The Incorporation of Nucleotides into Amino Acid Transfer Ribonucleic Acid

II. EVIDENCE FOR SEPARATE ENZYMATIC SITES FOR INCORPORATION OF ADENOSINE 5'-MONOPHOSPHATE AND CYTIDINE 5'-MONOPHOSPHATE*

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The preceding paper described the purification and some of the properties of an enzyme present in rabbit muscle extracts that catalyzes the incorporation of adenylic acid from adenosine triphosphate into the terminal position of amino acid transfer ribonucleic acid obtained from yeast (1). The data presented in this paper are concerned with the incorporation of cytidine 5'-monophosphate into this transfer RNA. Evidence is presented which suggests that there are two different enzymatic sites for ATP and CTP, which catalyze the incorporation of AMP and CMP, respectively, into amino acid transfer RNA. It is not yet known whether these sites exist on two separate proteins or on a single protein.

EXPERIMENTAL PROCEDURE

The method employed for preparing ATP was described (1). CMP was synthesized by the procedure of Hurwitz (2) with use of the isopropylidene derivative of cytidine (Aldrich Chemical Company). The conversion of CMP to CTP was accomplished enzymatically with a rabbit muscle preparation (3). The enzyme preparations, the RNA, and the assay for incorporation of nucleotide into RNA have been described (1).

Snake venom phosphodiesterase was prepared from crude Crotalus adamanteus venom (Ross Allen’s Reptile Institute) by the method of Felix, Potter, and Laskowski (4). RNA used as substrate for the CMP incorporation experiments was prepared by incubating, in a 0.2-ml reaction volume, 1 mg of RNA with phosphodiesterase in 0.01 M MgCl₂ and 0.05 M 2-amino-2-methyl-1,3-propanediol buffer at pH 9.5 at 15° or 21°. The reaction was stopped by immersion of the tubes in a 100° bath for 1 minute, and the RNA was reisolated by precipitation from a 0.5 M NaCl solution with 2 volumes of ethanol. For determination of optical density units liberated by phosphodiesterase, reaction mixtures contained 40 μmoles of 2-amino-2-methyl-1,3-propanediol buffer, pH 9.6, 8 μmoles of MgCl₂, 4.0 mg of RNA, and 0.55 unit of phosphodiesterase in a volume of 0.8 ml. Separate 0.1-ml aliquots were removed for assay of nucleotide incorporation activity and the degree of digestion. They were neutralized with 0.005 ml of 0.8 N HCl plus 1.0 μmole of potassium phosphate buffer, pH 7.0, and the reaction was stopped by immersion in a bath at 100° for 45 seconds. The pH of the cooled samples was readjusted with 0.005 ml of 0.8 N NaOH. For analysis of the optical density units liberated, the RNA was precipitated with 0.11 ml of 0.15% uranyl acetate in 1 N HClO₄ and centrifuged at 4000 × g for 10 minutes. The optical density of the acid-soluble material at 260 μ was determined, and the zero time control value was subtracted. The percentage of total optical density units digested was calculated as the acid-soluble optical density divided by the optical density of the RNA.

RESULTS

Previous work by other investigators with enzymes from Escherichia coli suggested that the incorporation of AMP and CMP from their respective nucleoside triphosphates was catalyzed by a single enzyme (5, 6). No attempt was made to distinguish between rat liver enzymes capable of incorporating AMP and CMP (7, 8). The effect of CTP on AMP incorporation and the converse, the effect of ATP on CMP incorporation, were therefore investigated with the rabbit muscle enzyme preparation (1) and yeast amino acid transfer RNA (9).

Inhibition of AMP Incorporation by Other Nucleoside Triphos- phates—The effect of unlabeled CTP, GTP, and UTP on the incorporation of AMP into RNA is indicated in Table I. These nucleoside triphosphates were tested in the presence of 10 mM Mg²⁺ to avoid limiting concentrations of the cation. It is apparent that the inhibition by CTP is more than that by GTP or UTP. A more extensive exploration of these CTP inhibition is indicated by the Lineweaver-Burk plot of the rates of AMP incorporation in the absence and presence of 1.0 mM CTP (Fig. 1). Substrate inhibition with ATP levels greater than 2.0 mM is evident. The Kₘ for ATP is 3.8 mM. CTP inhibition of AMP incorporation is competitive, and the Kᵢ for CTP is 0.87 mM. By the method of least squares, the intercepts for the values obtained without and with CTP are 0.064 and 0.071, respectively.

Reaction of RNA with Phosphodiesterase—In order to study the incorporation of CMP, prior digestion of the yeast amino acid transfer RNA was necessary (5, 10). The action of phosphodiesterase on the RNA is indicated in Fig. 2. At 10°, between 4 and 6% of the original optical density of the RNA was liberated as material not precipitable with 1 N perchloric acid.
TABLE I

Inhibition of AMP\textsuperscript{32} incorporation by other nucleoside triphosphates

Reaction vessels contained 16 μg of the DEAE-cellulose enzyme, 0.4 μmole of ATP\textsuperscript{32}, 2.0 μmoles of MgCl\textsubscript{2}, 2.5 μmoles of glycine buffer, pH 9.5, 1.0 μmole of phosphoenolpyruvate, 3 μg of phosphoenolpyruvate kinase, 0.1 mg of RNA, and the unlabeled nucleoside triphosphates in the concentrations indicated in a final volume of 0.2 ml; incubation was for 30 minutes at 37°.

<table>
<thead>
<tr>
<th>Concentration of added nucleoside triphosphate</th>
<th>Inhibition of AMP\textsuperscript{32} incorporation by unlabeled nucleoside triphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>CTP</td>
</tr>
<tr>
<td>1.0</td>
<td>31%</td>
</tr>
<tr>
<td>2.0</td>
<td>60%</td>
</tr>
<tr>
<td>3.0</td>
<td>76%</td>
</tr>
</tbody>
</table>

0.15% uranyl acetate. This percentage has not been corrected for the hyperchromic effect. The reaction was completed in 30 minutes. At 21°, a higher percentage of material was liberated initially and the reaction did not stop completely. At 37°, with the same amount of enzyme, the liberation of ultraviolet-absorbing material proceeded much more rapidly with no evidence of a limited reaction comparable to that at lower temperatures. Because of the limited reaction at 15°, this temperature was employed for digestion of the RNA used in most of the experiments to be reported.

Requirement of CTP for ATP Incorporation—With RNA that had previously reacted with phosphodiesterase at 15°, it was possible to demonstrate a requirement of nonradioactive CTP in order to obtain maximal AMP\textsuperscript{32} incorporation. In Fig. 3, almost maximal AMP\textsuperscript{32} incorporation was obtained with untreated RNA in 60 minutes. When CTP was added, the rate of incorporation into untreated RNA, but not the total incorporation, was decreased. With RNA previously incubated with diesterase, the total incorporation of AMP\textsuperscript{32} in the absence of CTP was approximately one-third of the original. When unlabeled CTP was added to the diesterase-treated RNA, there was an initial lag in the rate of AMP\textsuperscript{32} incorporation, after which the incorporation proceeded at a rate comparable to that obtained with the untreated RNA plus added CTP. The total incorporation was similar to that seen in the untreated material. It is apparent that in the diesterase-treated preparation between 8 and 9 mmoles of AMP were incorporated in the absence of CTP. This could be due either to incomplete digestion of the RNA or to contamination of either the ATP or the RNA by cytidine mono-, di-, or triphosphate. Contamination of the RNA with a cytidine nucleotide was possible, since this RNA was shown to contain trace amounts of ATP. One batch of RNA prepared with sodium dodecyl sulfate, ethanol precipitation, phenol treatment, and repeated ethanol precipitation still contained approximately 50 mmoles of ATP per mg, as estimated by the firefly assay and lack of ATP requirement in a serine-activating system (11).

Effect of p-Hydroxymercuribenzoate on Incorporation of AMP\textsuperscript{32} and CMP\textsuperscript{32}—In the previous paper (1), the stimulatory effect of HMB\textsuperscript{1} on AMP\textsuperscript{32} incorporation was mentioned. The effect on incorporation of both AMP\textsuperscript{32} and CMP\textsuperscript{32} is shown in Fig. 4.

\textsuperscript{1} The abbreviation used is: HMB, p-hydroxymercuribenzoate.
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FIG. 3. CTP requirement for AMP<sup>32</sup> incorporation into phosphodiesterase-treated RNA. Reaction mixtures contained 10 μmoles of 2-amino-2-methyl-1,3-propanediol buffer, pH 9.6, 2 μmoles of MgCl<sub>2</sub>, 0.1 mg of untreated or phosphodiesterase-treated RNA, 0.4 μmole of ATP<sup>32</sup>, 0.4 μmole of CTP where indicated, and 16 μg of the DEAE-cellulose enzyme in a final volume of 0.2 ml. Incubation was at 37°.

FIG. 4. Effect of HMB on the rate of AMP<sup>32</sup> and CMP<sup>32</sup> incorporation. Reaction mixtures contained 10 μmoles of 2-amino-2-methyl-1,3-propanediol buffer, pH 9.6, 2 μmoles of MgCl<sub>2</sub>, 2 μmoles of phosphoenolpyruvate, 3.0 μg of phosphoenolpyruvate kinase, 10 μmoles of KCl, 0.1 mg of untreated or phosphodiesterase-treated RNA, 0.4 μmole of ATP<sup>32</sup>, 0.4 μmole of CTP where indicated, and 18.6 μg of DEAE-cellulose enzyme protein in a final volume of 0.2 ml. Incubation was at 37°.

The DEAE-cellulose enzyme had lost some activity on prolonged storage, and the kinetics of incorporation of AMP<sup>32</sup> revealed a diphasic curve which was eliminated by preincubation of the enzyme for 30 minutes at 0° in 10<sup>-4</sup> M HMB for 30 minutes, in a final volume of 0.2 ml. Incubation was at 37°.

The requirement for cytidine diphosphate rather than cytidine triphosphate as the substrate for the incorporation reaction is indicated by the ability of inorganic pyrophosphate to remove radioactivity from RNA previously labeled with CMP<sup>32</sup>. For example, when RNA containing 46 mpmoles of labeled CMP per mg was incubated without pyrophosphate (in 0.01 M MgCl<sub>2</sub>-0.05 M 2-amino-2-methyl-1,3-propanediol buffer, pH 9.6, and with the DEAE-cellulose enzyme preparation), no CMP<sup>32</sup> was liberated in 60 minutes. With a 5 mM concentration of pyrophosphate, 8 mpmoles were liberated, whereas with 10 mM pyrophosphate, 9 mpmoles were liberated in 60 minutes. This confirms the finding of others (5–8) that the triphosphate derivative of cytidine is the substrate. The enzymatic activity may then be described as transfer RNA cytidylate pyrophosphorylase.

The requirement for AMP and CMP incorporation were done with enzyme preincubated with HMB.

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K<sub>m</sub> and K<sub>i</sub> for CTP and Effect of ATP on CMP Incorporation

With RNA preincubated with phosphodiesterase, CMP<sup>32</sup> incorporation was studied with CTP<sup>32</sup> as a substrate. The effect of concentration on the rate of incorporation was determined. The results are represented as a Lineweaver-Burk plot in Fig. 5.

FIG. 5. Lineweaver-Burk plot for CMP<sup>32</sup> incorporation. Reaction mixtures contained 5.0 μmoles of glycine buffer, pH 9.5, 2.0 μmoles of MgCl<sub>2</sub>, 1.0 μ mole of phosphoenolpyruvate, 3.0 μg of phosphoenolpyruvate kinase, 0.1 mg of phosphodiesterase-treated RNA, 2.2 μg of DEAE-cellulose enzyme protein, and increasing concentrations of CTP<sup>32</sup> from 0.0015 to 0.032 μmole in a final volume of 0.2 ml. ATP, 1.0 μmole, was present in one series of incubation mixtures. Incubation was at 37°.
The unusual diphasic curve shown in this figure has been observed in several experiments. The reason for this is not obvious, but several explanations for it will be discussed later.

The \( K_m \) values for CTP for both phases of the curve are relatively low. At the lower concentration of CTP, the \( K_m \) was 0.008 mM, and at the higher concentration, it was 0.037 mM. These \( K_m \) values are contrasted with the \( K_i \) of CTP in the AMP incorporation reaction of 0.87 mM, calculated from the data presented in Fig. 1.

Another unusual finding was the stimulatory effect of ATP on CMP incorporation. This is shown in the Lineweaver-Burk plot in Fig. 5. The break in the line with ATP addition was noted in several experiments. It generally occurred at the same point as in the CTP curve. This means that if an impurity in the CTP is the cause, it is not ATP. The reason for the stimulation by ATP is not obvious. Since the total incorporation of CTP\(^{32}\) in the absence of unlabeled ATP is no different than in its presence (data presented below), it seems unlikely that ATP stimulates by making available more sites for CTP incorporation. When the effect of CTP\(^{32}\) concentration on the rate of incorporation was determined in the presence of HMB, and the results recorded as a Lineweaver-Burk plot, the same diphasic curve was noted and the break occurred at the same CTP concentration as without HMB. A slight stimulation of the rate with HMB was apparent at all concentrations of CTP.

The finding of a \( K_i \) for CTP of an order of magnitude smaller than the \( K_m \) as well as the stimulatory effect of ATP on CMP incorporation as opposed to the inhibitory effect of CTP on AMP incorporation, suggested that two different enzyme sites exist in this preparation for incorporation of AMP and CMP.

**Rates of Incorporation of AMP and CMP with Different Enzyme Fractions**—A comparison of the rates of incorporation of AMP into untreated RNA, and of CMP into diesterase-treated RNA, with the enzyme preparation from four different stages of purification is presented in Fig. 6. To insure optimal rates of incorporation, the enzymes were preincubated with low concentrations of HMB at 0°C for 30 minutes, and 0.4 pmole of ATP\(^{32}\) or 0.04 pmole of CTP\(^{32}\) in a final volume of 0.2 ml. Incubation was at 37°C for 30 minutes for AMI\(^{32}\) and 15 minutes for CMP\(^{32}\) incorporation.

**Incorporation of AMP and CMP as a Function of pH**—With RNA predigested with phosphodiesterase, CMP\(^{32}\) incorporation was studied as a function of pH. The incorporation of AMP into untreated RNA was also examined as a function of pH. The data indicated in Fig. 7 for both curves were obtained with enzymes preincubated with 10⁻² M HMB at 0°C for 30 minutes. The DEAE-cellulose fraction shows a reversal of the relative rates of incorporation of AMP and CMP as compared to their rates catalyzed by the less well purified fractions. Similar results were obtained when the incubations were done in the absence of HMB. Although these results might be due to the purification of a degradation enzyme more specific for AMP than for CMP, experiments designed to demonstrate such an enzyme were unsuccessful (1). It is also possible that purification resulted in the loss of an inhibitor for CMP incorporation. A third explanation for these results is the presence of different enzymes for AMP and CMP incorporation.

**Lack of Competition between AMP and CMP for Incorporation into RNA**—If the incorporation of AMP and CMP were cat-
lyzed by a single enzyme site, then the rate of incorporation of both nucleotides in the same reaction vessel containing equal amounts of untreated and diesterase-treated RNA and saturating levels of nucleotides should be intermediate between the rates of AMP and CMP incorporation determined separately with the mixed RNA population. If, however, two enzymatic sites are present, then the rate of incorporation of both nucleotides should be the sum of the rates of each (13). The results of an experiment designed to determine which of these hypotheses is correct are shown in Fig. 8. The rate of CMP incorporation from CTP<sup>32</sup> was more rapid than the rate of AMP incorporation from ATP<sup>32</sup>, as was noted previously in Fig. 6. The rate of incorporation of nucleotide from CTP<sup>32</sup> and ATP<sup>32</sup> incubated together is equal to the sum of the separate rates rather than to some rate intermediate between the two. A control experiment in which labeled ATP was incubated in the presence of unlabeled CTP showed the inhibition previously described in Fig. 1, and a similar experiment with labeled CTP and unlabeled ATP showed the stimulation indicated in Fig. 5. The sum of these rates of incorporation is also equal to that of the combined incubations, as would be expected. This experiment provides the strongest evidence for the presence of two enzymatic sites involved in the incorporation of AMP and CMP into transfer RNA.

**Ratio of Total AMP to Total CMP Incorporated**—Previous workers have indicated that the terminal nucleotide sequence in amino acid transfer RNA was adenylic-cytidylic-cytidylic-RNA or A-C-C-RNA (5-8). The data relating the total AMP incorporated to the total CMP incorporated into yeast amino acid transfer RNA preincubated at 15<sup>°</sup> and at 21<sup>°</sup> with phosphodiesterase are shown in Table II. With preincubation at 15<sup>°</sup>, the maximal incorporation of CMP was 33 mmoles in the absence or presence of unlabeled ATP. The AMP incorporation in the presence of unlabeled CTP was complete in 8 to 9 hours, but not in 5<sup>1/2</sup> hours. The CMP to AMP ratio of 1.5 suggests that a portion of CMP<sup>32</sup> terminal residues were not removed by the phosphodiesterase. Preincubation at 21<sup>°</sup> with phosphodiesterase increased the incorporation of CMP in Experiment 7 and 8 to 46 mmoles, whereas 23 mmoles of AMP were incorporated in the presence of CTP with the same specimen of RNA. The CMP to AMP ratio of 2.0 was originally reported for the rat liver preparation (7) and has since been observed with other systems (5, 6). In Experiment 9, the decrease in both CMP and AMP, with, however, a persistent ratio of 2.0, suggests that this preparation of RNA was degraded more extensively than the others. The incorporation of AMP in the absence of CTP suggests that minimal amounts of CTP were present in either the RNA or the ATP and that the prolonged incubations allowed incorporation of this material.

**Nearest Neighbor Analysis**—Since ribonucleoside 5'-monophosphates are incorporated into the transfer RNA, and alkaline hydrolysis cleaves the phosphodiester bonds to liberate ribonucleoside 2'- or 3'-monophosphates, it is possible to recover P<sup>32</sup>,...
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TABLE III
Nearest neighbor analysis of diesterase-treated RNA incubated with
\(^{32}P\)-labeled and unlabeled nucleotides

The RNA in the reaction mixtures from Experiment 9, Table II, was precipitated with 0.4 \( \times \) \( \text{NaCl} \) and washed five times as described for the macromethod of assay (1). Carrier RNA, 3 mg, was added, and the RNA was extracted after neutralization with 9 ml of 0.5 \( \times \) \( \text{NaCl} \) at \( 100^\circ \) for 10 minutes. After re-extraction with 1 ml of 2 \( \times \) \( \text{NaCl} \) for 3 minutes at \( 100^\circ \), the RNA in the combined supernatants was precipitated with 2 volumes of ethanol, taken up in 1.0 ml of 0.5 \( \times \) \( \text{KOH} \), and incubated at \( 37^\circ \) for 10 hours. After neutralization with \( \text{HCIO}_4 \), centrifugation, and further acidification, the nucleotides were absorbed on 25 mg of Darco G-60, eluted, and separated by paper electrophoresis in 0.05 \( \times \) ammonium formate buffer, pH 3.5, for 5 hours with 800 volts. Nucleotides were eluted from the paper and counted.

<table>
<thead>
<tr>
<th>Nucleotide isolated after KOH digestion</th>
<th>Nucleotides added to the incubation mixture</th>
<th>% total counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTP(^{32})</td>
<td>CTP(^{32})</td>
</tr>
<tr>
<td>Cytidylic</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>Adenylic</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>Guanylic</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Uridylic</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

originally present in the 5' phosphate of the precursor, in the 2'- or 3'-phosphate of the neighboring nucleotide. Results of such an analysis are presented in Table III. When the ratio of incorporation of CMP to AMP was 2.0, almost 100% of the AMP was incorporated next to CMP. The ratio of 2.0 is compatible with the terminal sequence suggested by others (5-7) of adenyl-cytidyl-cytidyl-RNA. If this is assumed, then 50% of the CMP is external and has as a nearest neighbor CMP. From an average of the two experiments shown in Table III, it is possible to calculate the amounts of each nucleotide found as the nearest neighbor to the terminal CMP. Approximately 66% of the internal CMP is next to AMP, 18% next to GMP, 10% next to CMP, and the remainder next to UMP. This distribution is comparable to that observed in E. coli transfer RNA (6, 14). The fact that ATP does not alter either the total incorporation or the distribution of label between nearest neighbors is an argument against the suggestion that the stimulatory effect of ATP on CMP incorporation is due to an increase in the available sites for CMP incorporation.

DISCUSSION

Several lines of evidence presented in this paper support the concept that the incorporation of AMP and CMP into yeast transfer RNA is catalyzed by two separate enzymatic sites present in a partially purified rabbit muscle preparation. It has not yet been determined whether the separate sites are on the same or different proteins. The strongest evidence for separate sites is the demonstration that the rate of incorporation of AMP and CMP, when both labeled ATP and CTP were added together to the reaction mixture, is the sum of the rates of incorporation of AMP and CMP determined separately. This result would not be expected if both substrates were competing for the same enzymatic site. Further evidence for separate sites is the inhibition of AMP incorporation in the presence of CTP, as opposed to the stimulation of CMP incorporation in the presence of ATP. Also, the \( K_m \) for CTP is lower by an order of magnitude than the \( K_m \) for ATP in the AMP incorporation reaction. Finally, with some preparations of enzyme HMB has a marked stimulatory effect on AMP incorporation and little or no effect on CMP incorporation. The differences in the pH curves are small and of questionable significance as evidence for two enzymatic sites. Preliminary experiments with heating did not show any gross changes in the ratio of the rates of AMP and CMP incorporation with different degrees of inactivation. All of these findings could be interpreted as evidence for a single enzyme with two sites.

When the rates of incorporation of AMP and CMP are compared with the enzyme preparation at several stages of purification, however, differences are apparent. The purification data suggest that there may be two separate enzymes, but other interpretations, such as the loss of an inhibitor of CMP incorporation with purification, are possible. The difference between two sites on one enzyme and two separate enzymes must be clarified by an attempt to separate these activities. The specificity of the incorporation of the terminal nucleotides of amino acid transfer RNA is partially explained by the existence of different enzymatic sites for ATP and CTP. Three separate enzymatic sites corresponding to the terminal adenyl-cytidyl-cytidyl residues of transfer RNA may exist, possibly on one protein.

The explanation for the interesting diphasic curve noted in the Lineweaver-Burk plot of varying concentrations of CTP is not known. It is of interest that the break in the curve occurs at approximately the same substrate concentration when the data are plotted for a 15-minute, a 30-minute, and a 45-minute incubation, during which time the total CMP incorporation changes over 10-fold. This suggests that the change of slope is not due to a difference in the reaction of one species of RNA with no terminal CMP group, as opposed to a second species with one terminal CMP. Several other possible explanations exist for the diphasic curve. Two CTP molecules could react with the enzyme, one of which would facilitate the rate of reaction of the second (15). If the enzyme possessed two specific sites for CMP incorporation, it is possible that CTP on the second site could stimulate CMP incorporation by the first site. Also, some unknown impurity in the CTP might act as an activator. Finally, two different enzymes (16) or one enzyme with two identical interacting or nonidentical active sites (17) might show the same kinetics. Further purification of the enzyme and the CTP is required to decide which of these is the most probable.

The stimulatory effect of HMB remains unexplained. The inhibition of a degradative enzyme, specifically a phosphodies- terase, seemed a logical explanation, but an extensive investigation (1) revealed no evidence for such a contaminant. HMB is known to stimulate the activity of several enzymes (18-20). In this instance, if this is the explanation, the effect on AMP incorporation is much more pronounced than on CMP incorporation. ATP also stimulates the incorporation of CMP, and this suggests that there is an ATP site on the CMP-incorporating enzyme. Since the stimulatory effect of HMB on CMP incorporation occurs in the absence or presence of ATP, its site of action must be different from an ATP site. Obviously, further purification is required to resolve many of the interesting problems that have been described.
SUMMARY

The incorporation of adenosine 5'-monophosphate and cytidine 5'-monophosphate into yeast transfer ribonucleic acid catalyzed by an enzyme preparation from rabbit muscle has been studied. The following evidence is presented to support the concept that two separate enzymatic sites catalyze the incorporation of these two nucleotides. The rate of incorporation of AMP and CMP together is the sum of the rates of incorporation of each; CTP competitively inhibits the incorporation of AMP, whereas ATP stimulates the incorporation of CMP. The $K_i$ for CTP is more than an order of magnitude smaller than its $K_i$ in the AMP incorporation reaction; finally, $p$-hydroxymercuribenzoate has a much more pronounced stimulatory effect on AMP incorporation than on CMP incorporation. It is not yet apparent whether the two sites are on the same or different proteins.

REFERENCES
The Incorporation of Nucleotides into Amino Acid Transfer Ribonucleic Acid:
II. EVIDENCE FOR SEPARATE ENZYMATIC SITES FOR INCORPORATION OF ADENOSINE 5'-MONOPHOSPHATE AND CYTIDINE 5'-MONOPHOSPHATE
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