Phosphorylation and Regulation of CTP Synthetase from *Saccharomyces cerevisiae* by Protein Kinase A

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The phosphorylation and regulation of the *URA7*-encoded CTP synthetase (EC 6.3.4.2, UTP:ammonia ligase (ADP-forming)) from *Saccharomyces cerevisiae* by cAMP-dependent protein kinase (protein kinase A) were examined. Protein kinase A is the principal mediator of signals transmitted through the RAS/cAMP pathway in *S. cerevisiae*. The results of labeling experiments indicated that the phosphorylation of CTP synthetase was mediated by the RAS/cAMP pathway in vivo. In vitro, protein kinase A phosphorylated CTP synthetase at a serine residue with a stoichiometry consistent with one phosphorylation site per CTP synthetase subunit. Protein kinase A activity was dose- and time-dependent using CTP synthetase as a substrate. The dependence of protein kinase A activity on CTP synthetase cooperativity (*n* = 1.8) and the *K*ₘ value for CTP synthetase was 73 nm. Phosphorylation of CTP synthetase with protein kinase A resulted in the stimulation (190%) of activity. The mechanism of this stimulation included an increase in the *V*ₘₐₓ of the reaction with respect to UTP and ATP, a decrease in the *K*ₘ for ATP, and a decrease in the cooperative kinetic behavior of the enzyme. Phosphorylated CTP synthetase was less sensitive to product inhibition by CTP. Protein kinase C also phosphorylates and activates CTP synthetase. Phosphorylation of CTP synthetase with protein kinases A and C together resulted in an increase in CTP synthetase activity that was slightly greater than that obtained when the enzyme was phosphorylated with either protein kinase alone.

CTP synthetase (EC 6.3.4.2, UTP: ammonia ligase (ADP-forming)) is a glutamine aminotransferase that catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to the C-4 position of UTP to form CTP (1, 2). GTP activates the reaction by accelerating the formation of a covalent glutaminyl enzyme catalytic intermediate (2, 3). In the yeast *Saccharomyces cerevisiae*, CTP synthetase catalyzes the final step in the pyrimidine biosynthetic pathway (4). CTP synthetase is encoded by the *URA7* and *URA8* genes (4, 5). The deduced amino acid sequences of the open reading frames of the *URA7* and *URA8* genes show 78% identity and have predicted molecular masses of 64.7 and 64.5 kDa, respectively (4, 5). Purified native *URA7*- and *URA8*-encoded CTP synthetases exist as dimers that oligomerize to tetramers in the presence of their substrates, UTP and ATP (6, 7). Neither one of the *URA7* and *URA8* genes is essential provided that cells possess one functional gene encoding for CTP synthetase (4, 5). Phenotypic analysis of *ura7* and *ura8* mutants (5) and the characterization of the enzymological properties of the pure *URA7*- and *URA8*-encoded enzymes (6, 7) have shown that the two CTP synthetases are not functionally identical. In addition, the *URA7*-encoded CTP synthetase is 2-fold more abundant when compared with the *URA8*-encoded enzyme (7) and is responsible for the majority of the CTP synthesized *in vivo* (5).

The product of the CTP synthetase reaction, CTP, is required for the synthesis of RNA, DNA, dialglycoproteins, and membrane phospholipids (8). Thus, the level of CTP synthetase activity would be expected to play a major role in cell growth and physiology. In fact, data indicate that the expression of CTP synthetase and the relative synthesis of CTP play a role in the regulation of the pathways by which phospholipids are synthesized in *S. cerevisiae* (9).

CTP synthetase activity in *S. cerevisiae* is regulated by CTP product inhibition (6, 7) and by phosphorylation (10, 11). The phosphorylation of the *URA7*-encoded CTP synthetase by protein kinase C *in vitro* results in a stimulation of CTP synthetase activity (10, 11). Data from labeling experiments with mutant cells devoid of protein kinase C indicate that protein kinase C is not the only kinase involved in the phosphorylation of CTP synthetase (11). The examination of the deduced amino acid sequence of the CTP synthetase encoded by the *URA7* gene revealed that the enzyme has a potential target site for protein kinase A. Protein kinase A is the principal mediator of signals transmitted through the RAS/cAMP pathway in *S. cerevisiae* (12, 13). In this work, we demonstrated that the activation of the RAS/cAMP pathway can mediate the phosphorylation of CTP synthetase* in vivo*. Moreover, pure CTP synthetase was a substrate for protein kinase A, and the phosphorylation of CTP synthetase resulted in the stimulation of CTP synthetase activity. The mechanism of stimulation involved an increase in the *V*ₘₐₓ of the reaction and an increase of the enzyme affinity for ATP.

**EXPERIMENTAL PROCEDURES**

*Materials—* All chemicals were reagent grade. Growth medium supplies were purchased from Difco. Nucleotides, L-glutamine, phenylmethanesulfonyl fluoride, benzamidine, aprotinin, leupeptin, pepstatin, nitrocellulose paper, casein, histone (type III-S), phosphoamino acids, TPCK-trypsin,¹ alkaline phosphatase-agarose, and bovine serum albumin were purchased from Sigma. Protein kinase A catalytic subunit (bovine heart) and protein kinase C (rat brain) were purchased from Promega. Radiochemicals were purchased from DuPont NEN. Scintillation counting supplies were purchased from National Diagnostics, Inc. Protein assay reagent, molecular mass standards for SDS-polyacrylamide gel electrophoresis, and electrophoresis reagents were purchased.

¹ The abbreviation used is: TPCK, 1-tosylamido-2-phenylethyl chloromethyl ketone.

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chased from Bio-Rad. Protein A-Sepharose CL-4B was purchased from_phosphotyrosine, and the supernatant was again removed and retained. The gel slices were then incubated in water for 1 h at 37 °C. The mixture was then centrifuged, and the supernatant was collected and added to the previously retained supernatants. The combined supernatants were dried in vacuo. Samples were resuspended in 1 ml of water and dried again. This process was repeated four times. The samples were resuspended in 10 μl of 1% ammonium carbonate, clarified by centrifugation, and spotted on cellulose thin-layer chromatography plates (22). Separation of phosphorylase tides was accomplished by electrophoresis in 1% ammonium bicarbonate at 1000 V for 35 min, followed by ascending chromatography (in butanol:ethanol:acetic acid:water, 10:3:12:15) for 7 h (22). Dried plates were then subjected to phosphoimaging analysis.

Dephosphorylation of CTP Synthetase—Alkaline phosphatase attached to beaded agarose was used to dephosphorylate CTP synthetase. A 0.4-ml column of alkaline phosphatase-agarose (1,000 μmol/min/ml of resin) was equilibrated with 5 ml of chromatography buffer (50 mM Tris-HCl buffer, pH 8.0, 1 mM MgCl₂, 1 mM MnCl₂, and 10% glycerol) containing 0.1 mg/ml ovalbumin. Ovalbumin was included in the chromatography buffer to block nonspecific protein binding sites on the column. The column was then washed with 5 ml of chromatography buffer without ovalbumin. CTP synthetase was applied to the column and incubated for 10 min. The column was then washed with chromatography buffer to elute CTP synthetase.

Analysis of Kinetic Data—Kinetic data were analyzed according to the Michaelis-Menten and Hill equations using the EZ-FIT enzyme kinetic model fitting program (24). EZ-FIT uses the Nelder-Mead simplex and Marquardt/Nash nonlinear regression algorithms sequentially and tests for the best fit of the data among different kinetic models.

RESULTS AND DISCUSSION

Effect of the RAS/cAMP Pathway on the Phosphorylation of CTP Synthetase in Vivo—In S. cerevisiae, RAS proteins stimulate adenylyl cyclase activity and cAMP formation (12, 13). In turn, cAMP activates protein kinase A activity by causing the dissociation of the regulatory subunit from the catalytic subunit of the enzyme (12, 13). To examine whether the phosphorylation of CTP synthetase was mediated by protein kinase A in vivo, the extent of enzyme phosphorylation was measured in cells that were activated in the RAS/cAMP pathway. The RAS/cAMP pathway is known to be activated by the addition of glucose to nonfermenting cells (13, 16). Glucose triggers a rapid increase in cAMP levels and protein kinase A activity (13). OR8 cells bearing the single-copy plasmid pFL38-URA7 were grown in YEPA medium to attenuate the RAS/cAMP pathway (16). Cells were then incubated for 3 h with 32P, to detect phosphorylated CTP synthetase and [U-14C]-L-amino acid incorporation for the amount of CTP synthetase isolated. The RAS/cAMP pathway was then activated by the addition of 5% glucose to the growth medium (16). CTP synthetase was isolated by immunoprecipitation, and the amount of each label incorporated
We examined the phosphorylation of CTP synthetase by protein kinase A in vitro—We examined the phosphorylation of CTP synthetase by protein kinase A in vitro using the bovine heart catalytic subunit. The bovine heart protein kinase A catalytic subunit is structurally and functionally identical to the S. cerevisiae protein kinase A catalytic subunit (25). The protein kinase A preparation used in our studies was judged to be essentially pure as determined by SDS-polyacrylamide gel electrophoresis. This protein kinase A preparation phosphorylated casein with the activity stated by the manufacturer under the assay conditions used here.

To examine whether CTP synthetase was a substrate for phosphorylation by protein kinase A, we determined if protein kinase A catalyzed the incorporation of the γ-phosphate of [γ-32P]ATP into purified CTP synthetase. After the phosphorylation reaction, samples were subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. Autoradiography of the nitrocellulose paper showed that CTP synthetase was a substrate for protein kinase A. The position of [γ-32P]labeled CTP synthetase on the nitrocellulose paper was confirmed by immunoblot analysis. Protein kinase A activity was dependent on the concentration of protein kinase A (Fig. 2A) and the time of the reaction (Fig. 2B) using CTP synthetase as substrate. The dependence of protein kinase A activity on the concentration of CTP synthetase was examined. Protein kinase A activity did not follow typical saturation kinetics. Instead, the data followed a sigmoidal kinetic pattern (Fig. 3). The analysis of the data according to the Hill equation yielded a Hill number of 1.8. This cooperativity was consistent with the observation that native CTP synthetase exists as a dimer (6). The $K_m$ value for CTP synthetase was 11 μg/ml (73 nm for the dimeric enzyme). Based on its relative $K_m$ value, CTP synthetase was an excellent substrate for the protein kinase A reaction (26).

To determine the stoichiometry of the phosphorylation of CTP synthetase by protein kinase A, the phosphorylation reaction was carried out to completion. At the point of maximum phosphorylation, protein kinase A catalyzed the incorporation of 0.57 mol of phosphate/mol of dimeric CTP synthetase. The purified CTP synthetase was already phosphorylated to some extent (10). Therefore, we examined the stoichiometry of the reaction using dephosphorylated CTP synthetase. Accordingly, CTP synthetase was treated with alkaline phosphatase to dephosphorylate the enzyme. Protein kinase A catalyzed the incorporation of 1.3 mol of phosphate/mol of the dephosphorylated dimeric CTP synthetase. These results suggested that there is one phosphorylation site per CTP synthetase subunit. Protein kinase A (27–29) is a serine/threonine-specific protein kinase. To examine which amino acid residues of CTP synthetase served as substrates for phosphorylation by protein kinase A, we determined if protein kinase A catalyzed the incorporation of the γ-phosphate of [γ-32P]ATP into purified CTP synthetase. After the phosphorylation reaction, samples were subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. Autoradiography of the nitrocellulose paper showed that CTP synthetase was a substrate for protein kinase A. The position of [γ-32P]labeled CTP synthetase on the nitrocellulose paper was confirmed by immunoblot analysis. Protein kinase A activity was dependent on the concentration of protein kinase A (Fig. 2A) and the time of the reaction (Fig. 2B) using CTP synthetase as substrate. The dependence of protein kinase A activity on the concentration of CTP synthetase was examined. Protein kinase A activity did not follow typical saturation kinetics. Instead, the data followed a sigmoidal kinetic pattern (Fig. 3). The analysis of the data according to the Hill equation yielded a Hill number of 1.8. This cooperativity was consistent with the observation that native CTP synthetase exists as a dimer (6). The $K_m$ value for CTP synthetase was 11 μg/ml (73 nm for the dimeric enzyme). Based on its relative $K_m$ value, CTP synthetase was an excellent substrate for the protein kinase A reaction (26).

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Protein Kinase A Phosphorylation of Yeast CTP Synthetase

**Fig. 3.** Dependence of protein kinase A activity on CTP synthetase concentration. Panel A, protein kinase A (0.25 nmol/min/ml) and [γ-32P]ATP were incubated with the indicated concentrations of CTP synthetase for 10 min. Following the incubations, samples were subjected to SDS-polyacrylamide gel electrophoresis, autoradiogram analysis, and autoradiography. A portion of an autoradiograph showing the position of CTP synthetase is shown. Lane 1. 14C-labeled protein molecular mass standards (from top to bottom) phosphorylase b (97.4 kDa) and bovine serum albumin (69 kDa). Lanes 2–10, concentrations of CTP synthetase indicated in panel B. Panel B, the incorporation of phosphate into CTP synthetase was determined by scintillation counting of phosphorylated enzyme excised from the immunoblot. The curve drawn was the result of the analysis of the data according to the Hill equation.

32P-Labeled CTP synthetase was also subjected to digestion with TPCK-trypsin, followed by thin-layer electrophoresis and chromatographic analysis. The protease digestion yielded one major phosphopeptide and some additional minor phosphopeptides (Fig. 4B).

**Effect of Phosphorylation on CTP Synthetase Activity**—We questioned whether the phosphorylation of CTP synthetase by protein kinase A altered CTP synthetase activity. To do this, CTP synthetase was phosphorylated by protein kinase A, and CTP synthetase activity was subsequently measured. In these experiments, CTP synthetase activity was measured with subsaturating concentrations of ATP and UTP (6). In this manner, we could simultaneously monitor for stimulatory or inhibitory effects of phosphorylation on CTP synthetase activity. Phosphorylation of CTP synthetase by protein kinase A resulted in a dose-dependent stimulation of CTP synthetase activity (Fig. 5). Maximum stimulation (190%) of CTP synthetase activity was obtained when the enzyme was phosphorylated with 1 nmol/min/ml protein kinase A (Fig. 5). The shortening of the Hill equation since the purified enzyme is already partially phosphorylated (10).

**Effect of Phosphorylation on the Kinetics of CTP Synthetase Activity**—Kinetic analyses were performed to further characterize the effects of protein kinase A phosphorylation of CTP synthetase on its activity in vitro. As indicated above, the CTP synthetase that we purified was already partially phosphorylated (10). Consequently, our kinetic studies on the native enzyme reflected the property of this phosphorylated state of the enzyme. We were unable to perform kinetic studies on the dephosphorylated form of CTP synthetase since alkaline phosphatase treatment of the purified enzyme resulted in the loss of its activity (10). CTP synthetase displays positive cooperative kinetics with respect to UTP and ATP (6). The cooperative kinetic behavior of the enzyme toward one substrate increases when the other substrate is present in the enzyme assay at a subsaturating concentration (6). We examined whether phosphorylation of CTP synthetase by protein kinase A altered these kinetic properties. The kinetic experiments were performed with saturating concentrations of glutamine, GTP, and magnesium ions. The effect of phosphorylation on the dependence of CTP synthetase activity on UTP was examined using subsaturating and saturating concentrations of ATP (Fig. 6). Phosphorylation of CTP synthetase resulted in an increase in the apparent V_max of the reaction with respect to UTP when the activity was measured with subsaturating or saturating concentrations of ATP (Table I). The effect of phosphorylation on the apparent V_max was more evident when the activity was measured with the subsaturating ATP concentration (Fig. 6). Phosphorylation of CTP synthetase did not affect the apparent K_m values or the Hill numbers for UTP when the activity was measured with subsaturating or saturating concentrations of ATP (Fig. 6 and Table I).

The effect of phosphorylation on the dependence of CTP synthetase activity on ATP using subsaturating and saturating concentrations of UTP was then examined. Phosphorylation of CTP synthetase resulted in a decrease in the apparent K_m.
values for ATP and a modest increase in the apparent $V_{\text{max}}$ values for the reaction (Fig. 7 and Table I). In addition, the phosphorylation of the enzyme resulted in a decrease of the positive cooperative kinetic behavior of the enzyme toward ATP when compared with the native enzyme (Fig. 7 and Table I). The effects of phosphorylation on the kinetic properties of the enzyme were more evident when CTP synthetase activity was measured with the subsaturating UTP concentration (Fig. 7).

GTP stimulates native CTP synthetase activity by increasing the $V_{\text{max}}$ of the reaction and decreasing the $K_m$ value for glutamine (6). The enzyme also exhibits negative cooperative kinetics toward glutamine as reflected in Hill numbers of less than 1 (6). We examined if phosphorylation by protein kinase A influenced these kinetic properties. Kinetic experiments were performed with saturating concentrations of UTP, ATP, and magnesium ions. Phosphorylation of the enzyme did not have a significant effect on the apparent $V_{\text{max}}$ and $K_m$ values of the enzyme with respect to glutamine when measured in the absence or presence of GTP (Table I). However, the phosphorylation of CTP synthetase abolished the negative cooperative behavior of the enzyme with respect to glutamine (Table I). The effect of phosphorylation on the stimulation of CTP synthetase activity by GTP was examined using saturating concentrations of all substrates. Phosphorylation of CTP synthetase resulted in a small decrease in the apparent activation constant ($K_a$) for GTP (Table I).

**Effect of Phosphorylation on the Inhibition of CTP Synthetase Activity by CTP** —An important biochemical property of the URA7-encoded CTP synthetase is the inhibition of its activity by CTP (6). The inhibition of CTP synthetase activity by CTP regulates the cellular concentration of CTP in growing cells (6, 9). We questioned whether phosphorylation affected this property. The phosphorylated and native forms of CTP synthetase were inhibited by CTP in a dose-dependent manner when activity was measured with 0.5 mM ATP (Fig. 8A) and 1 mM ATP (Fig. 8B). However, the protein kinase A phosphorylated enzyme was less sensitive to CTP inhibition when compared with the native enzyme (Fig. 8 and Table I). The decrease in the sensitivity of the enzyme to CTP inhibition was more evident when CTP synthetase activity was measured with the subsaturating ATP concentration of 0.5 mM (Fig. 8A).

CTP inhibits CTP synthetase activity by increasing the positive cooperativity of the enzyme toward UTP and increasing the apparent $K_m$ value for UTP (6). We questioned what effect phosphorylation of CTP synthetase had on this kinetic property. In these experiments, the concentration of ATP was held constant at 1 mM, and the other substrates in the reaction were saturating. The apparent $V_{\text{max}}$ value of the phosphorylated enzyme measured in the presence of CTP was greater than that of the native enzyme measured in the presence of CTP (Fig. 9 and Table I). Phosphorylation of CTP synthetase with protein kinase A did not have a significant effect on the cooperative kinetic behavior of the enzyme toward UTP or the apparent $K_m$ value for UTP (Fig. 9 and Table I).

**Effects of Protein Kinases A and C on CTP Synthetase Activity** —CTP synthetase is also phosphorylated and activated by protein kinase C (10, 11). In contrast to protein kinase A, data indicate that protein kinase C phosphorylates CTP synthetase on multiple sites (10). A comparison of the phosphopeptide

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**Table I**

| Substrate, activator, or inhibitor | Native CTP synthetase | Phosphorylated CTP synthetase |
|-----------------------------------|-----------------------|-----------------------------|
|                                  | $V_{\text{max(app)}}$ | $K_m(app)$ | Hill | $K_a(app)$ | IC_{50} | $V_{\text{max(app)}}$ | $K_m(app)$ | Hill | $K_a(app)$ | IC_{50} |
| UTP$\text{a}$                    | 0.76                  | 0.08       | 1.5  |               |         | 1.19                  | 0.08       | 1.7  |               |         |
| UTP$\text{b}$                    | 2.50                  | 0.05       | 1.0  |               |         | 2.99                  | 0.04       | 1.0  |               |         |
| ATP$\text{c}$                    | 1.08                  | 0.16       | 2.2  |               |         | 1.48                  | 0.12       | 1.6  |               |         |
| ATP$\text{d}$                    | 2.11                  | 1.44       | 2.7  |               |         | 2.61                  | 0.98       | 1.8  |               |         |
| Glutamine$\text{e}$              | 2.50                  | 0.85       | 1.9  |               |         | 3.02                  | 0.60       | 1.0  |               |         |
| Glutamine$\text{f}$              | 1.10                  | 0.50       | 0.8  |               |         | 1.16                  | 0.50       | 1.0  |               |         |
| Glutamine$\text{g}$              | 2.50                  | 0.34       | 0.9  |               |         | 3.01                  | 0.28       | 1.0  |               |         |
| CTP$\text{a}$                    | 15.3                  | 0.12       | 0.12 | 0.21          | 0.31    |                      |            |     |               |         |
| CTP$\text{b}$                    | 0.22                  |            |     |               |         |                      |            |     |               |         |

$\text{a}$ Kinetic constants determined with 0.5 mM ATP.

$\text{b}$ Kinetic constants determined with 2 mM ATP.

$\text{c}$ Kinetic constants determined with 1 mM ATP.

$\text{d}$ Kinetic constants determined with 0.1 mM CTP.

$\text{e}$ Kinetic constants determined with 2 mM UTP.

$\text{f}$ Kinetic constants determined with 0.5 mM UTP.

$\text{g}$ Kinetic constants determined with 0.5 mM ATP.

$\text{h}$ Kinetic constant determined with 0.1 mM ATP.
maps of CTP synthetase phosphorylated with protein kinase A (Fig. 4B) and protein kinase C (10) indicated that the target sites for these kinases differed. With this in mind, we examined CTP synthetase activity after the enzyme had been used as a substrate by both protein kinases A and C. In these experiments, CTP synthetase was incubated with protein kinases A and C together under the phosphorylation conditions that maximally stimulated CTP synthetase activity. Following incubation, CTP synthetase activity was measured using subsaturating concentrations of ATP and UTP. As described above for the phosphorylation by protein kinase A and previously for the phosphorylation by protein kinase C (10, 11), the phosphorylation of CTP synthetase resulted in a 190 and 280% stimulation of activity, respectively (Fig. 10A). When CTP synthetase was phosphorylated with protein kinases A and C together, CTP synthetase activity was stimulated 360% (Fig. 10A). Similar results were obtained when CTP synthetase was first incubated with one of the protein kinases followed by the incubation of the enzyme with the other protein kinase. The combined effect of protein kinases A and C on CTP synthetase activity was only slightly greater than that caused by either one of the protein kinases alone. This result may be expected if the mechanism by which each kinase stimulated CTP synthetase was the same. Indeed, protein kinases A and C (11) stimulate CTP synthetase activity by similar mechanisms.
We also examined the effects of protein kinases A and C on CTP synthetase that was dephosphorylated by treatment with alkaline phosphatase. As described previously (10), the dephosphorylation of CTP synthetase resulted in an 80% decrease in CTP synthetase activity (Fig. 10B). Although the alkaline phosphatase-treated CTP synthetase could be rephosphorylated with protein kinases A and C (10), only 25 and 40% of the native enzyme activity, respectively, was recovered after rephosphorylation (Fig. 10B). In addition, phosphorylation of the enzyme by protein kinases A and C together did not restore the native CTP synthetase activity (Fig. 10B). The same result was observed when the dephosphorylated enzyme was incubated sequentially with the protein kinases. Full activation of CTP synthetase activity by protein kinases A and C may require additional protein kinase phosphorylations (29, 31). In this light, there are potential phosphorylation sites for casein kinase II (32) within the amino acid sequence of CTP synthetase.

Concluding Discussion—The level of CTP synthetase activity plays an important role in the growth and metabolism of S. cerevisiae (5, 6, 9), as well as in higher eukaryotic organisms (33–43). In this study, we demonstrated that the URA7-encoded CTP synthetase was phosphorylated and stimulated by protein kinase A. The results of labeling experiments using cells that were activated in the RAS/cAMP pathway indicated that protein kinase A mediated the phosphorylation of CTP synthetase in vivo. The mechanism of the stimulation of CTP synthetase activity involved an increase in the $V_{\text{max}}$ of the reaction with respect to UTP and ATP and an increase in the affinity for ATP by the enzyme. Phosphorylation of the CTP synthetase also reduced the positive cooperative kinetics of the enzyme with respect to ATP and its negative cooperative kinetics with respect to glutamine. In addition, phosphorylation caused the synthetase to be less sensitive to inhibition by CTP. The effects of phosphorylation on the kinetic properties of the enzyme were most dramatic when the ATP concentration was subsaturating. It has been suggested that the stimulation of CTP synthetase activity by phosphorylation may be a mechanism by which the cell regulates CTP synthesis when the cellular levels of ATP are limiting (11).

In S. cerevisiae, protein kinase A is the enzyme through which the RAS/cAMP pathway mediates its signal for cell growth (12, 13). Stimulation of RAS activity and the subsequent elevation of protein kinase A activity are associated with rapid cell growth and enhanced metabolic activity of the cell (12, 13). The regulation of CTP synthetase activity by protein kinase A may represent a mechanism by which this signal transduction pathway mediates CTP synthesis and cell growth.

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