (18), as implemented by MacDNASis software.

**Protein Expression in E. coli—**The E. coli strain Y1089 was lysogenized with A isolates #9 or #20, and induction of protein expression was accomplished with 1 mM IPTG in LB medium as described (19). For SDS-PAGE, bacteria were treated with 10% trichloroacetic acid, centrifuged, washed with 1% trichloroacetic acid, and lysed in the presence of 10% trichloroacetic acid with glass beads. After centrifugation, the pellet was boiled for 5 min in SDS-PAGE sample buffer containing EDTA and a protease inhibitor mixture and centrifuged prior to electrophoresis.

**Isolation of Nuclei, Cell Fractionation, and Nuclear Subfractionation—**The S. cerevisiae strain BJ2168 was used for preparation of nuclei according to Ref. 20. Cell fractions were obtained from different layers of a Ficoll 400 step gradient that is used in the final step of isolation of yeast nuclei and are virtually identical to those described in Ref. 13. Nuclear subfractionation was done according to Ref. 13.

**RESULTS**

**Monoclonal Antibodies against 47- and 49-kDa Proteins in the Nucleus—**During the preparation of monoclonal antibodies against nucleus- and nucleolus-enriched fractions from yeast, we identified four monoclonals that reacted with proteins of apparent molecular masses 47 and 49 kDa (Fig. 1). mAbs C65, D61, and D62 resulted from a screen for nuclear-specific monoclonal antibodies, whereas monoclonal 31F5 resulted from a screen for nucleolar-specific monoclonals (see “Experimental Procedures”).

Isolated yeast nuclei are characterized by prominent SDS-PAGE bands corresponding to histones and the nuclear protein Nop1p, whereas in nucleolar preparations, Nop1p is further enriched, but histones are depleted (Fig. 1). mAb 31F5 recognizes the 47- and 49-kDa proteins present in yeast nuclei and nucleoli prepared from isolated nuclei (Fig. 1). The band at 47 kDa appears to consist of a doublet of closely migrating bands. D61 recognizes the 47-kDa band, but the 49-kDa band only weakly. C65 reacts with the 47-kDa band, but does not recognize a 49-kDa band (Fig. 1), even after long exposures of the Western blot (not shown). Like mAb 31F5, D62 recognizes two pairs of closely migrating protein bands that have apparent molecular masses of 47 and 49 kDa (Fig. 1).

The immunologic reactivities of mAbs 31F5 and D62 suggest the recognition of shared epitopes on related proteins. Results with C65 suggest monospecific immunoreactivity. The pattern of immunologic reactivity of mAb D61 appears distinct from the other three insofar as the epitope is recognized avidly in one protein, but to a reduced extent in the other. Also, 31F5 appears similar to D62. Three explanations for these findings are: (i) the existence of distinctly different proteins of similar molecular masses; (ii) the presence of one protein with multiple post-translational modifications, each of which is recognized by a mAb; or (iii) a combination in which two (or more) similar proteins share post-translational modifications.

To compare the reactivities of the mAbs, nuclear proteins were separated on two-dimensional NEPHGE-SDS gels and probed by immunoblotting. NEPHGE was used in the first dimension instead of isoelectric focusing, because it gave better separation of immunoreactive proteins (data not shown). Interestingly, mAbs C65, D61, and D62 react with distinct, but overlapping, sets of proteins on two-dimensional gels (Fig. 2). D62 reacts with the largest number of proteins (Fig. 2C). C65 and D61 each reacts with a smaller number of proteins, each of which appears to be recognized by D62 (Fig. 2, A and B). The detection of multiple proteins suggests the presence of different isoforms of the same mobility on SDS-PAGE gels.

To assess the similarity of 31F5 and D62, immunoblots from
two-dimensional gels were compared (Fig. 2, E and F). The patterns of reactivity appear identical, suggesting that 31F5 and D62 recognize the same epitope. The immunoblotting results from E. coli lysogens are consistent with this (see below and Fig. 5). These data suggest that the monoclonals fall into three classes of immunologic reactivity.

**Immunofluorescence Localization**—To evaluate the intracellular distribution of the 47- and 49-kDa proteins, we performed indirect immunofluorescence localization using procedures described previously (13). Immunofluorescence signals obtained with the mAbs were compared with staining with DAPI, which intercalates into DNA and marks the distribution of chromatin and mitochondrial DNA in the cell. All four monoclonal antibodies give a primarily, but not exclusively, nuclear immunofluorescence staining pattern (Fig. 3, A, D, G, and J). In certain dividing cells, mAb 31F5 reveals a faint trail of staining between nuclei, which corresponds to a narrow isthmus-like connection between nuclei in a dividing pair of cells at the end of mitosis (Fig. 3A, upper right). This faint trail of staining also stains with DAPI. A faint cytosolic staining is visible, more in some cells than others, and is most readily seen with mAb C65 (Fig. 3D). Staining with the secondary antibody alone is not responsible for this faint cytosolic signal (not shown). In some cases, the mAb staining patterns appear larger than the DAPI staining region of the cell. This is due to immunostaining within the nucleolus, which is not stained with DAPI. Images less bright than those shown in Fig. 3 reveal a punctate, intranuclear staining pattern throughout the nucleus (not shown). Fig. 3 shows results obtained with two yeast strains of different genetic backgrounds, a W303 diploid and a protease deficient strain BJ2168. Considering the intracellular localization of the 47- and 49-kDa proteins, it is not surprising that monoclonal antibodies prepared against isolated nuclei and nucleoli react with these proteins.

**Cloning of LYS20 and a Related Gene**—To characterize the immunoreactivity of the monoclonal antibodies at a molecular level, we elected to screen a yeast expression library with one of the mAbs (see “Experimental Procedures”). mAb 31F5 was chosen for this because it, like D62, strongly recognized multiple proteins. Immunoblotting of a yeast expression library with mAb 31F5 yielded five independent full-length clones (Fig. 4A). The insert from one positive clone, #9, was subcloned and sequenced in its entirety (see “Experimental Procedures,” DNA sequence not shown). The 2639 nucleotides of DNA sequence data obtained matched exactly the sequence on chromosome IV between 132,967 and 135,605 and contained the open reading frame YDL182w. This sequence has recently been shown to encode a homocitrate synthase isozyme and has been named LYS20 (4). Two additional positive clones, #15 and #25, were found to overlap with #9.

Interestingly, two different positive clones, #20 and #39, carried the open reading frame YDL131w, between 227,393 and 228,715 on chromosome IV (Fig. 4A). The insert from one positive clone, #9, was subcloned and sequenced in its entirety (see “Experimental Procedures,” DNA sequence not shown). The 2639 nucleotides of DNA sequence data obtained matched exactly the sequence on chromosome IV between 132,967 and 135,605 and contained the open reading frame YDL182w. This sequence has recently been shown to encode a homocitrate synthase isozyme and has been named LYS20 (4). Two additional positive clones, #15 and #25, were found to overlap with #9.

![Fig. 2. Reactivities of monoclonal antibodies toward 47- and 49-kDa proteins separated in two dimensions.](http://www.jbc.org/)

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| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Immunoblotting with C65 (A), D61 (B), D62 (C), or silver staining (D) were done on one set of gels run in parallel. Comigrating spots recognized by more than one mAb are marked (Ç, A, or ◆). A similar pair of blots were probed with monoclonals D62 (E) and 31F5 (F). In A–C and E some immunoreactive protein remained at the origin of the first dimension gel and appears on the left-hand side of each blot. | | | | | |
a single band on SDS gels. Lys20p and its homologue are 90% identical (over 428 positions). The LYS20 gene is approximately 90% identical to YDL131w at the nucleotide level (over 1287 nucleotides). The predicted isoelectric points for Lys20p and its homologue YDL131w are 6.9 and 5.9, respectively. The two previously characterized homocitrate synthase isozymes have isoelectric points of 5.8 and 4.9, the more acidic of which is down-regulated in the presence of lysine (2). Prosite analyses of Lys20p and its homologue revealed the presence of multiple potential phosphorylation sites, but indicated that neither protein contained an amino-terminal mitochondrial presequence or a canonical nuclear localization sequence.

LYS20 and YDL131w Gene Products Expressed in E. coli—We wished to show that 31F5 recognizes the products of the LYS20 and YDL131w genes carried on the inserts in clones #9 and #20, respectively, and not β-galactosidase fusion proteins. It is also valuable to demonstrate that these gene products migrate at 47 and 49 kDa as predicted by their primary structure. Also, considering that immunoscreening a yeast expression library yielded two classes of positives, and that certain mAbs react with a group of proteins on Western blots, only a subset of which were recognized by other mAbs, it is likely that not all of the mAbs recognize the same gene product. Thus, we wished to further characterize the immunoreactivity of the other three antibodies. λ lysogens were prepared for heterologous protein expression in E. coli, and Western blotting was done with mAbs C65, D61, D62, and 31F5 (see “Experimental Procedures”).

A 47-kDa band was detected by mAb 31F5 in an extract from an IPTG-induced E. coli lysogen carrying the insert present in clone #9 (Fig. 5). This band exactly comigrates with the 47-kDa band present in samples of yeast nuclei. The 47-kDa band was not detected in an IPTG-induced extract from a lysogen carrying a clone for the putative acetyl-coenzyme A synthetase 2,† which was used as a control (Fig. 5). India ink staining of the blot reveals a band of 47 kDa present in samples from lysogen #9, but not from the control (data not shown). This argues that clone #9 was isolated during immunoscreening by virtue of the expression of the 47-kDa protein.

In a similar experiment, a 49-kDa band was detected by mAb 31F5 in extracts from a lysogen carrying clone #20 (Fig. 6). The 49-kDa band exactly comigrates with the 49-kDa band present in yeast nuclei. The 49-kDa band was not detected in an IPTG-induced extract from E. coli strain Y1089 that was not lysogenized, which was used as a control (Fig. 6). Several immunoreactive proteins less than 49 kDa are also visible in Fig. 6. Two experiments with independently isolated lysogens of clone #20 yielded the same lower molecular mass immunoreactive bands. We attribute these bands to proteolysis of the 49-kDa YDL131w gene product. Proteolysis

†S. Chen, J. E. Dove, J. S. Brockenbrough, and J. P. Aris, unpublished results.
is not uncommon in SDS-PAGE lysates of induced lysogens and has apparently occurred despite precautions taken to reduce proteolysis during sample preparation (see “Experimental Procedures”). It was not possible to prepared IPTG-induced protein lysates from clone #39, which also carries YDL131w. Two independent lysogens of clone #39 spontaneously

**Fig. 4.** Summary of results from expression library screening. A, location on chromosome IV of open reading frames designated YDL182W (LYS20) and YDL131W in the Saccharomyces genome data base. Clones #9, #15, #20, #25, and #39 were obtained by screening a yeast genomic library in Agt11 with mAb 31F5 (see “Experimental Procedures”). Insert from #9 was sequenced in its entirety. Other positives were mapped by partial sequencing of insert ends, and only approximate coordinates are given. B, alignment of the predicted amino acid sequences of the YDL182w and YDL131w gene products.

**Fig. 5.** Expression of clone #9 in E. coli. E. coli strains harboring λ lysogens from clone #9 and a control clone (C) were induced with IPTG, and protein extracts were analyzed by immunoblotting with mAbs 31F5, C65, D61, and D62. Yeast nuclei (Nu) were probed in parallel with 31F5. The open square designates a lower molecular mass immunoreactive protein.

**Fig. 6.** Expression of clone #20 in E. coli. E. coli strains harboring λ lysogens from clone #20 and a control clone (C) were induced with IPTG, and protein extracts were analyzed by immunoblotting with mAbs 31F5, C65, D61, and D62. Yeast nuclei (Nu) were probed in parallel with 31F5. Open squares designate lower molecular mass immunoreactive proteins.
shown). DAPI staining of cells shown in Fig. 7 reveals a significant reduction in staining that appeared most pronounced for C65, noticeably reduced the nuclear immunofluorescence staining intensity (Fig. 7). This reduction was most pronounced for C65, which reacts with Lys20p. The reduction seen with D61 is not shown). The absence of reaction of D61 could be due to the absence of formation of the necessary epitope in E. coli (e.g. absence of post-translational modification). Like 31F5, mAb D62 reacts with lower molecular mass bands from clones #9 and #20 (Figs. 5 and 6). The fact that same low molecular mass bands are recognized by 31F5 and D62 is consistent with the notion that 31F5 and D62 recognize the same epitope.

**Lysine Reduces Nuclear Immunofluorescence Staining Intensity**—The localization of homocitrate synthase to the nucleus contradicts previous studies that suggest cytosolic and/or mitochondrial localization. To generate additional evidence that homocitrate synthase is detected by the mAbs during immunofluorescence localization, we performed the localization with cells grown in the presence of lysine. Additionally, it is possible that the localization of homocitrate synthase is dependent on carbon source and/or presence of lysine. For instance, growth of yeast in YPD, which is routinely used for immunofluorescence localization, does not induce maximal proliferation of mitochondria, and YPD contains lysine, which is known to repress expression of one homocitrate synthase isofrom (2). Growth in glycerol-lactate medium in the absence of lysine induces proliferation of mitochondria and expression of homocitrate synthase, which should provide the optimum conditions under which to detect mitochondrial localization.

To test these possibilities, yeast were grown in dextrose or glycerol-lactate medium, with or without lysine and examined by immunofluorescence localization. Growth in either carbon source and/or presence of lysine. The results presented in Figs. 5 and 6 indicate that mAb 31F5 does not detect a β-galactosidase fusion protein, which would be predicted to have a molecular mass greater than 116 kDa. The 47- and 49-kDa proteins expressed in E. coli also reacted with monoclonal D62 (Figs. 5 and 6). C65 reacts with the 47-kDa protein expressed in E. coli, but not the 49-kDa protein, which is consistent with the reactivity of C65 against yeast nuclear proteins (Fig. 1). C65 also reacted with certain E. coli proteins in control samples, indicating nonspecific reactivity with the antibody (Fig. 5). Interestingly, D61 did not react with either Lys20p or the YDL131w gene product expressed in E. coli (Figs. 5 and 6), even after long exposures of film (data not shown). The absence of reaction of D61 could be due to the absence of formation of the necessary epitope in E. coli (e.g. absence of post-translational modification). Like 31F5, mAb D62 reacts with lower molecular mass bands from clones #9 and #20 (Figs. 5 and 6). The fact that same low molecular mass bands are recognized by 31F5 and D62 is consistent with the notion that 31F5 and D62 recognize the same epitope.

Characterization of 47- and 49-kDa Proteins by Cell and Nuclear Fractionation—To address the intracellular localization of the 47- and 49-kDa proteins with an additional technique, subcellular fractions were analyzed by Western blotting (Fig. 8). The fractions employed are: purified nuclei (Nu), mAb D77 recognizes the nuclear protein Nop1p. Monoclonals C65, D61, and D62 react with 47- and 49-kDa proteins. mAb B47 recognizes the nuclear protein Nop1p. mAb C56 recognizes the plasma membrane ATPase Pma1p. Nop1p (open circle) and histones (closed circles) are indicated.
D61 recognizes the 47-kDa isoform most highly enriched in the nuclear fraction (Fig. 8). mAb D61 also recognizes the 49-kDa protein, but only very weakly (as seen in Fig. 1). Some 47- and 49-kDa protein bands are present in the high density membrane fraction, which contains endoplasmic reticulum, plasma membrane, some vacuolar membrane, and a small amount of nuclear fragments and nuclear envelope (20). The non-nuclear reactivity is most readily seen with mAb D62, but the majority of the 47- and 49-kDa proteins are found in the nuclear fraction. To provide a basis for comparison, three other mAbs were used: mAb D77 was used to detect the 38-kDa nucleolar protein Nop1p; mAb B47 was used to detect the 67-kDa nucleolar protein Nsr1p; and the mAb C56 was used to detect the 100-kDa integral plasma membrane protein Pma1p (Fig. 8).

Nuclear fractionation experiments were undertaken to evaluate the intranuclear localization of the 47- and 49-kDa proteins (Fig. 9). This approach is valuable because certain nuclear proteins exhibit a typical extraction behavior (13). For example, histones are extracted from nuclei by DNase I digestion and high salt treatment, whereas the nucleolar proteins Nop1p and Nsr1p are not. Nop1p and Nsr1p are liberated by exposure to high salt. The three monoclonals, C65, D61, and D62, recognize 47- and 49-kDa proteins, less than half of which are released by DNase I digestion and EDTA (Fig. 9). The majority of the 47- and 49-kDa proteins are present in the pellet fraction (Fig. 9). Of this, the majority is released by exposure of the pellet to 0.5 M NaCl. Similar behavior is exhibited by the nucleolar proteins Nop1p and Nsr1p (Fig. 9). This suggests that the majority of the 47- and 49-kDa proteins present in the nucleus are not freely diffusible, and a significant portion are tightly bound and can only be extracted from nuclei with DNase digestion and high salt.

**DISCUSSION**

We have generated four monoclonal antibodies specific for homocitrate synthase in yeast. Homocitrate synthase catalyzes the first committed step in the α-aminoacidopatway for lysine biosynthesis. Recently, Ramos et al. (4) have characterized a gene (YDL182w) encoding an isozyme of homocitrate synthase and have named it LYS20. These four monoclonal antibodies, C65, D61, D62, and 31F5, recognize Lys20p, which migrates at 47 kDa. The mAbs D62, D61, and 31F5 also recognize the 49-kDa YDL131w gene product, but D61 does so only very weakly. The YDL131w gene product is predicted to be slightly larger than Lys20p and to possess an amino acid sequence 90% identical to Lys20p.

Our studies using monoclonal antibodies have localized homocitrate synthase to the nucleus. Cell fractionation and immunofluorescence labeling experiments indicate that the majority of homocitrate synthase in yeast is present in the nucleus. The localization of homocitrate synthase to the nucleus is unexpected given the generally accepted view that homocitrate synthase is present in the mitochondrion and cytoplasm in *S. cerevisiae* (6, 7). One explanation for this discrepancy is that a percentage of homocitrate synthase is not localized in the nucleus and accounts for the previous findings. This is consistent with the results reported herein insofar as we estimate that the majority, but not all, of homocitrate synthase is localized to the nucleus. However, we view it equally likely that previous studies were done in such a manner as to compromise the structural integrity of the nucleus. A widely cited report (6) localizing homocitrate synthase to mitochondria is based on experiments in which mitochondria were isolated by differential centrifugation of a cell-free extract, which yields only a crude preparation. This was done under conditions that we have previously found to be incompatible with isolating intact nuclei from yeast (11, 20). Specifically, Betterton et al. (6) lysed spheroplasts in pH 7.4 buffer, without Mg2+, using sonication. We have found previously that isolation of intact nuclei in good yield requires pH 6.5, at least 1 mM Mg2+, and gentle lysis conditions (11, 20). The “post-nuclear supernatant” obtained by Betterton et al. (6) using differential centrifugation, and the mitochondria obtained from it, are not likely to be free of nuclear fragments and/or constituents. The nuclear fractionation experiments presented herein show that a portion of homocitrate synthase does not freely diffuse out of the nucleus and appears to be associated with a sedimentable nuclear subfraction. This is consistent with the view that a crudely prepared “mitochondria” fraction may contain nuclear fragments. Thus, the observation that homocitrate synthase is present in the nucleus in *S. cerevisiae* may simply have been overlooked, because the techniques used in previous studies could not adequately differentiate between the mitochondrion and other compartments, such as the nucleus.

Previous studies of homocitrate synthase and lysine biosynthesis have shown that two isoforms of this enzyme are present in *S. cerevisiae* (2). LYS20 and its homologue YDL131w undoubtedly encode the two isozymes of homocitrate synthase present in yeast. Both the LYS20 gene and its homologue are located on chromosome IV, suggesting that they arose via gene duplication (24). Neither Lys20p, nor the homologous protein, which contains an amino-terminal extension of 14 amino acids, is predicted to have an amino-terminal mitochondrial localization signal. Neither Lys20p, nor its homologue, is predicted to contain a nuclear localization sequence (NLS). This raises the question as to the mechanism by which homocitrate synthase is transported to the nucleus. The 47-kDa size of Lys20p is near the upper limit for diffusion across the nuclear pore complex, but could readily accumulate in the nucleus if bound to a nuclear component (21). Alternatively, nuclear transport of Lys20p may be explained either by “piggyback” transport in association with a nuclear protein, or the presence of an atyp-
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