Regulation of Profilin Localization in Saccharomyces cerevisiae by Phosphoinositide Metabolism*

(Received for publication, July 24, 1995, and in revised form, September 15, 1995)

Darin B. Ostrander‡, Jessica A. Gorman§, and George M. Carman¶

From the ‡Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903 and ¶Department of Microbial Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000

Profilin is an actin- and phosphatidylinositol 4,5-bisphosphate-binding protein that plays a role in the organization of the cytoskeleton and may be involved in growth factor signaling pathways. The subcellular localization of profilin was examined in the yeast Saccharomyces cerevisiae. Immunoblot analysis showed that profilin was localized in both the plasma membrane and cytosolic fractions of the cell. Actin was bound to the profilin localized in the cytosol. The association of profilin with the membrane was peripheral and mediated through interaction with phospholipid. The phospholipid dependence of profilin for membrane binding was examined in vitro using pure profilin and defined unilamellar phospholipid vesicles. The presence of phosphatidylinositol 4,5-bisphosphate in phospholipid vesicles was required for maximum profilin binding. Moreover, the binding of profilin to phospholipid vesicles was dependent on the surface concentration of phosphatidylinositol 4,5-bisphosphate. The subcellular localization of profilin was examined in vivo under growth conditions (i.e. inositol starvation of ino1 cells and glucose starvation of respiratory deficient cells) where plasma membrane levels of phosphatidylinositol 4,5-bisphosphate were depleted. Depletion of plasma membrane phosphatidylinositol 4,5-bisphosphate levels resulted in a translocation of profilin from the plasma membrane to the cytosolic fraction. Profilin translocated back to the membrane fraction from the cytosol under growth conditions where plasma membrane levels of phosphatidylinositol 4,5-bisphosphate were replenished. These results suggested that phosphoinositide metabolism played a role in the localization of profilin.

Profilin is a ubiquitous actin- and PIP$_2^{±}$-binding protein found in eucaryotic cells (1). It is believed that profilin assists in the regulation of the reorganization of the microfilament cytoskeleton (2). Profilin competes with other actin-binding proteins for globular actin (3), catalyzing the exchange of nucleotide phosphates on monomeric actin, thereby activating actin for polymerization (4).

Profilin has also been shown to bind anionic phospholipids (5). However, at physiological salt concentrations, profilin binds exclusively to PIP$_2$ (6). Profilin cannot bind to actin and PIP$_2$ simultaneously (7), as the actin- and PIP$_2$-binding sites are sufficiently close as to impart mutually exclusive binding (7). The interaction of profilin with PIP$_2$ may regulate the availability of profilin for interaction with actin (5). Another role proposed for profilin's association with PIP$_2$ is to prevent the phospholipase C$_1$-mediated hydrolysis of PIP$_2$ (8), and thus the generation of the second messengers inositol trisphosphate and diacylglycerol (9). However, tyrosine phosphorylation of phospholipase C$_1$ allows hydrolysis of PIP$_2$ even in the presence of profilin (10).

We are using the yeast Saccharomyces cerevisiae as a model eucaryote to study profilin. The gene (PFY1) encoding for profilin has been cloned from S. cerevisiae (11). S. cerevisiae profilin resembles profilins from higher eucaryotes in size (~15 kDa), ability to bind actin, PIP$_2$, and polyproline, and inhibit ATP hydrolysis by monomeric actin (11–13). Moreover, profilin is required for the proper organization of the actin cytoskeleton into actin cables, which occur at regions of active growth and for proper maintenance of cell polarity (12). It is unclear whether these roles for profilin are related to its ability to bind actin and/or PIP$_2$ (13). The physiological relevance of profilin's ability to bind polyproline is also unclear (13).

The fact that binding to actin and PIP$_2$ are mutually exclusive in profilin, and that actin is localized in the cytosol (14) whereas PIP$_2$ is localized in the plasma membrane (15), raises the suggestion that profilin is localized in these two compartments of the cell. In this report we demonstrated that profilin was indeed localized to the plasma membrane and cytosolic fractions of S. cerevisiae. The majority of profilin was bound to plasma membranes. However, under growth conditions where the plasma membrane levels of PIP$_2$ were depleted, profilin was found to translocate from the membrane to the cytosol. Profilin translocated back to the membrane fraction from the cytosol under growth conditions where plasma membrane levels of phosphatidylinositol 4,5-bisphosphate were replenished. The results of our studies were consistent with the notion that the metabolism of PIP$_2$ played a role in the localization of profilin.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were reagent grade. Growth medium supplies were purchased from Difco Laboratories. ATP, phospholipids, phenylmethanesulfonyl fluoride, benzamide, aprotonin, leupeptin, pepstatin, and bovine serum albumin were obtained from Sigma. Protein assay reagent was purchased from Bio-Rad. SDS-polyacrylamide gel electrophoresis and immunochemical reagents were from Life Technologies, Inc. Sephadex G-50 superfine was purchased from Pharmacia Biotech. Octyl-$β$-D-glucopyranoside (octyl glucoside) was from Calbiochem. Cent
Localization of Yeast Profilin

Methods

Strains and Growth Conditions—Strain W303-1A (MATa ade2-1, his3-11, 15, leu2-3,112, trpl-1, ura3-1, can1-100) was from Rodney Rothstein and used for profilin localization studies. Strains on the translocation of profilin in response to changes in phosphoinositide metabolism were performed with the ino1 strain MC13 (MATa ino1-13 lys2) (16) and respiratory deficient strain SDO44, which was selected on ethidium bromide medium (17) using strain Y294 (MATa his3-11,12 leu2-3,112 ura3-52 trpl-289) (18). Cells were grown to the exponential phase of growth at 30 °C in synthetic complete medium with 2% glucose as the carbon source (17). The growth medium for ino1 cells also contained 75 μM inositol. Profilin was purified from strain SDO17 (MATa his3, leu2-3,112, trpl ura3, pfr1-LEU2) bearing the multiplicity plasmid pDO3. Plasmid pDO3 was derived from plasmid pH101 (19) and contains the open reading frame of profilin by Sephadex G-50 superfine column chromatography (32) or by affinity adsorbent. The constructions of strain SDO17 and plasmid pDO3 will be described elsewhere. Cells used for the purification of profilin were grown in synthetic complete medium containing 2% raffinose as the carbon source. Profilin production was induced by the addition of 2% galactose to the growth medium.

Purification of Profilin and the Preparation of Anti-profilin Antibodies—Profilin was purified to apparent homogeneity using polyproline affinity chromatography as described by Haarer et al. (12). Antiserum to profilin was raised in New Zealand White rabbits by standard procedures (20). Anti-profilin antibodies were purified from the antiserum as described by Olimsted (21) using nitrocellulose-bound profilin as an affinity adsorbent.

Preparation of Subcellular Fractions—All steps were carried out at 5 °C. Cell extracts were prepared by disruption with glass beads using a Bead-Beater (BioSpec Products) in buffer A (50 mM Tris-HCl (pH 7.5), 1 mM Na2EDTA, 0.3 M sucrose, 10 mM 2-mercaptoethanol, 0.5 mM g-mercaptoethanol, and 5 mM benzamide) as described previously (22). Unbroken cells and glass beads were removed by centrifugation at 1,500 × g for 5 min. Mitochondrial (23) and microsomal (22) fractions were collected from cell extracts by differential centrifugation. The postmitochondrial supernatant was used as the cytosolic fraction. The cytosolic fraction was concentrated with a Centricon-10 concentration filter. Plasma membranes were isolated by the method of Serrano (24) as modified by Monk et al. (25). Measurement of plasma membrane (vanadate-sensitive) ATPase activity (26) was used to assay the purity of plasma membranes. Vanadate-sensitive ATPase activity accounted for 90% of the total ATPase activity in plasma membranes. Membrane fractions were incubated in buffer A to a volume equivalent to that of the cytosolic fraction.

Electrophoresis and Immunoblotting—SDS-polyacylamide gel electrophoresis (27) was performed with 10–20% gradient slab gels. Immunoblot assays (28) were performed with anti-profilin antibodies and anti-actin antibodies (12) using a chemiluminescent detection system (29). The density of bands on immunoblots were quantified by scanning densitometry. Protein molecular mass standards were phosphorylase b (92.5 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.3 kDa).

Preparation of Phospholipid Vesicles—Large (average diameter = 90 nm) unilamellar phospholipid vesicles were prepared in 50 mM Tris-HCl buffer (pH 8.0) by removing octyl glucoside from octyl glucoside/phospholipid (molar ratio of 15:1) mixed micelles (30) by Sephadex G-50 superfine column chromatography (31, 32). Pure phospholipids and phospholipids extracted (33) from yeast plasma membranes were used for the preparation of phospholipid vesicles. The phospholipid composition of the vesicles is indicated in figure legends.

Analysis of Profilin Binding to Plasma Membranes and Phospholipid Vesicles—Affinity-purified profilin (5 μg) was incubated for 30 min at room temperature with the indicated concentrations of plasma membranes or phospholipid vesicles in 50 mM Tris-HCl buffer (pH 8.0) at a total volume of 0.5 ml. Following incubation, the profilin bound to plasma membranes or phospholipid vesicles was separated from unbound profilin by Sephadex G-50 superfine column chromatography (32) or by centrifugation at 100,000 × g for 1 h. Both methods for the separation of bound and unbound profilin gave the same results. The amount of bound and unbound profilin was quantitatively determined by scanning densitometry of immunoblots using pure profilin to generate a standard curve. The curve was linear over the concentration range of 50–5000 ng of profilin. The lower limit of sensitivity for the binding assay was 6 μg of profilin/mg of phospholipid phosphate. Profilin binding assays were conducted in triplicate with an average standard deviation of ± 3%. The amount of profilin bound to membranes and phospholipid vesicles was normalized to phospholipid phosphate.

Analysis of Protein and Phospholipid Phosphate—Protein was determined by the method of Bradford (34) using bovine serum albumin as the standard. The phosphate concentration of membranes and phospholipid vesicles was determined by the method of Bartlett (35).

RESULTS

Subcellular Localization of Profilin—Affinity-purified anti-profilin antibodies were prepared and used to examine the subcellular localization of profilin. The anti-profilin antibodies could be used to immunoprecipitate profilin from cell extracts (data not shown) and to analyze profilin by immunoblotting (Fig. 1A). Immunoblot signals were optimized by analyzing a number of antigen and antibody concentrations and were in the linear range of detectability. Profilin was localized to the total membrane and cytosolic fractions of the cell (Fig. 1A). About 80% of the total amount of profilin was associated with membranes; whereas the remainder was associated with the cytosolic fraction (Fig. 1B). Subcellular membrane fractions were isolated and examined for the association of profilin. Essentially all of the membrane-associated profilin was found with...
the plasma membrane fraction (Fig. 1C).

We next questioned whether the association of profilin to membranes was integral or peripheral. Peripheral membrane proteins are associated with membranes through noncovalent interactions and can be released by treatment with high ionic strength. Integral membrane proteins are more tightly associated and cannot be released by changes in ionic strength. Membranes were treated with NaCl, and the amount of profilin remaining bound to the membrane was analyzed. Treatment with NaCl resulted in a dose-dependent dissociation of profilin from the membranes (Fig. 2). Maximum dissociation was obtained with 0.1 M NaCl. Thus, profilin was a peripheral membrane protein.

Since profilin is an actin-binding protein (1), we questioned whether the cytosolic-associated profilin was bound to actin in the cytosol. Profilin was purified from the cytosolic fraction by affinity chromatography with polyproline-Sepharose (12). The polyproline-Sepharose chromatography procedure was modified such that the column was not washed with buffer containing 3 M urea prior to the elution of profilin with 6 M urea (12). In this way, if profilin was bound to actin in the cytosol, it should remain bound to actin until dissociated from the polyproline-Sepharose column with 6 M urea. The actin- and polyproline-binding sites on profilin are not the same (13). The purified profilin preparation was subjected to immunoblot analysis using affinity-purified anti-actin antibodies. Indeed, actin was present in the profilin preparation purified from the cytosolic fraction (Fig. 3).

Binding of Pure Profilin to Plasma Membranes and Phospholipid Vesicles Prepared from Plasma Membrane Phospholipids—The nature of profilin’s association with the plasma membrane was examined by binding studies using pure profilin and plasma membranes. Plasma membranes were washed with 0.5 M NaCl to remove profilin and other peripheral membrane proteins. Pure profilin was then incubated with the salt-washed plasma membranes, and the amount of profilin which bound to the membranes was analyzed. Profilin bound to plasma membranes in a concentration-dependent manner (Fig. 4). Maximum profilin binding was obtained with 80 μg/ml phospholipid phosphate. The concentration of plasma membranes that gave half-maximum binding was 30 μg/ml of phospholipid phosphate. These results indicated that the association of profilin with plasma membranes was not through interaction with other peripheral membrane proteins.

Phospholipids were extracted from plasma membranes and used for the preparation of phospholipid vesicles. These vesicles were used to examine profilin binding using phospholipid phosphate concentrations similar to that used in the experiment shown in Fig. 4. Profilin bound to these phospholipid vesicles in a concentration-dependent manner similar to that found with the salt-washed plasma membranes (Fig. 5). Profilin binding to these vesicles saturated at 80 μg/ml phospholipid phosphate and half-maximum binding was at 38 μg/ml phospholipid phosphate. The results of this experiment indicated that the association of profilin with plasma membranes was not through interaction with integral membrane proteins. Thus, the association of profilin with the plasma membrane was through interaction with membrane phospholipids.

Effect of Phospholipid Vesicle Composition on Profilin Binding—We examined the effect of phospholipid composition on the ability of profilin to bind vesicles. Phosphatidylcholine vesicles were prepared with phospholipids known to be present in the plasma membranes of S. cerevisiae (36, 37). Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidate are plasma membrane phospholipids that are also localized in most cellular organelles (37). PIP and PIP₂ are minor phospholipids localized in the plasma membrane (15). The only phospholipid vesicles that showed the ability to bind a significant amount of profilin were those containing PIP₂ (Fig. 6). The addition of PIP to PC:PIP₂ vesicles did not have a major effect on profilin binding (Fig. 6). Binding to PC:PIP₂ vesicles was dependent on the concentration of profilin (Fig. 7). The Kᵣ value for profilin was 0.13 μM.

Profilin Binding to PC:PIP₂ Vesicles Follows Surface Dilution Kinetics—Lipid-dependent enzymes, which bind their substrates at a membrane surface, follow surface dilution kinetics.
parameters of lipid-dependent enzymes (38, 39). Profilin was face and bulk concentrations of lipid when defining kinetic (38). Surface dilution kinetics takes into account both the sur-

pure profilin (5 µg) was incubated with the indicated concentrations of phospholipid vesicles. The amount of profilin bound to the phospholipid vesicles was determined as described under “Experimental Procedures.” The data shown are representative of two independent experiments.

Profline to PC:PIP2 vesicles were incubated with the indicated phospholipid vesicles. The amount of profilin bound to the phospholipid vesicles was determined as described under “Experimental Procedures.” The data shown are representative of two independent experiments.

Effect of phospholipid composition on the binding of profilin to phospholipid vesicles. Pure profilin (5 µg) was incubated with the indicated phospholipid vesicles. The amount of profilin bound to the phospholipid vesicles was determined as described under “Experimental Procedures.” The molar ratio of the phospholipids in the vesicles was indicated. The final phospholipid concentration (100 µg/ml phospholipid phosphate) of all vesicles was the same. The data shown are representative of two independent experiments.

Effect of profilin concentration on the binding of profilin to PC:PIP2 vesicles. Pure profilin (5 µg) was incubated with PC:PIP2 vesicles with the indicated surface concentrations of PIP2. The final phospholipid concentration of the vesicles was 100 µg/ml phospholipid phosphate. The amount of profilin bound to phospholipid vesicles was determined as described under “Experimental Procedures.” The data shown are representative of two independent experiments.

Profline to PC:PIP2 vesicles was indeed dependent on the surface concentration of PIP2, (i.e. at a bulk vesicle concentration of 100 µg/ml phospholipid phosphate, Figs. 4 and 5). Profilin binding to PC:PIP2 vesicles was indeed dependent on the surface concentration of PIP2 (Fig. 8). The binding dependence of profilin to PIP2 appeared cooperative with a Hill number of 2. However, these data do not rule out a bimolecular reaction. The concentration of PIP2 that gave half-maximum binding was 8.5 mol %.

Effect of Inositol Starvation on the Subcellular Localization of Profilin—We sought evidence that the association of profilin with the plasma membrane was dependent on the concentration of PIP2 in vivo. The availability of the ino1 mutant facilitated experiments to address this question. Strain MC13 is auxotrophic for inositol due to a mutation in the INO1 gene encoding for inositol-1-phosphatesynthase (40, 41). When ino1 cells are deprived of inositol, they undergo changes in their metabolism which ultimately result in a loss of cell viability, commonly referred to as “inositol-less death” (42, 43). Among the early biochemical consequences of inositol starvation of ino1 cells is a 6-fold decrease in the plasma membrane levels of PIP and PIP2 (36). PIP and PIP2 account for 0.6 and 2 mol %, respectively, of the total plasma membrane phospholipids in S. cerevisiae (36). PIP and PIP2 are derived from inositol via phosphatidylinositol synthase and the phosphoinositide kinases (44). Plasma membrane-associated phosphatidylinositol 4-kinase has been shown to be inhibited during inositol starvation of ino1 cells (36). Inositol-supplemented ino1 cells were grown to the exponential phase of growth. Cells were washed and then incubated for 4 h in fresh growth medium lacking inositol. As reported previously (43), the inositol-starved cells grew equally as well as the control inositol-supplemented cells during the 4-h incubation period. Following the incubation, the association of profilin with the membrane and cytosolic fractions of the cells was analyzed. This analysis showed that the percentage of profilin bound to membranes decreased from 83% to 66% upon inositol starvation (Fig. 9). At the same time, the amount of profilin associated with the cytosol increased from 17% to 34% (Fig. 9).

Effect of Glucose Starvation on the Subcellular Localization of Profilin—To further support the notion that PIP2 metabo-
Localization of Yeast Profilin

Phospholipids found in mellar phospholipid vesicles. The vesicles were prepared with the phospholipids from S. cerevisiae plasma membranes. Under the conditions of our experiments, binding of profilin to these vesicles was dependent on the presence of PIP₂. The dissociation constant for profilin binding to PC:PIP₂ vesicles (0.13 μM) was in the range reported for profilin from higher eucaryotes (5, 8). In addition, profilin binding to PC:PIP₂ vesicles was dependent on the surface concentration of PIP₂. Thus, profilin followed surface dilution kinetics (39), a characteristic common to lipid-dependent enzymes that bind lipids at a membrane surface (38).

The physiological relevance of the dependence of profilin binding on the surface concentration of PIP₂ was addressed by two independent approaches using S. cerevisiae strains where the plasma membrane levels of PIP₂ are known to fluctuate under certain growth conditions. Inositol starvation of ino1 cells (36, 43) and glucose starvation of respiratory deficient cells (45) were used to deplete plasma membrane levels of PIP₂. After a 4-h starvation period of ino1 cells for inositol, a small but reproducible amount of profilin was found to translocate from membranes to the cytosol. Inositol starvation also causes fluctuations in the plasma membrane levels of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinerine, and phosphatidate, and PIP (36). However, it was unlikely that the translocation of profilin was due to changes in these phospholipids. None of these phospholipids had a significant effect on binding of pure profilin to vesicles. In a more dramatic fashion, glucose starvation of respiratory deficient cells over a 20-min period caused a significant amount of profilin to translocate from membranes to the cytosol. Moreover, the addition of glucose back to glucose-starved cells caused profilin to translocate back to membranes from the cytosol. These translocations occurred too rapidly to be attributed to a genetic mechanism. Taken together, these results raised the suggestion that the plasma membrane concentration of PIP₂ played a role in the regulation of profilin localization. Our studies did not rule out the possible regulation of profilin localization by other plasma membrane-associated components or other cellular factors (e.g. cytoskeleton), which could have changed in response to inositol starvation of ino1 cells or glucose starvation of respiratory deficient cells.

In summary, this work addressed the fundamental question of where profilin was localized in the cell. We demonstrated that profilin was localized to the plasma membrane and cytosolic fractions and that profilin binding to the membrane was dependent on PIP₂. It is known that PIP₂ levels change under...
various growth conditions. Our studies showed that conditions which affect PIP2 metabolism played a role in profilin's localization. Future studies will be directed toward gaining insight into the role PIP2 metabolism plays in profilin function.

Acknowledgments—We thank Susan S. Brown for providing us with anti-actin antibodies. We also acknowledge Virginia M. McDonough and Thom LaVoie for helpful suggestions in the preparation of this paper.

REFERENCES

1. Machesky, L. M., and Pollard, T. D. (1993) Trends Cell Biol. 3, 381–385
2. Theriot, J. A., and Mitchison, T. J. (1993) Cell 75, 825–838
3. Pantaloni, D., and Carlier, M. (1993) Cell 75, 1007–1014
4. Goldschmidt-Clermont, P. J., Machesky, L. M., Doberstein, S. K., and Pollard, T. D. (1991) J. Cell Biol. 113, 1081–1089
5. Arai, K., and Lindberg, U. (1985) J. Cell Biol. 104, 1449–1460
6. Machesky, L. M., Goldschmidt-Clermont, P. J., and Pollard, T. D. (1990) J. Biol. Chem. 265, 1575–1578
7. Metzler, W. J., Bell, A. J., Ernst, E., Lavoie, T. B., and Mueller, L. (1994) J. Biol. Chem. 269, 4620–4625
8. Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J., and Pollard, T. D. (1990) Science 247, 1575–1578
9. Berridge, M. J. (1987) Biochim. Biophys. Acta 907, 33–45
10. Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G., and Pollard, T. D. (1992) Science 255, 1231–1233
11. Machesky, L. M., Goldschmidt-Clermont, P. J., and Pollard, T. D. (1990) J. Cell Biol. 110, 105–114
12. Lassing, I., and Lindberg, U. (1985) J. Cell Biol. 100, 7077–7085
13. Haffey, M. J., Stevens, J. T., Terry, B. J., Dorsky, D. I., Crumpacker, C. S., Wietstock, S. M., Ruyechen, W. T., and Field, A. K. (1988) J. Cell Biol. 105, 949–958
14. Kilmartin, J. V., and Adams, E. M. (1984) J. Biol. Chem. 259, 4620–4625
15. Bullock, M. J., and Wortman, J. R. (1984) J. Biol. Chem. 259, 14688–14694
16. Culbertson, M. R., and Henry, S. A. (1975) J. Biol. Chem. 250, 5936–5942
17. Rose, M. D., Winston, F., and Heiter, P. (1990) Genetics 123, 911–917
18. Field, A. K., Stahl, J. C., Smith, M. I., and Field, A. K. (1988) J. Biol. Chem. 263, 1081–1089
19. Haffey, M. L., Stevens, J. T., Terry, B. J., Dorsky, D. I., Crumpacker, C. S., Wietstock, S. M., and Field, A. K. (1988) J. Cell Biol. 100, 9013–9020
20. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Olimsted, J. B. (1981) J. Biol. Chem. 256, 11955–11957
22. Fischl, A. S., and Carman, G. M. (1983) J. Biol. Chem. 258, 304–311
23. Bélédieu, G., Mangnall, D., Tung, B., Westley, J., and Getz, G. S. (1978) J. Biol. Chem. 253, 4555–4565
24. Serrano, R. (1988) Methods Enzymol. 157, 533–544
25. Monir, B. C., Kurtz, M. B., Maranin, J. A., and Perlin, D. S. (1991) J. Bacteriol. 173, 6626–6636
26. Koland, J. G., and Hammes, G. G. (1986) J. Biol. Chem. 261, 5936–5942
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Haid, A., and Sussa, M. (1983) Methods Enzymol. 96, 192–205
29. Schaap, A. P., Akhavan, H., and Romano, L. J. (1989) Clin. Chem. 35, 1863–1864
30. Mimms, L. T., Zampighi, G., Nolz, Y., Tanford, C., and Reynolds, J. A. (1981) Biochemistry 20, 833–840
31. Green, P. R., and Bell, R. M. (1975) J. Cell Biol. 64, 23–40
32. Henry, S. A., Atkinson, K. D., Kolat, A. J., and Culbertson, M. R. (1977) Biochemistry 16, 466–468
33. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
34. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
35. Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
36. Nickels, J. T., Jr., Buxeda, R. J., and Carman, G. M. (1994) J. Biol. Chem. 269, 11018–11024
37. Zinser, E., Speck, E., Kohlhoven, S. D., Paltauf, F., and Daum, G. (1991) J. Bacteriol. 173, 2026–2034
38. Carman, G. M., Deems, R. A., and Dennis, E. A. (1995) J. Biol. Chem. 270, 18711–18714
39. Deems, R. A., Eaton, B. R., and Dennis, E. A. (1975) J. Biol. Chem. 250, 9013–9020
40. Donahue, T. F., and Henry, S. A. (1981) J. Biol. Chem. 256, 7077–7085
41. Dock, J. D., and Henry, S. A. (1989) J. Biol. Chem. 264, 1274–1283
42. Henry, S. A., Atkinson, K. D., Kolat, A. J., and Culbertson, M. R. (1977) J. Bacteriol. 130, 472–484
43. Becker, G. W., and Lester, R. L. (1977) J. Biol. Chem. 252, 8684–8691
44. Paltauf, F., Kohlhoven, S. D., and Henry, S. A. (1992) in The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression (Jones, E. W., Pringle, J. R., and Broach, J. R., eds) pp. 415–500, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
45. Talwalkar, R. T., and Lester, R. L. (1973) Biochim. Biophys. Acta 306, 412–421
46. Buxeda, R. J., Nickels, J. T., Jr., and Carman, G. M. (1993) J. Biol. Chem. 268, 6248–6255