Alternative Start Sites in the Saccharomyces cerevisiae GLR1 Gene Are Responsible for Mitochondrial and Cytosolic Isoforms of Glutathione Reductase*

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To combat oxidative damage, eukaryotic cells have evolved with numerous anti-oxidant factors that are often distributed between cytosolic and mitochondrial pools. Glutathione reductase, which regenerates the reduced form of glutathione, represents one such anti-oxidant factor, yet nothing is known regarding the partitioning of this enzyme within the cell. Using the bakers' yeast Saccharomyces cerevisiae as a model, we provide evidence that a single gene, namely GLR1, encodes both the mitochondrial and cytosolic forms of glutathione reductase. A deletion in GLR1 drastically increases levels of oxidized glutathione in these two subcellular compartments. The GLR1 gene has two in-frame start codons that are both used as translation initiation sites. Translation from the first codon generates the mitochondrial form that includes a mitochondrial targeting signal, whereas translation from the second codon produces the cytosolic form that lacks this sequence. Our results indicate that the sequence context of the two AUG codons influences the efficiency of translation initiation at each site, which in turn affects the relative levels of cytosolic and mitochondrial GLR1p. This method of subcellular distribution of glutathione reductase may be conserved in mammalian cells as well.

The abundant tripeptide glutathione (γ-glutamylcysteinylglycine) plays a pivotal role in the oxidative stress defense systems of the cell. Glutathione is a ubiquitous thiol that maintains the intracellular redox state by reducing cellular disulfide bonds and detoxifying damaging molecules such as xenobiotics and heavy metals (1). Glutathione also serves as a reductive cofactor for anti-oxidant enzymes such as glutaredoxins and glutathione peroxidases (1, 2). The anti-oxidant function of glutathione depends upon its redox-active thiol group that becomes oxidized when glutathione reduces target molecules. Two molecules of reduced glutathione (GSH) are thereby converted to glutathione disulfide (GSSG). The enzyme glutathione reductase catalyzes the reduction of GSSG back to GSH, therefore it plays a critical role in the cellular defense against oxidative damage as well. Glutathione reductase (GR) is a flavin-containing oxidoreductase similar in sequence and structure to thioredoxin reductase. It contains a redox-active disulfide in its active site and requires NADPH for its catalytic activity. GR is found in many types of organisms including bacteria, plants, and yeast, as well as higher eukaryotes such as mice and humans. However, the intracellular localization of GR is not completely understood.

Because mitochondria are an important source and target of oxidative damage, it would seem critical to have anti-oxidant factors such as GR housed in this organelle along with other cellular locations. In eukaryotes, anti-oxidant factors typically have both mitochondrial and cytosolic versions that are often encoded by different genes. For example, the matrix of the mitochondria harbors a manganese-containing superoxide dismutase (SOD2), whereas the cytosol contains a Cu,Zn superoxide dismutase (SOD1) (3). Both yeast and mammalian cells express two different thioredoxin reductases as well as numerous thioredoxins and glutaredoxins that are specifically targeted to the mitochondria or the cytosol (4–9). There are also different mechanisms for the generation of cytosolic and mitochondrial NADPH, an essential cofactor for both thioredoxin reductase and GR. In the cytosol, NADPH is primarily supplied by the pentose phosphate pathway (10, 11), whereas in the mitochondria, NADPH is either provided by an NADH kinase (e.g. Saccharomyces cerevisiae POS5) (12) or by NADP⁺-dependent isocitrate dehydrogenase as in mammalian cells (13). Another method by which anti-oxidant enzymes may be distributed between the cytosol and mitochondria is through dual targeting of a single gene product. For example, the Cu,Zn-SOD1 polypeptide is targeted to both the cytosol and the intermembrane space of mitochondria (14).

As with other anti-oxidant factors, GR is clearly expected to reside in the mitochondria and cytosol. Reduced GSH is synthesized in the cytosol and can be transported into the mitochondria (15), but the GSSG formed in the matrix is unable to exit this compartment (16). Therefore, the GR-mediated regeneration of GSH must take place inside the mitochondria as well as in the cytosol. In mammals and yeast, only a single GR-encoding gene has been identified yet GR activity has been detected in both the cytosol and mitochondria of mammalian cells (17). The question remains as to how GR is localized to both compartments.

Our objective was to use the S. cerevisiae as a model system targeting signal; SD, synthetic-defined medium; FS, frameshift; WT, wild type.
tions were performed by the lithium acetate procedure (21). Yeast trans-
supplemented with 2% glucose or minimal synthetic-defined medium
maintained at 30°C. Identical residues are **highlighted** in black, and similar residues are **outlined in gray**. For the eukaryotic sequences, the two methionines encoded by the two in-frame start codons are shown in boldface. The predicted cleavage site for the MTS in yeast Glr1p is designated by an arrow (1).

**Experimental Procedures**

**Strains and Plasmids**—S. cerevisiae strains used in this study were BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and BY4741 glr1Δ::kanMX4 obtained from Research Genetics. Strains were maintain-
ted at 30°C on either enriched yeast extract peptone medium supplemented with 2% glucose or minimal synthetic-defined medium (SD) supplemented with the appropriate amino acids (20). Yeast trans-
formations were performed by the lithium acetate procedure (21).

The **GLR1** CEN plasmid pCO113 was originally constructed as a green fluorescent protein (GFP) fusion with one copy of GFP fused to the C terminus of Glr1p. The **GLR1** gene was amplified from WT and introduced into two SacII sites at +1449. After mutagenesis, the plasmid was digested with SacII and religated to remove the GFP tag located from the indicated strains. Strains utilized were as follows: WT, BY4741; glr1Δ, BY4741 glr1Δ; glr1Δ + vector, glr1Δ transformed with pAA1; and glr1Δ + GLR1, glr1Δ transformed with pCO113. Activity is reported as units per milligram of protein in the extract. One unit of enzyme activity = 1.0 μmol DTNB reduced/min at 25°C in the presence of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The total glutathione (GSH + GSSG) was calculated from total GSH and GSSG levels in each extract. For western blots, the percent of oxidized glu-

**GR Activity Assays**—GR activity assays were performed in SD medium with 2% galactose so that cyto-

**GR Activity Assays**—GR activity assays, yeast cells were grown aerobically to mid-log phase in glucose SD medium and then fraction-
ated into IMS and mitochondrial fractions. GR activity was measured using the glutathione reductase assay kit from Sigma, which is a colorimetric assay monitoring the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). 140–300 μg of protein was used directly in the assay. For mitochondrial fractions, 30–70 μg of extract was diluted to 200 μl in assay buffer with 1% Tween 20 and vortexed for 1 min prior to analysis.

**FIG. 1**. Sequence alignment of human, mouse, and S. cerevisiae and **E. coli**. The amino acid sequences of the N termini of human, mouse, yeast and **E. coli** were aligned using ClustalW. Identical residues are **highlighted in black**, and similar residues are **outlined in gray**. For the eukaryotic sequences, the two methionines encoded by the two in-frame start codons are shown in **boldface**. The predicted cleavage site for the MTS in yeast Glr1p is designated by an arrow (1).
GSH/GSSG Assays—Total glutathione and oxidized glutathione were measured by the DTNB-GR recycling assay (25, 26). Cells were grown to mid-log phase in glucose SD medium and fractionated into mitochondria and PMS fractions as described above. Freshly made extracts were deproteinized by the addition of 10% 5-sulfosalicylic acid to a final concentration of 1% and incubated on ice for 30 min. Precipitated protein was then removed by centrifugation at 13,000 × g for 5 min. Measurement of total GSH and GSSG in acidified extracts was conducted by spectrophotometric analysis of DTNB reduction as described previously (26).

Immunoblotting Techniques—Yeast extracts were subjected to electrophoresis on a 12% SDS-polyacrylamide gel and analyzed by Western blotting using an anti-Glr1p antibody diluted to 1:10,000 and a secondary anti-rabbit IgG (Amersham Biosciences) diluted to 1:12,500. For Glr1p antibody production, S. cerevisiae Glr1p was purchased from Sigma and used to prepare rabbit-generated anti-Glr1p antibodies (Cosmilo Biologicals, Reamstown, PA). PMS fractions were monitored by using antibodies directed against cytochrome b_{2} (diluted to 1:10,000) in the IMS and Mas2 (diluted to 1:25,000) in the matrix (kind gifts of R. Jensen). Detection employed the ECL kit (Amersham Biosciences) used according to the manufacturer’s specifications. Protein concentrations were determined using the Bradford method (Bio-Rad) with bovine serum albumin as the calibration standard.

RESULTS

Glr1p Subcellular Localization—S. cerevisiae is reported to contain a single GR-encoding gene, namely GLR1 (27). A single GR-encoding gene has also been identified in mammalian species such as humans and mice (28, 29). A comparison of the amino acid sequences of GR from human, mouse, and yeast to that of Escherichia coli GR reveals that the eukaryotic homologues all have a 19–60 amino acid extension at the N terminus of the protein that precedes the homology region to E. coli GR (Fig. 1). For the mammalian sequences, this extension includes two in-frame start codons with an abundance of arginine residues in between. Previous researchers have proposed that these mammalian N termini encode a putative mitochondrial targeting signal (MTS) (28, 29). The N-terminal sequence in the yeast Glr1p protein also includes two in-frame start codons and exhibits the hallmarks of a MTS including an abundance of positively charged and hydroxylated amino acids with no negatively charged residues (30, 31). The putative cleavage site for mitochondrial processing of yeast Glr1p is between Met-17 and Ser-18 as determined by TargetP, version 1.0 (32). The presence of this putative MTS in S. cerevisiae Glr1p would suggest that the protein is exclusively targeted to the mitochondria.

To address the localization of Glr1p in yeast, we assayed for GR activity in cell lysates that were resolved into crude mitochondria and largely cytosolic (PMS) fractions. As seen in Fig. 2A, GR activity is found in both the mitochondria and cytosolic fractions of wild-type (WT) cells. Furthermore, both compartments have similar concentrations of GR activity, although mitochondrial levels are somewhat higher with ~35% more activity/mg protein compared with the PMS fraction. As seen in Fig. 2A, deletion of GLR1 results in the loss of GR activity in both compartments. When the GLR1 gene is reintroduced on a CEN plasmid in glr1Δ strains, activity in both compartments is restored to wild-type levels. These results indicate that GLR1 is responsible for both the cytosolic and mitochondrial forms of GR.

The levels of total glutathione as well as the ratio of oxidized (GSSG) to reduced (GSH) glutathione were measured in WT and glr1Δ strains. As shown in Fig. 2B, the amounts of total glutathione (GSH + GSSG) in both the mitochondria and cytosol are similar for WT and glr1Δ strains. When the ratio of oxidized to reduced was examined, the mitochondria of WT strains exhibited a higher percentage of oxidized glutathione (~9%) compared with the cytosol (~0.4%) (Fig. 2C). A similar situation has been reported for the cytosol and mitochondria of mammalian cells (9, 33). This result is expected because mitochondria are a major source of reactive oxygen species in the cell, which would lead to a higher proportion of GSSG within this compartment. Upon comparison of the glr1Δ mutant to WT, we found that glr1Δ has a much greater percentage of oxidized glutathione in both the cytosol (~57%) and the mitochondria (~41%) (Fig. 2C). Because the consequence of a loss of GLR1 is a vast increase in oxidized glutathione in both com-
partments, this provides further evidence that GLR1 encodes both the mitochondrial and cytosolic isoforms of GR in yeast.

To confirm that Glr1p is localized to both the cytosol and mitochondria, we conducted a Western blot analysis of the PMS (largely cytosolic) and mitochondrial fractions of WT and glr1/H9004 strains. Bakers’ yeast GR was purchased from Sigma and used to prepare anti-Glr1p antibodies. This antibody recognizes a major species of ~50 kDa that corresponds to Glr1p because it is seen in WT cells but not in glr1/H9004 mutants (Fig. 3A, top panel, lanes 1 and 2). This antibody also occasionally recognizes a minor contaminant that migrates slightly faster than Glr1p, but this does not reflect Glr1p because it is also observed in glr1/H9004 strains. In Fig. 3A, lanes 3–6, identical cell equivalents of PMS and crude mitochondria were analyzed to approximate the proportion of Glr1p protein that localizes to the different cellular compartments. These results indicate that Glr1p is predominantly found in the cytoplasm but a small fraction of total Glr1p is also seen in mitochondria. A titration of the PMS fraction (lanes 7–9) reveals that the total amount of Glr1p present in the mitochondria represents ~5–10% of cytosolic Glr1p. Given that mitochondria constitute ~3% of the total cell volume under these growth conditions (34), it appears that the concentration of Glr1p is roughly equivalent in the two compartments. This result is in accordance with the activity assays in Fig. 2A that demonstrate that GR activity levels are similar in the mitochondria and cytosol.

To identify the mitochondrial compartment that houses Glr1p, we fractionated the mitochondria into IMS and matrix components. The results shown in Fig. 3B indicate that Glr1p is specifically localized to the matrix as determined by co-localization with the mitochondrial processing protease, Mass2p.

Glr1p Distribution in Translation Initiation Mutants—As shown in Table I, the GLR1 gene has two in-frame start codons corresponding to Met-1 and Met-17. Translation from the first AUG codon would produce a 483 amino acid, 53.4-kDa protein including the putative MTS. Translation from the second AUG codon would produce a 467 amino acid, 51.6-kDa protein that lacks the MTS. Through site-directed mutagenesis studies, we tested whether these potential translation start sites had any role in determining Glr1p subcellular distribution.

Several different mutants were designed with variations in the translation initiation sequences (Table I). First, the putative MTS was deleted by removing amino acids 1–16 (Δ1–16). We also mutated the two potential start sites, creating M1L and M17L single mutants and an M1L/M17L double mutant. As shown in the Western blot in Fig. 4A, lanes 1–6, the Δ1–16 and M1L mutants both have no detectable mitochondrial Glr1p, although cytosolic Glr1p is still present. Together, these results suggest that translation from the first AUG codon produces the mitochondrial Glr1p isoform and that amino acids 1–16 are important for mitochondrial targeting. Furthermore,
the abundant expression of cytosolic Glr1p in the MIL mutant indicates that in the absence of AUG1, AUG17 is translated efficiently.

Conversely, mutation of the second AUG codon (M17L) generates the opposite result. This mutant has very low levels of cytosolic Glr1p (Fig. 4A, lanes 7–8). In fact, the cytosolic isoform of M17L Glr1p runs higher on the Western blot than the mitochondrial protein. We believe that this band actually corresponds to the long mitochondrial form that has not been processed and is trapped in the cytoplasm. This phenomenon may occur because the mitochondrial import process for Glr1p is overwhelmed by the increased levels of the mitochondrial isoform in this mutant, creating a backlog of Glr1p proteins waiting to be imported. These results indicate that in the absence of AUG17, translation from AUG1 exclusively generates the long form of the protein that is either imported into the mitochondria and processed or trapped in the cytosol as the unprocessed form. AUG1 and AUG17 are indeed the only possible start sites for Glr1p because a double mutant (M1L/M17L) lacking both start sites results in loss of GR activity in both the PMS and the mitochondria (Fig. 5).

**Glr1p Distribution in Frameshift Mutants**—Based on our results with the MIL and M17L mutants, it appears that either of these two start codons can be used to initiate translation of Glr1p. However, even though the second AUG codon is translated in the MIL mutant, this does not necessarily mean that the ribosome naturally initiates at this site in the wild-type GLR1 mRNA. Typically, translational initiation of eukaryotic mRNAs proceeds via the “scanning model” in which the ribosome binds to the 5′ end of the mRNA and migrates linearly, initiating translation at the first AUG codon it encounters (35). Therefore, in the case of the MIL mutant, the ribosome might initiate at AUG17 only because it is the first available initiation site. Results with the MIL mutant did not exclude the possibility that both cytosolic and mitochondrial Glr1p normally result from initiation at AUG1 with the shorter cytosolic Glr1p produced from processing of the longer form, as has been described for yeast fumarase (36–38).

To more definitively determine whether the second start codon of GLR1 is utilized when the first start codon is still intact, two different frameshift (FS) mutations were introduced between AUG1 and AUG17. For the FS1 and FS2 mutants, translation from AUG1 produces a nonsense polypeptide that is 9 amino acids and 52 amino acids long, respectively (Table I). If AUG1 is the only start site employed, Glr1p should be absent from both the cytosol and mitochondria in both mutants. However, as shown in Fig. 4B, lanes 1–6, both frameshift mutants still express Glr1p in the cytosol but the protein is not detected in the mitochondria. Therefore, translation must be initiating at AUG17. Taken together, the data from the translation initiation and frameshift mutants suggest that both start codons are naturally used as initiation sites in vivo with translation from AUG1 generating mitochondrial Glr1p, whereas translation from AUG17 produces the cytosolic isoform of the protein.

As another means of assaying the effects of all of these mutations on Glr1p distribution, we monitored GR enzymatic activity in the cytosol and mitochondria. As seen in Fig. 5, GR activity closely correlates with the level of Glr1p protein, i.e., mitochondrial GR activity is increased in the M17L mutant and reduced in Δ1–16, MIL, FS1, and FS2 mutants. Yet there remains some residual mitochondrial activity in the latter mutants that is not detected by Western blots. This may represent GR activity from the PMS or other cellular compartments that contaminate the crude mitochondrial prep.

**AUG Codon Context and Translation Initiation Efficiency**—Although both start sites are utilized in vivo, it appears that translation initiation at AUG17 is preferred over AUG1 because the cytosolic isoform of Glr1p is the predominant form of the protein in the cell. According to the scanning model for translation, this result is unexpected because the first AUG codon in an mRNA sequence is ordinarily preferred as the ribosome initiation site (35). However, “leaky scanning” or translation from downstream AUG codons may occur when the sequence context of the first AUG codon is unfavorable (39). To investigate whether leaky scanning was occurring in Glr1p translation, we examined the sequences surrounding each AUG codon. These flanking sequences, also called the Kozak sequence, have been shown to influence initiation efficiency (40, 41). In yeast, the preferred consensus sequence is 5′-AU/
Y1A(A/U)/AUG UCU-3' with the A in position −3 (in boldface) being the most highly conserved of the residues surrounding the AUG codon (42). As shown in Table II, AUG17 more closely matches this consensus sequence, notably having an A in the critical −3 position, whereas AUG1 contains a U at this site. This finding suggests that the relatively poor utilization of AUG1 may be due to the absence of an A in position −3.

To test this hypothesis, we introduced an A residue at position −3 with respect to AUG1 creating the U−3A(M1) mutant (Tables I and II). This single mutation resulted in a large increase in the levels of mitochondrial Glr1p (Fig. 4C, lane 4), indicative of a dramatic increase in translation initiation at AUG1. Furthermore, this increase in mitochondrial Glr1p is accompanied by a decrease in cytosolic Glr1p in the U−3A(M1) mutant. This result is most obvious when equal protein levels are analyzed for mitochondrial and cytosolic extracts (Fig. 4C, bottom panel, compare lanes 1 and 3). The results obtained from Western blot analysis were also confirmed in enzymatic assays of GR activity (Fig. 5). However, initiation at AUG17 is still evident in the U−3A(M1) mutant because a substantial level of Glr1p still accumulates in the cytosol (Figs. 4C, lane 3, and 5). The majority of this cytosolic protein indeed results from initiation at AUG17 because a double U−3A(M1)/M17L mutant abolishes production of the major cytosolic species of Glr1p (Fig. 4C, lane 5). A minor species corresponding to the unprocessed mitochondrial isoform (ω) is also detected in the cytosol of the U−3A(M1) and U−3A(M1)/M17L mutants (Fig. 4C, top panel, lanes 3 and 5). As is the case with the M17L mutant (Fig. 4C, lane 7), the increased levels of polypeptide initiated at AUG1 may not be efficiently imported into the mitochondria, resulting in accumulation of a portion of the long unprocessed isoform in the cytosol. Overall, these results suggest that improvement of the AUG1 sequence context reduces but does not completely preclude translation from AUG17 by limiting the occurrence of leaky scanning (see “Discussion”).

**DISCUSSION**

In this study, we provide evidence that yeast Glr1p is localized to both the mitochondria (~5–10%) and the cytosol (~90–95%). Although the cytosolic isoform is predominant in terms of total cellular levels, the concentration of Glr1p in each compartment appears to be similar when the volume of the cytosol versus the mitochondria is taken into account. When considering the generation and distribution of GSH within the cell, the importance of having a mitochondrial version of Glr1p becomes clear. GSH is exclusively synthesized in the cytosol (15) and is then transported into the mitochondria, presumably via organic anion carriers (43). Within the mitochondria, GSH re-
levels of mitochondrial Grl1p (translated from AUG1) increase with a concomitant decrease in cytosolic levels (translated from AUG17). Presumably, leaky scanning is reduced in this mutant as more ribosomes initiate translation at AUG1 rather than AUG17. Therefore, this result suggests that the long mitochondrial form and the short cytosolic form are translated from the same mRNA that includes both start codons.

Although leaky scanning appears to be the main factor controlling the dual distribution of yeast GR, we cannot rule out other factors that could contribute to localization of this protein including slow mitochondrial import and processing. Under certain conditions, we see a small percentage of the unprocessed mitochondrial isoform in the cytosol. This phenomenon is significantly more pronounced in mutants with increased levels of mitochondrial Grl1p (e.g. M17L, U. A(M1)); however, it is still apparent in WT Grl1p (see Figs. 3A, lane 4, and A–C, lanes 1). It is possible that the mitochondrial import of Grl1p may be somewhat inefficient, such that a small fraction of the polypeptide folds before import and remains trapped in the cytosol. This type of mechanism has been described for the localization of yeast fumarase (Fum1p). A portion of the FUM1 translation product is processed and fully imported into the mitochondria, whereas the majority is released back into the cytosol after processing, presumably because of rapid folding of the polypeptide into an import-incompetent state (37, 38). It is possible that a similar mechanism contributes to incomplete mitochondrial uptake of Grl1p, although our results indicate that leaky scanning translation is the driving force for Grl1p distribution.

Can the mechanism for dual targeting of GR described here be extrapolated to other organisms as well? An examination of the gene sequences of mammalian GRs provides some clues to be extrapolated to other organisms as well? An examination of the second AUG codons for both mammalian GRs for both human and mouse GRs lack this critical residue. In contrast, the second AUG codons for both mammalian GRs (AUG1) are still the most critical in determining translation efficiency (39, 41). As shown in Table III, the AUG1 sequences include the putative MTS, whereas translation from the second AUG codon could encode the shorter cytosolic isoform. The preferred AUG context sequence for mammalian genes (5′-GCC A/G/C AUG C-3′) is somewhat different from that of S. cerevisiae, although the purine at position −3 (in boldface) is still the most critical in determining translation efficiency (39, 41). As shown in Table III, the AUG1 sequences for both human and mouse GRs lack this critical residue. In contrast, the second AUG codons for both mammalian GRs (AUG44 in human GR and AUG27 in mouse GR) have the appropriate residue (an A or G) at this important position. Based on this analysis alone, the second AUG is predicted to be the preferential start site for translation of mammalian GR, just as we have demonstrated here for yeast Grl1p. Therefore, the leaky scanning mechanism for translation may be the conserved driving force across eukaryotes that ensures the dual targeting of GR to both the mitochondria and cytoplasm.

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REFERENCES

1. Jamieson, D. J. (1998) Yeast 14, 1511–1527
2. Carmel-Harel, O., and Storz, G. (2000) Annu. Rev. Microbiol. 54, 439–461
3. Fridovich, I. (1989) J. Biol. Chem. 264, 7761–7764
4. Pedrazz, J. R., Kosmidou, E., Miranda-Vizuete, A., Gustafsson, J. A., Wright, A. P., and Syprou, G. (1999) J. Biol. Chem. 274, 6396–6373
5. Rodriguez-Manzaneque, M. T., Tamarit, J., Belli, G., Ros, J., and Herrero, E. (2002) Mol. Biol. Cell 13, 1109–1121
6. Pedrazz, J. R., Porras, P., Martinez-Galisteo, E., Padilla, C. A., Miranda-Vizuete, A., and Barrena, J. A. (2002) Biochim. J. 364, 617–623
7. Miranda-Vizuete, A., Dandimopoulos, A. E., and Syprou, G. (2000) Antioxid. Redox Signal. 2, 811–810
8. Lundberg, M., Johansson, C., Chandra, J. Enskoss, M., Jacobsson, G., Ljung, J., Johansson, M., and Holmgren, A. (2001) J. Biol. Chem. 276, 26269–26275
9. Gladyshev, V. N., Liu, A., Novoselov, S. V., Krysan, K., Sun, Q. A., Kryukov, V. M., Kryukov, G. V., and Lou, M. F. (2001) J. Biol. Chem. 276, 30374–30380
10. Johnsr, H., Krems, B., Kotter, P., and Entian, K. D. (1996) Mol. Gen. Genet. 252, 456–464
11. Pandolfi, P. P., Sinati, F., Fisi, R., Mason, P., Grosveld, F., and Luzzatto, L. (1995) EMBO J. 14, 5299–5310
12. Oufett, C. E., and Culotta, V. C. (2003) EMBO J. 22, 2015–2024
13. Jo, S. H., Son, M. K., Koh, H. J., Lee, S. M., Song, I. H., Kim, Y. O., Lee, Y. S., Jeong, K. S., Kim, W. B., Park, J. W., Song, B. J., Huh, T. L., and Huie, T. L. (2003) J. Biol. Chem. 276, 1616–16176
14. Sturtz, L. A., Diekert, K., Jensen, L. T., Lill, R., and Culotta, V. C. (2001) J. Biol. Chem. 276, 38084–38089
15. Griffith, O. W., and Meister, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4668–4672
16. Olafsdottir, K., and Reed, D. J. (1988) Biochim. Biophys. Acta 964, 377–382
17. Mhembha, F., Heubron, A., Raes, M., and Remacle, J. (1985) Biochim. Biophys. Acta 838, 211–220
18. Muller, E. G. (1996) Mol. Biol. Cell 7, 1805–1813
19. Trotter, E. W., and Grant, C. M. (2000) EMBO Rep. 1, 184–188
20. Sherman, F., Pink, G. R., and Lawrence, C. W. (1978) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Gietz, R. D., and Schiestl, R. H. (1991) Yeast 7, 253–263
22. Robb, A. E., Srinivasan, M., McCaffery, J. M., and Jensen, R. E. (2001) J. Biol. Chem. 152, 401–410
23. Daum, G., Bohn, P. C., and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033
24. Jensen, L. T., and Culotta, V. C. (2000) Mol. Cell. Biol. 20, 3918–3927
25. Anderson, M. E. (1985) Methods Enzymol. 113, 548–555
26. Cuzin, J. W., and Kaiser, C. A. (1999) Nat. Cell Biol. 1, 130–135
27. Collinson, L. P., and Dawes, I. W. (1995) Gene (Amst.) 152, 123–127
28. Kelner, M. J., and Montoya, M. A. (2000) Biochim. Biophys. Res. Commun. 269, 366–368
29. Tamura, T., McIsaac, H. W., Smith, C. V., and Hansen, T. N. (1997) Biochem. Biophys. Res. Commun. 237, 419–422
30. Neupert, W. (1997) Annu. Rev. Biochem. 66, 863–917
31. Schatz, G., and Butow, R. A. (1983) Cell 32, 316–318
32. Emanuelsson, O., Nielsen, N., Brunak, S., and von Heijne, G. (2000) J. Mol. Biol. 300, 1005–1016
33. Lenton, K. J., Therriault, H., and Wagner, J. R. (1999) Anal. Biochem. 274, 125–130
34. Stevens, B. J. (1977) Biol. Cell 28, 37–56
35. Kozak, M. (1978) Cell 15, 1109–1123
36. Stein, L., Peleg, Y., Even-Ram, S., and Pines, O. (1994) Mol. Cell. Biol. 14, 4770–4778
37. Knox, C., Sass, E., Neupert, W., and Pines, O. (1998) J. Biol. Chem. 273, 25474–25491
38. Siss, E., Blachinsky, K., Karnaule, S., and Pines, O. (2001) J. Biol. Chem. 276, 4611–46117
39. Kozak, M. (2002) Gene (Amst.) 299, 1–34
40. Kozak, M. (1994) Nature 370, 241–246
41. Kozak, M. (1986) Cell 44, 283–292
42. Cigan, A. M., and Donahue, T. F. (1987) Gene (Amst.) 50, 1–18
43. Chen, Z., and Losh, L. H. (1996) J. Pharmacol. Exp. Ther. 265, 608–618
44. Green, R. C., and O’Brien, P. J. (1970) Biochim. Biophys. Acta 197, 31–39