The Regulation of Purine Utilization in Bacteria

V. INHIBITION OF PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITIES AND PURINE UPTAKE IN ISOLATED MEMBRANE VESICLES BY GUANOSINE TETRAPHOSPHATE*

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SUMMARY

The ability of rel+ cell strains of Escherichia coli to take up nucleosides and bases and convert them to the corresponding ribonucleoside 5'-triphosphates is qualitatively dependent upon amino acids, much the same as the ability to accumulate RNA. Restriction of uptake in this case occurs under the same conditions that elicit guanosine 5'-diphosphate, 2'- or 3'-diphosphate (ppGpp) accumulation and both phenomena are reversed by addition of chloramphenicol. The P-ribosyl-PP-dependent transport of purine nucleosides and bases into membrane vesicles is inhibited by ppGpp, as are membrane-bound purine phosphoribosyltransferase activities. The degree of inhibition differs for different substrates; for purines, inhibition approximates the uptake restrictions observed in whole cells. Enzyme and membrane preparations obtained from rel+ and rel- cells were equally inhibited by ppGpp.

Purified soluble 6-hydroxy purine phosphoribosyltransferase, which mediates membranous uptake of 6-hydroxy purines, is inhibited by ppGpp. Inhibition is not strictly competitive, but appears to be less severe at high P-ribosyl-PP levels. Adenine phosphoribosyltransferase was only mildly inhibited by ppGpp.

The inhibitory effects of ppGpp on purine transport due to inhibition of purine phosphoribosyltransferase can account for purine uptake restrictions observed under different physiological conditions in whole cells.

Recent evidence suggests that Escherichia coli cells possess a capacity for a pleiotropic regulatory response to amino acid starvation affecting metabolically diverse reactions (2-7). This regulatory pattern can be made dependent upon a single gene product since the amino acid-dependent phenotypes seen in rel+ cells are abolished in rel- mutants (8), suggesting a common factor mediating this pleiotropic response. Since most of the cellular responses affected during stringency are inhibited and since the rel+ allele is dominant over rel- (9), rel+ strains might form a pleiotropic inhibitor (4, 6, 7, 10).

It is possible that guanosine 5'-diphosphate, 2'- or 3'-diphosphate (formerly called MSI and now abbreviated ppGpp) might be such an inhibitor (10-12). Physiological concentrations of ppGpp have been shown to inhibit two steps in de novo purine biosynthesis, IMP dehydrogenase (6, 7) and adenylosuccinate synthetase (7), as well as to give limited inhibition of RNA polymerase (13).

Our concern here is whether ppGpp inhibition might account for the amino acid dependent of the uptake of purines and pyrimidines. The uptake and incorporation of 5-fluorouracil is strongly amino acid-dependent in rel+ strains (14). Uracil uptake is also amino acid-dependent in rel+ cells but not in rel- strains (5). This dependence is exerted at a reaction prior to UTP formation since the triphosphates are not labeled by exogenous uracil (5). Amino acid control of RNA accumulation (15, 16) and ppGpp accumulation (11) are dependent upon the ability to change an amino acid to its cognate tRNA. The same applies to the amino acid dependence of labeling of ribonucleoside 5'-triphosphates with uracil (17), guanine or guanosine (19). Quantitative estimates of the degree of restriction may vary with the particular amino acid deficiency as well as the duration of starvation (18). In general, however, the labeling of the corresponding ribonucleoside 5'-triphosphates during the stringent response is reduced about 100-fold for uracil (5, 18), 10- to 20-fold for guanine or guanosine (19), and about 2-fold for adenosine (18).

Studies on transport in E. coli membrane vesicles have led to the elucidation of the probable mechanism of purine transport although pyrimidine transport in vesicles has not yet been studied in detail. Purine uptake in E. coli is catalyzed by membrane-localized purine phosphoribosyltransferases (20-22). These enzymes catalyze the transfer of ribose 5'-monophosphate from phosphoribosylpyrophosphate to the purine base, releasing purine nucleoside 5'-monophosphate inside the membrane vesicle (20, 22). The transport of guanine and hypoxanthine is catalyzed by an enzyme specific for the 6-hydroxy purines (23, 24) while adenosine uptake is catalyzed by a separate enzyme.
(20, 22). It appears likely that purine nucleosides are first hydrolyzed, then taken up by the purine phosphoribosyltransferase mechanism in *E. coli* (23).

In this paper we note that the ability of ppGpp directly to inhibit purified purine phosphoribosyltransferases and purine uptake by isolated membrane vesicles is correlated with the amino acid dependence of cellular utilization of purines and purine nucleosides.

**METHODS AND MATERIALS**

**Bacterial Strains**—Unless specified, *E. coli* K-12 wild type, rel', is employed throughout this study: the strain was obtained from Dr. I. Saleman, National Institute of Arthritis and Metabolic Diseases. The 58-161 pair of rel' and rel', both metabolic auxotrophs, as well as prototrophic strain NP 29 (valyl-tRNA synthetase) have been described earlier (11).

**Growth of Organisms**—In general, cells were cultured in media making them dependent upon exogenous purines by supplementation with adenine (PAT medium) (20, 22). Experiments with NP 29 cells were carried out in 32P-labeled Tris minimal medium supplemented with 0.2% casamino acids (25) and tritiated uridine or adenosine as described in the legend to Fig. 1. Extraction of cells, thin layer chromatographic resolution of the ribonucleoside 5'-triphosphate pools and radioactivity measurements were performed as described previously (29).

**Enzyme Preparation and Assay**—The preparation methods and assay for soluble adenine phosphoribosyltransferase (30) and 6-hydroxy purine phosphoribosyltransferase (24) are as described earlier.

**Purine Uptake by Isolated Membrane Vesicles**—Membrane-localized guanine and hypoxanthine activities were measured in a manner analogous to that used for the purified enzyme (21, 24). With isolated membranes, guanine could not be used as a substrate in the membrane filter assay because of high blank values due to nonspecific binding of guanine to Millipore filters, so hypoxanthine was used instead. Membrane-localized adenine phosphoribosyltransferase was assayed by the filter assay (21). The membrane vesicles themselves were isolated by the procedure of Kaback (26) as described for this system (21).

**Chemicals and Isotopes**—The identities of purchased radioactive compounds were established as previously (20). The preparative methods for ppGpp and 32P-labeled ppGpp from strain NP 29 have been published (19). Preparations used were at least 90% pure. The major contaminants migrate as ppG2p and ppG3p after borate-phosphate two-dimensional thin layer chromatography (12).

**RESULTS**

Implication of ppGpp as Inhibitor of Transport in Vivo—Available evidence suggests that diminished nucleoside and nucleobase transport is correlated with high levels of ppGpp during restricted biosynthesis of tRNA in stringent but not in relaxed strains of *E. coli*. The starting point here was whether reversal of ppGpp accumulation by chloramphenicol also restores the cellular capacity to label purine and pyrimidine nucleoside 3'-triphosphates with exogenous precursors despite a persisting amino acid charging block. Fig. 1 shows that it does.

When strain NP 29 is shifted to 40°, and the activity of its temperature-sensitive valyl-tRNA synthetase is restricted (15, 16), an abrupt accumulation of ppGpp occurs (right panel). There is an equally abrupt lowering of pulse labeling of UTP and CTP with [PH3]uridine (left panel) and a similar but less severe effect on adenosine labeling of ATP and GTP (middle panel). Chloramphenicol addition at restrictive temperatures (arrows) results in rapid depletion of the ppGpp pool and a rapid restoration in the ability to pulse label UTP and CTP with uridine, an ATP and GTP with adenosine (dashed lines). The kinetics of pulse labeling shows an overshoot substantially above rates of labeling at permissive temperatures.

These observations support the possibility of a direct inhibitory effect of ppGpp on the transport of purines and pyrimidines as well as their nucleosides.

**Effect of ppGpp on Purine Uptake by Membrane Vesicles**—Fig. 2A shows that ppGpp addition to membrane vesicles does give mild inhibition of [14C]adenine uptake and substantially more
with the following specific activities: [3H]adenine (60.1 mCi per mmole), [3H]hypoxanthine (60.1 mCi per mmole) and the incubation continued for another 10 min. The vesicular products before chromatography of a portion of the reaction mixtures (0.02 ml) containing 0.12 mg of membrane protein, labeled purine, 80 mM Tris-HCl (pH 7.8), 2 mM magnesium acetate, 4 mM KMgEDTA, and the concentration of ppGpp indicated were previously incubated at 37°C for 10 min. To begin the uptake reaction, 0.1 mM (final concentration) [3H]adenine (68 mCi per mmole) or [3H]hypoxanthine (60.1 mCi per mmole) was added and the incubation continued for another 10 min. The reactions were terminated by a 20-fold dilution in 0.5 M NaCl, filtered, dried, and counted in a gas flow spectrometer. The value of the control samples not containing P-ribose-PP was subtracted from each series and usually amounted to about 15% of the observed activity at low ppGpp levels. The results are plotted as the fraction of uptake activity observed with ppGpp (V) divided by the activity in the absence of ppGpp (V0). A, adenine uptake, V0 = 1.54 nmoles of adenine taken up per 0.1 ml per 10 min; ▲, hypoxanthine uptake, V0 = 1.40 nmoles of hypoxanthine taken up per 0.1 ml per 10 min. B, the effect of ppGpp on adenine, hypoxanthine, and guanine phosphoribosyltransferase activities of isolated membrane vesicles. Reaction mixtures (0.02 ml) containing 0.12 mg of membrane protein, labeled purine, 80 mM Tris-HCl (pH 7.8), 2 mM magnesium acetate, 4 mM KMgEDTA, and the concentration of ppGpp indicated were previously incubated for 10 min at 37°C. The enzyme reaction was initiated by the addition of MgP-ribose-PP (1 mM final) and the incubation continued for another 10 min. The rates of the reactions are linear during this time (20, 24). The reactions were terminated by the addition of 3 μles of KPO4/TA and the reaction mixtures were boiled for 1 min to liberate intravesicular products before chromatography of a portion of the reaction mixture. Labeled purines were used at 0.2 mM (final) with the following specific activities: [3H]hypoxanthine (60.1 mCi per mmole), [3H]adenine, 51.1 mCi per mmole; [3H]guanine, 36.1 mCi per mmole). The parameters V and V0 are defined as in A. □, AMP synthesis, V0 = 1.06 nmoles per 10 min; ▲, IMP synthesis, V0 = 0.74 nmoles per 10 min; ○, GMP synthesis, V0 = 1.28 nmoles per 10 min.

Fig. 3 (center). Effect of ppGpp on soluble adenine and guanine phosphoribosyltransferase activities. Reaction mixtures (20 μl) containing either 5 μg of adenine phosphoribosyltransferase or 10 μg of guanine-hypoxanthine phosphoribosyltransferase were incubated as in Fig. 2B. The adenine enzyme was apparently homogeneous after acrylamide gel electrophoresis and had a specific activity of 8 to 14 amoles of AMP formed per min per mg of protein. The guanine enzyme was approximately 25% pure and had a specific activity of 4 to 7 amoles of GMP formed per min per mg of protein. Both enzymes gave <2% degradation of 0.2 mM AMP or GMP after incubating for 1 hour at 37°C; the same was true for incubations under similar conditions with P-ribose-PP alone at 1 to 5 mM. The guanine-specific enzyme did not detectably degrade [3P]ppGpp at 1 μM, or at 0.2 mM after 30 min at 37°C under reaction conditions in the presence or absence of enzyme substrates. For each enzyme series plotted, a control value (minus P-ribose-PP) was subtracted from each experimental point; this blank usually amounted to <1% of experimental activities at low ppGpp concentrations. The V/V0 values are defined as in Fig. 2. □, adenine phosphoribosyltransferase activity; ▲, guanine phosphoribosyltransferase activity.

Fig. 4 (right). The effect of P-ribose-PP concentrations on guanine phosphoribosyltransferase activity. Enzyme assays were performed as in Fig. 2B, but using 0.05-ml reaction mixtures containing 0.5 μg of enzyme (specific activity 1.0 μ mole of GMP formed per min per μg), 1 mM MgCl2, 0.4 mM [3H]guanine at a specific activity of 52 mCi per mmole and appropriately diluting 4 mM LiP-ribose-PP to initiate the reaction after a 15-min preliminary incubation. This seemingly anomalous P-ribose PP concentration dependence observed at 50 μM ppGpp was observed in several experiments. PRPP, P-ribose-PP.

Severe inhibition of 6-hydroxy purine uptake ([3H]hypoxanthine was used here in place of guanine for technical reasons). It seems likely that this inhibition is due to a direct effect of ppGpp rather than a degradation product since 30 min incubations of 0.2 mM [3P]ppGpp under otherwise identical conditions did not result in detectable ppGpp hydrolysis.

More severe inhibition of 6-hydroxy purine transport than of adenine transport into vesicles is consistent with the fact that in ret" cells, guanine labeling of GTP is more amino acid-dependent than adenine labeling of ATP.

Vesicle preparations have membrane bound purine phosphoribosyltransferase activity which produces reaction products outside the vesicle as well as transporting a portion inside the vesicle. For example, at 37°C, only about 25% of the AMP formed is inside the vesicle (21, 23). Therefore, we have measured the effect of ppGpp on total membrane bound purine phosphoribosyltransferase activities, presented in Fig. 2B. The figure shows that ppGpp inhibits total membrane-bound purine phosphoribosyltransferase activities with a concentration dependence similar to the inhibition of uptake. Again, inhibition of phosphoribosylation of both 6-hydroxy purines (hypoxanthine or guanine) by ppGpp is more severe than with adenine. The concentration of ppGpp giving 50% inhibition of either uptake or phosphorylation of 6-hydroxy purines is 0.2 to 0.5 mM (depending upon the particular assay) while for adenine this value is in excess of 3 mM.

Since there is strong evidence linking the vesicular transport of purines to the activities of purine phosphoribosyltransferases (20-24), we have also measured the effect of ppGpp on soluble purified enzyme preparations.

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Effect of ppGpp on Purified, Soluble Adenine and Guanine Phosphoribosyltransferase Activities—As can be seen from Fig. 3, ppGpp severely inhibits adenylate and guanylate formation catalyzed by both 6-hydroxy purine-specific and the adenine-specific phosphoribosyltransferase. As with the membrane preparations of purine phosphoribosyltransferase, ppGpp inhibits the adenine-specific activity less than the 6-hydroxy purine-specific enzymatic activity.

Comparison of Fig. 3 with Fig. 2 reveals that ppGpp is a slightly more potent inhibitor of the purified enzymes than of membrane preparations of enzyme activity or of transport activity. With the purified soluble enzyme preparations, the concentration of ppGpp giving 50% inhibition of GMP formation is about 85 μM and the corresponding value for AMP formation is 1.5 mM.

Effect of P-ribose-PP Concentration on ppGpp Inhibition of Guanine Phosphoribosyltransferase Activity—An attempt was made to see whether ppGpp inhibition of the 6-hydroxy purine phosphoribosyltransferase was competitive with P-ribose-PP concentrations. Fig. 4 indicates that the inhibition follows complex kinetics. While not strictly competitive, more severe ppGpp inhibition is obtained at the concentration of P-ribose-PP decreases below 1 mM. Similar complex kinetic data have been obtained earlier with other inhibitors of the guanine enzyme and the adenine enzyme (20, 21, 23, 24).

Effects of ppGpp and ATP on Uptake and Phosphoribosyltransferase Activity for Several Purine Bases and Nucleosides—Since ATP is normally the major constituent of the nucleotide pools and is an effector of phosphoribosyltransferase activities (20, 21), it seemed of value to relate the effect of ppGpp to the effects of ATP on vesicular phosphoribosyltransferase and transport activities. Since purine nucleoside uptake has been postulated to occur by hydrolysis to purine bases followed by purine phosphoribosyltransferase-linked transport (23), it seemed also desirable to check the effect of ppGpp on vesicular transport of purine nucleosides.

Table I shows the residual fraction of uptake or phosphoribosyltransferase activity relative to the activity in the absence of inhibitor (V/V0) for three purine bases and their nucleosides.

<table>
<thead>
<tr>
<th>Base or nucleoside</th>
<th>Membrane uptake</th>
<th>Membrane PRPTase a</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>5 mM PPTP b</td>
<td>Plus 1.25 mM ATP</td>
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<tr>
<td></td>
<td>cpm</td>
<td>cpm</td>
</tr>
<tr>
<td>Adenine</td>
<td>4305</td>
<td>834</td>
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<tr>
<td></td>
<td>3136</td>
<td>0.73</td>
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<tr>
<td></td>
<td>2735</td>
<td>0.57</td>
</tr>
<tr>
<td>Adenosine</td>
<td>4973</td>
<td>748</td>
</tr>
<tr>
<td></td>
<td>2735</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>2735</td>
<td>0.57</td>
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<tr>
<td>Hypoxanthine</td>
<td>1254</td>
<td>843</td>
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<tr>
<td></td>
<td>507</td>
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<td></td>
<td>507</td>
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<tr>
<td>Inosine</td>
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<td>503</td>
<td>0.38</td>
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<tr>
<td></td>
<td>503</td>
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</tr>
<tr>
<td>Guanine</td>
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<tr>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Guanosine</td>
<td>571</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>0.33</td>
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<td></td>
<td>174</td>
<td>0.33</td>
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</table>

a For nucleosides, phosphoribosyltransferase measurement is the measurement of nucleosides (labeled in the purine moiety) converted to the nucleoside monophosphate after reaction with both nucleoside phosphorylase and phosphoribosyltransferase.

b PRPTase; phosphoribosyltransferase.

The addition of ppGpp inhibits uptake of 6-hydroxy purines and their nucleosides to a similar degree which is more severe than with adenine or adenosine. Conversely, ATP addition inhibits adenosine and adenosine uptake more severely than does ppGpp, but ATP is a relatively more mild inhibitor of uptake of the 6-hydroxy purine group.

Activities of Vesicular Transport and Purine Phosphoribosyltransferase Obtained from rel+ and rel− Bearing Strains—We have compared activities of vesicles derived from relaxed and stringent strains in order to see if the purine phosphoribosyltransferase-linked transport system was not altered by mutation of the rel gene.

Enzymes and membrane vesicles were prepared from E. coli K-12 rel+ wild type, 58-161 met- rel+, and 58-161 met- rel−. All cultures were grown from small inocula on VBC medium (20–22) for 14 hours to exponential phase and then transferred to Medium A (20–22) supplemented with methionine (10 mg per liter) for 1 hour. The cells were washed twice in Medium A; then grown for another 20 min either in the presence or absence of methionine and harvested by centrifugation. The cells were resuspended in 10 mM Tris-Cl (pH 8) at 0° and portions taken for the fractionation of the phosphoribosyltransferases as previously described (20–22) or for the isolation of membrane vesicles (31, 26).

Table II shows the effect of ppGpp on enzyme activities and uptake rates from these strains. Apparently enzymes and membranes prepared from the several strains grown under conditions which did and did not include amino acid starvation do not differ in their ability to be inhibited by ppGpp. These data are compatible with rel+ being dominant over rel− in merodiploids (9).

Effects of ppGpp on Vesicular Transport of Pyrimidines—We have examined the effects of ppGpp on uracil and cytosine uptake although relatively little information is available on vesicular uptake of pyrimidines. Fig. 5 shows P-ribose-PP-dependent uracil uptake is much more severely restricted by high concentrations of ppGpp than P-ribose PP-dependent cytosine uptake. Although partial inhibition of cytosine uptake is more sensitive to low concentrations of ppGpp, the maximum inhibition obtained is only about 40 to 50%. Uracil uptake (Fig. 5) is less sensitive to low concentrations of ppGpp than 6-hydroxy purine phosphoribosyltransferase, Fig. 2A. On the other hand, high concentrations of ppGpp (2.5 mM) inhibit uracil uptake more completely than hypoxanthine uptake.

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DISCUSSION

In these experiments we have asked whether ppGpp might account for inhibition of conversion of extracellular purines and
TABLE II
Effect of ppGpp on uptake and phosphoribosyltransferase activity in three strains of Escherichia coli

Specific activities and recoveries of activities were comparable (+/- 35%) among the various cell preparations.

| Fractional activity observed in presence of 3 mM ppGpp (activity at 0 ppGpp equals 1.0) |
|----------------------------------|---------------------------------|---------------------------------|
|                                 | K. coli K-12                   | K. coli 38-10 relr             |
|                                  | -Methionine                   | -Methionine                   |
| Guanine phosphoribosyltransferase activity | 0.13<sup>a</sup>              | 0.19<sup>b</sup>              |
| Hypoxanthine phosphoribosyltransferase activity | 0.17<sup>a</sup>              | 0.14<sup>b</sup>              |
| Adenine phosphoribosyltransferase activity | 0.25<sup>a</sup>              | 0.25<sup>a</sup>              |
| Hypoxanthine uptake              | 0.20                           | 0.22                           |
| Adenine uptake                  | 0.58                           | 0.58                           |

<sup>a</sup> Purified soluble enzyme.
<sup>b</sup> Membrane enzyme.

Our results indicate that the addition of ppGpp is inhibitory to purine phosphoribosyltransferase activities measured in three ways: (a) as vesicular transport of purines, (b) as membrane-bound activities, and (c) as the activity of purified soluble enzyme preparations. We infer that the restriction of the cellular ability to take up purines during the stringent response could be accounted for by ppGpp inhibition of phosphoribosyltransferase activities.

This inference is supported by several observations. The strongest indication is the close correlation between the accumulation of ppGpp and the inhibition of conversion of exogenous purines to cellular purine nucleotides. This correlation appears to hold for the uptake of purines, their nucleosides, and for uracil or uridine.

Fig. 1 shows the accumulation of ppGpp during amino acid starvation is correlated with restricted uptake. Fig. 1 shows further that ppGpp accumulation is reversed by the action of chloramphenicol, as is the uptake restriction, although restrictive temperatures are maintained. This also confirms earlier observations (27) showing that chloramphenicol inhibits ppGpp accumulation under these conditions.

Both uptake restriction and ppGpp accumulation can also be made dependent upon the rel<sup>+</sup> allele since rel<sup>-</sup> cells do not accumulate ppGpp (11, 25) or restrict uptake (5, 6) during amino acid starvation. However, this genetic dependence is not obligatory for either phenomenon. When relaxed strains are made to accumulate ppGpp, as by direct carbon source starvation or diauxie (25, 28-30), they also show uptake restriction (29). Consistent evidence presented here (Table II) shows that the phosphoribosyltransferases and membranes from both rel<sup>+</sup> and rel<sup>-</sup> strains are similar in their response to ppGpp.

However, there are reasons for exercising caution before concluding that these experiments show a cellular mechanism for transport regulation. The degree of inhibition depends upon the concentrations of both ppGpp and P-ribose-PP (Fig. 4). The effective levels of ppGpp are within the physiological concentration range (0.1 to 0.4 mM during exponential growth, 2 to 4 mM during the stringent responses). However, we can find no published estimates of the intracellular levels of P-ribose-PP, either during normal growth or during amino acid starvation. In Salmonella, there is no change in the level of P-ribose-PP synthetase during amino acid starvation (31), nor does ppGpp have any specific inhibitory effect on P-ribose-PP synthetase activity. Fig. 4 shows that if changes in P-ribose-PP concentration did occur, they well might alter the inhibitory effects of ppGpp on phosphoribosyltransferase activities. The kinetically complex interaction between ppGpp and purified guanine phosphoribosyltransferase is not unique to the ppGpp effect. Similarly complex inhibition has been obtained with 5'-nucleotides in these systems (20, 24). These considerations emphasize the need to examine the simultaneous effects of all the major nucleotide pool constituents on transport using concentrations characteristic of normal growth as well as characteristic of the stringent response. Table I shows that the predominant nucleotide, ATP, does have a rather specific effect on the adenine enzyme and on adenine uptake. Conversely, ppGpp has relatively specific effects on the 6-hydroxy purine enzyme, as does GTP (24).

During stringency ATP and GTP cannot account for inhibition of phosphoribosyltransferase-linked transport systems since these pools do not increase during the stringent response (4, 6, 32). There is evidence that ppGpp mediates these pool changes in purine nucleosides to the corresponding intracellular ribonucleoside 5'-triphosphates observed during the stringent response. Available evidence suggests that this restriction probably occurs at the level of formation of the purine 5'-monophosphate, since adenine- and guanine-supplemented cells undergoing the stringent response do not accumulate sizeable amounts of either GMP, GDP, AMP, or ADP. We have therefore focused our attention on the effects of ppGpp on a class of enzymes which convert purines to the corresponding purine nucleoside 5'- monophosphate and whose activity in membrane vesicles has been shown to be responsible for membrane transport of purines and their nucleosides (20-24).

1 R. Switzer, personal communication.
by direct inhibition of IMP dehydrogenase and adenylosuccinate synthetase activities (6, 7). Figs. 2 and 3 reveal that ppGpp interacts somewhat differently with membrane-bound as compared to soluble preparations of phosphoribosyltransferases. The effects of ppGpp on the membrane-bound enzyme more closely parallel the effects on transport. The fact that both preparations are inhibited by ppGpp further supports the identity of membrane catalysis and transport using yet another effector (21). Yet, differences in the degree of ppGpp inhibition of enzymes prepared in different transport regulation are not compelling, the evidence is highly suggestive. It would seem compelling evidence might be obtained from characterization of mutants in which the amino acid dependence of purine transport is abolished under all growth conditions. Nevertheless, ppGpp inhibition approximately mimics whole cell behavior since adenine phosphoribosylation is less severely inhibited than reactions using 6-hydroxy purines as substrates.

Although the arguments favoring a regulatory role for ppGpp as a negative effector of phosphoribosyltransferase-linked purine transport regulation are not compelling, the evidence is highly suggestive. It would seem compelling evidence might be obtained from characterization of mutants in which the amino acid dependence of purine transport is abolished under all growth conditions.

It is worth noting that the range of reactions so far possibly regulated by ppGpp is extended beyond those participating in de novo synthetic pathways to include so-called “scavenging” pathways for utilization of previously formed bases.

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