Inhibition of Cellular Growth by Increased Guanine Nucleotide Pools

CHARACTERIZATION OF AN ESCHERICHIA COLI MUTANT WITH A GUANOSINE KINASE THAT IS INSENSITIVE TO FEEDBACK INHIBITION BY GTP*

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In Escherichia coli the enzyme guanosine kinase phosphorolyses guanosine to GMP, which is further phosphorolysed to GDP and GTP by other enzymes. Here I report that guanosine kinase is subject to efficient feedback inhibition by the end product of the pathway, GTP, and that this regulation is abolished by a previously described mutation, gsk-3, in the structural gene for guanosine kinase (Hove-Jensen, B., and Nygaard, P. (1989) J. Gen. Microbiol. 135, 1263–1273). Consequently, the gsk-3 mutant strain was extremely sensitive to guanosine, which caused the guanine nucleotide pools to increase dramatically, thereby initiating a cascade of metabolic changes that eventually led to growth arrest.

By isolation and characterization of guanosine-resistant derivatives of the gsk-3 mutant, some of the crucial steps in this deleterious cascade of events were found to include the following: first, conversion of GMP to adenine nucleotides via GMP reductase, encoded by the guaC gene; second, inhibition of phosphoribosylpyrophosphate synthetase by an adenine nucleotide, presumably ADP, causing starvation for histidine, tryptophan, and pyrimidines, all of which require PRPP for their synthesis; third, accumulation of the regulatory nucleotide guanosine 5′,3′-bispyrophosphate (ppGpp), a general transcriptional inhibitor synthesized by the relA gene product in response to amino acid starvation.

In Escherichia coli, purine nucleotides can be synthesized de novo from simple precursor metabolites, or they can be derived from exogenous nucleosides or nucleobases via the so-called salvage pathways (reviewed in Ref. 1). Purine auxotrophic mutants can utilize exogenous guanosine by either of two pathways (Fig. 1). One involves direct phosphorylation of guanosine to GMP by guanosine-inosine kinase (ATP:guanosine 5′-phosphotransferase (EC 2.7.1.73)) encoded by the gsk gene. For simplicity I shall hereafter refer to this enzyme simply as guanosine kinase. The other pathway is initiated by phosphorylation of guanosine to guanine and ribose 1-phosphate catalyzed by purine nucleoside phosphorylase encoded by the deoD gene. Subsequently guanine is phosphoribosylated to GMP by either guanine phosphoribosyltransferase or hypoxanthine phosphoribosyltransferase, encoded by the gpt and hpt genes, respectively.

Curiously, the latter two-step pathway predominates in vivo, even though it is energetically more costly. If, however, the deoD gene is inactivated by mutation, guanosine can only be utilized via the guanosine kinase reaction, which seems to be inefficient (2). Thus, a purine-auxotrophic purE deoD mutant strain cannot grow with guanosine as the sole source of purines, apparently because too little GMP is formed by guanosine kinase to furnish the supply of both guanine nucleotides and adenine nucleotides. In line with this interpretation, Hove-Jensen and Nygaard (2) isolated from a purE deoD strain a number of mutant derivatives that were able to grow with guanosine as the sole source of purines and showed that these strains had acquired mutations in the gsk gene, which apparently improved the efficiency of guanosine kinase.

Fortuitously I discovered that one of these mutant alleles, gsk-3, paradoxically gave rise to a severe guanosine sensitivity when transduced into a wild type strain. Here I report that the apparent inefficiency of the guanosine kinase reaction is because of a tight feedback inhibition of the enzyme by GTP and that this regulation is abolished by the gsk-3 mutation. Thus, the extreme guanosine sensitivity of the gsk-3 mutant was found to be caused by an uncontrolled swelling of the guanine nucleotide pools in response to guanosine addition. Considering the ubiquitous roles of guanine nucleotides in vital cellular processes such as protein synthesis, RNA metabolism, and cell cycle control, I decided to investigate how such an increase of the guanine nucleotide pools interferes with cellular metabolism and growth.

The present results show that the swelling of the guanine nucleotide pools was propagated to the adenine nucleotide pools, which restricted the synthesis of the central intermediary metabolite PRPP to impose starvation for pyrimidines and for the amino acids histidine and tryptophan, all of which require PRPP for their synthesis (reviewed in Ref. 3). The starvation for histidine and tryptophan induced the accumulation of the regulatory nucleotide ppGpp, which is synthesized from GTP by the relA gene product, ppGpp synthetase I (for review see Ref. 4). ppGpp is the key mediator of the so-called “stringent response” to amino acid starvation and serves to inhibit the synthesis of rRNA, tRNA, and some mRNAs when substrates for protein synthesis are limiting.

The ability to induce the synthesis of ppGpp is usually considered beneficial to bacterial cells, as it enables them to adjust more rapidly to changing growth conditions. However, the guanosine sensitivity of the gsk-3 mutant was found to be relieved by a deletion of the relA gene, indicating that the metabolic problems caused by guanosine addition was aggravated to an intolerable level by the accumulation of ppGpp.

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1 The abbreviations used are: PRPP, phosphoribosylpyrophosphate; ppGpp, guanosine 5′,3′-bispyrophosphate.
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This finding may explain how the gsk-3 mutant could originally be selected for its ability to grow on guanosine, because this selection was performed on a relA mutant strain (2).

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids**—The bacterial strains used in this study are all derivatives of *E. coli* K12 and are listed in Table I. The plasmid pGSS235 was a kind gift from Dr. John R. Guest. It contains the *gusC* gene cloned on a 1370-base pair EcoRI-BclI fragment into pBR322 between the EcoRI and BamHI sites (10).

**Genetic Techniques**—Generalized transductions with lysates of bacteriophage P1vir were performed as described in Ref. 6. Minimal medium plates contained AB minimal medium (11) solidified with 2% Difco Bacto agar and supplemented with 0.2% glucose, 100 μg/ml proline, and 1 μg/ml of thiamine. Rich medium was Luria broth.

**Measurements of PRPP and Nucleotide Pools**—Bacteria were grown with shaking in Tris-buffered medium (12) with the phosphate concentration lowered to 0.3 mM. The medium was supplemented with 0.2% glycerol as carbon source and with proline and thiamine at 100 μg/ml respectively. At an optical density of 0.9, 35 ml of liquid culture was also severely inhibited by guanosine and was unaffected by guanosine (Table II). Growth of CN1932 in nucleoside (Fig. 2a). Exposure to guanosine, however, was not lethal but only bacteriostatic; the viability of CN1932 was not reduced after several hours of incubation with the nucleoside (data not shown). Interestingly, CN1932 was not sensitive to inosine, the other substrate of guanosine kinase (Fig. 1), presumably because the gsk-3 enzyme functions less efficiently with this nucleoside as substrate.

To trace the mechanisms by which guanosine affects nucleotide metabolism and cellular growth, I measured how the nucleotide pools of the gsk-3 mutant change in response to...
of the antibiotics. The adk CN1932 (Table II). However, this was probably because salvage uridine alone did not eliminate the guanosine sensitivity of starvation. Supplementation with the pyrimidines uracil or depletion of the UTP pool (Fig. 3), a sign of severe pyrimidine also impaired the synthesis of pyrimidines, as indicated by the (data not shown).

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| Straina | Genotypeb | Reference/source/constructionc |
|---------|-----------|--------------------------------|
| CF1651 | [MG1655] ΔrelA251:kan | |
| CN1524 (CSH26) | [CSH26] | |
| CN1607 (H0677) | [S0003] deoD udp gsk-3 purC202::Tn10 | |
| CN1861 (S0607) | [CSH26] hpt deoD | |
| CN1879 (S01726) | [S0003] purE deoD adk-2 zbb-2419::Tn10 | |
| CN1881 | [CSH26] adk-2 zbb-2419::Tn10 | |
| CN1892 (H0602) | [S0003] deoD udp::Tn5 gsk-3 | |
| CN1910 | [CSH26] hpt deoD adk-2 zbb-2419::Tn10 | |
| CN1932 | [CSH26] gsk-3 | |
| CN1936 | [CSH26] hpt deoD gsk-3 | |
| CN1995 | [CSH26] gsk-3 ΔrelA251::kan | |
| CN2031 | [CSH26] gsk-3 udp::Tn5 | |
| CN2079 | [CSH26] hpt deoD gsk-3 guaC2079 | |
| CN2124 (NK6732) | [MG1655] leu-82::Tn10 | |
| CN2127 | [CSH26] gsk-3 leu-82::Tn10 | |
| CN2133 | [CSH26] gsk-3 guaC2079 | |
| CN2174 (S01172) | [S0003] purE deoD apt leu-1 gsk-1-1 | |
| CN2176 | [CSH26] gsk-3 hpt deoD guaC2079 | |
| CN2178 | [CSH26] gsk-3 hpt deoD guaC2079 | |
| CN2176 | [CSH26] gsk-3 hpt deoD guaC2079 | |
| CN2178 | [CSH26] gsk-3 hpt deoD guaC2079 | |

a Original strain designations are given in parenthesis.

b [CSH26]: F- λ ara Δ(gpt-pro-lac) thi,
[S0003]: F- λ metB rpsL relA spoF supF lamB,
[MG1655]: F- λ IN(rrnD-rrnE)1 rph-1.

* Unselected markers are shown in parenthesis. Resistance to tetracycline or kanamycin was scored on Luria broth medium containing 15 μg/ml of the antibiotics. The adk-2 marker was scored by its temperature sensitivity (t) at 42 °C. The hpt marker gives rise to a requirement for δ-aminolevulenic acid (40 μg/ml). Guanosine sensitivity (GRs) was scored on glucose minimal medium containing 30 μg/ml of guanosine.

Table II

Effect of guanosine and various supplements on the growth of the gsk-3 mutant and its relatives on glucose minimal medium

| Strain | Addition to medium |
|--------|--------------------|
|        | None | Guanosine | Guanosine + His | Guanosine + Trp | Guanosine + His + Trp | Guanosine + uracil | Guanosine + uridine |
| CN1524 (gsk-1') | + | + | + | + | + | + | |
| CN1932 (gsk-3) | + | - | - | - | - | - | |
| CN2031 (gsk-3 udp::Tn5) | + | + | + | + | + | + | |

It is noteworthy that the purine deoxyriboseuridylate nucleotides also increased dramatically in response to guanosine addition (Fig. 3). Within 3 min after the addition of guanosine to CN1932, the GTP pool increased 3-fold, whereas the PRPP pool increased by a factor of six. Most noteworthy, the regulatory nucleotide, ppGpp, also accumulated during the first 10 min after guanosine addition, signaling a restriction on the amino acid supply for protein synthesis. Later on, the GTP pool continued to increase to very high levels, whereas the PRPP pool increased even further.

PRPP is a central precursor metabolite that is required for the synthesis of purines, pyrimidines, and nicotinamide coenzymes as well as for the amino acids histidine and tryptophan (reviewed in Ref. 3). The dramatic reduction of the PRPP pool and the concomitant induction of ppGpp synthesis suggested that guanosine might impose starvation for histidine and tryptophan by restricting the availability of PRPP. In support of this notion, supplementation with histidine and tryptophan enabled the gsk-3 mutant to form colonies on minimal medium in the presence of guanosine, whereas no growth was observable in the presence of either amino acid alone (Table II). Moreover, the addition of histidine and tryptophan together with guanosine almost eliminated the accumulation of ppGpp (data not shown).

Eventually the guanosine-induced decrease of the PRPP pool also impaired the synthesis of pyrimidines, as indicated by the depletion of the UTP pool (Fig. 3), a sign of severe pyrimidine starvation. Supplementation with the pyrimidines uracil or uridine alone did not eliminate the guanosine sensitivity of CN1932 (Table II). However, this was probably because salvage of uracil by uracil phosphoryltransferase requires PRPP, and exogenous uridine is predominantly phosphorylated to uracil by uridine phosphorylase encoded by the udp gene (reviewed in Ref. 14). A derivative of CN1932 carrying a disrupted udp gene, CN2031 (Table I), was indeed found to be rescued by supplementation with uridine (Table II), which could now be salvaged exclusively by direct phosphorylation to UMP by uridine kinase.

It is generally considered that the most important physiological inhibitor of PRPP synthetase is an adenine nucleotide, presumably ADP (3). However, guanine nucleotides can act as competitive inhibitors of PRPP synthetase in vivo (15) and apparently to some extent in vitro as well (16). The finding that the ATP pool in the gsk-3 mutant remained fairly constant during the first 10 min after guanosine addition (Fig. 3) suggests that the initial dramatic decrease of the PRPP pool was caused by the increase of one or more of the guanine nucleotide pools. After 10 min, the pool of ATP, and presumably of ADP as well, started to increase in parallel with the GTP pool, most likely because of conversion from GMP via the guaC pathway (Fig. 1). This increase of the adenine nucleotide pools probably contributed to the further reduction of the PRPP pool seen at later times.
dATP (reviewed in Ref. 17), but the feedback regulation was apparently not sufficiently tight to prevent the marked swelling of the dGTP and dATP pools under conditions of increased substrate pools. This may have been because the pool of ATP, which is an activator of the enzyme, was also very large.

In contrast to the gross disturbances of nucleotide metabolism seen in the $gsk-3$ mutant, only minor changes occurred in the nucleotide pools of the isogenic $gsk^+$ strain CN1524 in response to guanosine addition (Fig. 4). Apart from a slight and transient increase of the GTP and ATP pools, the only notable feature was a marked swelling of the PRPP pool initiating approximately 10 min after guanosine addition. This increase, which did not affect the growth of CN1524 significantly (Fig. 2a), may be explained by the fact that this strain, like all the others described in this work, carries a deletion of the $gpt$ gene encoding guanine phosphoribosyltransferase (see “Discussion”).

The $gsk-3$ Mutation Renders Guanosine Kinase Resistant to Inhibition by GTP—The dramatic increase of the guanine nucleotide pools in the $gsk-3$ strain compared with its $gsk^+$ parent suggested that the mutation did indeed increase the efficiency of guanosine kinase, as proposed by Hove-Jensen and Nygaard (2). However, these investigators did not find a significantly higher level of enzymatic activity in extracts of the mutant strain and, thus, suggested that the improvement of the mutant enzyme was of a qualitative nature. The data of the previous section suggested that the primary effect of the mutation might be to eliminate feedback inhibition of guanosine kinase by one or more of the guanine nucleotides.

To test this possibility I investigated how the addition of GTP affected the activity of guanosine kinase in crude extracts of CN1861 and CN1932, a pair of isogenic strains carrying the $gsk^+$ and $gsk-3$ alleles, respectively (Table I). In addition, these strains contain a mutation of the $deoD$ gene to eliminate competition from the purine phosphorylase reaction. As shown in Fig. 5, the activity of the wild type guanosine kinase was almost eliminated by the addition of 2 mM of GTP to the assay, whereas the activity of the mutant enzyme was only reduced by
approximately 20%. The physiological concentration of GTP is approximately 1 mM (18), so it is likely that the inhibition observed here in vitro is responsible for the apparent inefficiency of the wild type guanosine kinase in vivo. Conversely, the apparent desensitization of the enzyme to this inhibition by the \textit{gsk-3} mutation satisfactorily accounts for the uncontrolled swelling of the GTP pool in the mutant strain.

In the experiments shown in Fig. 5 there was a 2-fold higher activity in the mutant extract than in the wild type extract even in the absence of GTP, in apparent contradiction to the finding of an essentially unchanged level of enzymatic activity in the original \textit{gsk-3} isolate (2). However, during the performance of these experiments, I observed that the absolute levels of enzymatic activity were somewhat variable because of a marked instability of particularly the wild type enzyme in the crude extract. In other experiments more similar levels of activity were obtained for the two strains, in agreement with the previously published results.

In light of these problems, it should be stressed that these experiments have been repeated several times, always by dividing the extracts and adding GTP to one half immediately before assaying. Irrespective of the absolute levels obtained, the inhibition by GTP were always more than 90% for the wild type and less than 20% for the mutant enzyme. Thus, I believe that the guanosine-sensitive phenotype of the \textit{gsk-3} mutant was caused by this qualitative difference, not by an increase of enzymatic activity, which in any event was less than 2-fold. In agreement with this supposition, I have observed that introduction of a multicopy plasmid containing the wild type \textit{gsk} gene, pGSK2 (19), does not give rise to guanosine sensitivity.

\textbf{Guanosine Sensitivity of the gsk-3 Mutant Is Abolished by a Mutation in the guaC Gene Encoding GMP Reductase—}To gain further insight into the mechanisms by which the increased

\textbf{FIG. 4. Changes in the nucleotide pools of the \textit{gsk}^+ strain, CN1524, after the addition of guanosine to 30 \mu g/ml at time zero.} In this experiment dGTP was not clearly resolved from inorganic pyrophosphate; however, the size of the dGTP pool was probably unaffected by guanosine addition because the amount of radioactivity in the combined spot remained essentially constant (data not shown).

\textbf{FIG. 5. Effect of GTP on the guanosine kinase activity in crude extracts of the strains CN1861 (\textit{gsk}^+) and CN1936 (\textit{gsk}-3).} In each case, the extract was divided in two immediately before the assay, and 2 mM GTP was added to one half. The conversion of [8-\textsuperscript{14}C]guanosine to guanine nucleotides was monitored by thin layer chromatography as described under “Experimental Procedures.”
guanine nucleotide pools inhibit PRPP synthesis and cellular growth, I attempted to isolate guanosine-resistant derivatives of the gsk-3 strain CN1932. Such mutants were readily isolated at a frequency of $7 \times 10^{-6}$; however, an initial characterization of seven guanosine-resistant mutants revealed that the responsible mutation mapped to the region of the gsk gene and resulted in a loss of guanosine kinase activity (data not shown).

To avoid repetitive selection of such simple loss of function mutations in the gsk gene, I isolated guanosine-resistant derivatives of the gsk-3 strain, CN1936, in which both the gpt and hpt genes were inactivated by mutation (Table I). I observed that strains in which salvage of guanosine is completely blocked by triple mutations in the gpt, hpt, and gsk genes (Fig. 1) fail to form colonies on rich medium for reasons that are not entirely clear. Thus, mutant derivatives of CN1936 that had become resistant to guanosine because of mutations in the gsk gene could easily be identified and discarded on account of their inability to form colonies on Luria broth medium.

One guanosine-resistant mutant, CN2079, that was not rich medium-sensitive and did not appear to carry a mutation in the vicinity of the gsk locus was selected for further characterization. By P1 transduction, the mutation responsible for the guanosine resistance was mapped to the approximate position of the guaC gene at 4 min on the genomic map (data not shown). Furthermore, CN2079 became sensitive to guanosine upon transformation with a plasmid, pGS235, that carries a functional guaC gene flanked by 209 and 121 base pairs of upstream and downstream sequence, respectively (10). These results indicated that the guanosine resistance of CN2079 was caused by a loss of function mutation in the guaC gene, which could be complemented by a functional gene copy in trans. The guaC mutation of CN2079, now designated guaC2079, was moved into CN1932 by P1 transduction, and as expected, the resulting strain, CN2133 (Table I), was resistant to guanosine. In addition, CN1932 could also be made resistant to guanosine by introduction of the guaC23 allele, a previously characterized loss of function mutation in the guaC gene (20). Thus, blocking the conversion of guanine nucleotides to adenine nucleotides by mutational inactivation of the guaC gene prevented the fatal effects of guanosine on the gsk-3 mutant.

In liquid medium, growth of the gsk-3 guaC2079 strain, CN2133, was only slightly retarded by guanosine addition (Fig. 2b), even though the nucleotide pools were grossly distorted (Fig. 6). As observed for CN1932, the GTP pool increased more than 3-fold within 3 min accompanied by a decrease of the PRPP pool, again indicating that the initial very rapid decrease of the PRPP pool was caused by the accumulation of one or more of the guanine nucleotides. Interestingly the GTP pool did not increase further, perhaps because the ATP pool, which was initially unaffected by guanosine in CN1932, decreased precipitously in the guaC derivative to reach 10% of its original size after 10 min. Being unable to be synthesized from GMP via the guaC reaction, the reduction of the ATP pool was probably caused by an inhibition of the purine de novo synthesis pathway by the concurrent decrease of the PRPP pool as well as the accumulation of GMP, which is an inhibitor of PRPP amidotransferase, the purF gene product (21).

The lack of inhibition of PRPP synthetase by adenine nucleotides presumably caused the PRPP pool to increase again after 3 min to recover its original size within 30 min. Apparently, the prolonged and complete repression of the PRPP pool observed in the parent strain CN1932 required the combined accumulation of both guanine nucleotides and adenine nucleotides. Most noteworthy, guanosine addition only caused a slight and transient accumulation of ppGpp, coincident with the short period of PRPP pool depression. Furthermore, the recovery of the PRPP pool prevented induction of pyrimidine starvation, thus avoiding the severe depletion of the UTP pool observed in the parent strain. Eventually, after 60 min, the purine nucleotide pools returned to normal, whereas the UTP and PRPP pools increased even further. In other experiments the normalization of the purine nucleotide pools took even longer, occurring only after more than 120 min after guanosine addition. The mechanisms responsible for these later adjustments of the nucleotide pools are not understood.

Guanosine Sensitivity of the gsk-3 Mutant Is Abolished by the prs-1 Mutation—The previous results indicated that a decisive factor in mediating guanosine sensitivity was a restriction of PRPP synthesis caused by swelling of the pools of both guanine and adenine nucleotides, among which ADP is a particularly potent allosteric inhibitor of PRPP synthetase (15, 22). To obtain further support for this interpretation, I introduced into
CN1932 by P1 transduction a previously characterized mutation, prs-1, which renders PRPP synthetase insensitive to inhibition by ADP (9). As anticipated, the resulting strain, CN2178 (Table I), was resistant to guanosine, indicating that PRPP synthetase is indeed the crucial regulatory target affected by the guanosine-induced swelling of the purine nucleotide pools.

CN2178 formed relatively small colonies on glucose minimal plates, and guanosine supplementation seemed to increase the colony size (data not shown), as if purine synthesis and growth of this strain were slightly limited by the mutant prs-1 allele. However, the growth rate of CN2178 in liquid medium was fairly normal and was hardly affected by guanosine addition (Fig. 2b), even though the GTP pool within the first 10 min increased as dramatically as in the parent strain CN1932 (compare Figs. 3 and 7). The PRPP pool of CN2178, however, was only slightly and transiently reduced at 3 min and had nearly recovered at 10 min, in marked contrast to the severe reduction of the PRPP pool seen in the guanosine-sensitive parent strain during the same time interval. The data of the previous sections suggested that the initial rapid decrease of the PRPP pool was caused by accumulation of a guanine nucleotide, so this finding indicates that the prs-1 mutation renders PRPP synthetase insensitive not only to inhibition by ADP but also to inhibition by guanine nucleotides. In line with the lack of PRPP restriction, there was hardly any accumulation of ppGpp during the first 10 min to signal starvation for histidine or tryptophan. Moreover, de novo synthesis of adenine nucleotides presumably continued at a considerable rate in the absence of PRPP restriction, which may explain that the ATP pool in CN2178 reproducibly doubled within the first 10 min in marked contrast to the initial constancy of this pool in CN1932.

After 10 min, the purine nucleotide pools continued to increase at a slowing rate, whereas the PRPP pool started to decrease, perhaps because the purine pools increased to such high levels that even the mutant PRPP synthetase started to be inhibited. At the same time, ppGpp started to accumulate to reach a moderately high level, which might signal a partial restriction of histidine and tryptophan synthesis. Finally, it is noteworthy that the prs-1 mutant just like the guaC2079 mutant, managed to avoid the severe depletion of the UTP pool observed in the guanosine-sensitive parent strain.

**Mutational Inactivation of the relA Gene Confers Guanosine Resistance upon a gsk-3 Mutant**—It seemed paradoxical that the gsk-3 mutation gave rise to guanosine sensitivity in the CN1524 background, considering that it was originally selected for its ability to permit growth of a purE deoD mutant on guanosine. The difference, however, did not seem to be related to the purine prototrophy of CN1524. The gsk-3 mutation also gave rise to guanosine sensitivity when transduced into a purE derivative of CN1524 (data not shown). Having observed the marked induction of ppGpp synthesis in CN1932 in response to guanosine and noting that the gsk-3 allele was originally selected in a relA mutant strain (2), I realized that ppGpp might aggravate the guanosine-induced disturbances of nucleotide metabolism to an intolerable extent.

To test this hypothesis, a relA null allele, ΔrelA251::kan (5), was introduced into CN1932 by P1 transduction. Without exception, all Kan<sup>R</sup> transductants became resistant to guanosine, indicating that a functional relA gene is indeed required for the guanosine-sensitive phenotype of the gsk-3 strain.

In liquid medium, the growth of one such gsk-3ΔrelA251::kan transductant, CN1995 (Table I), was initially unaffected by guanosine addition, but after 1.5 h, the growth rate was somewhat reduced (Fig. 2b). The initial response of the nucleotide pools of CN1995 to guanosine addition was very similar to that of its relA<sup>−</sup> parent strain, except for the lack of ppGpp accumulation (Fig. 8). Within 3 min, the GTP pool increased 3-fold, whereas the PRPP pool decreased abruptly; thus, the guanine nucleotide responsible for the initial decrease of the PRPP pool was apparently not ppGpp. However, the PRPP pool in CN1995 did not decrease to the same extent as in CN1932 (Fig. 3), which may explain why CN1995 at later times managed to maintain a fairly normal UTP pool, in marked contrast to the parent strain.

**DISCUSSION**

**Feedback Regulation of Guanosine Kinase**—The present results indicate that guanosine kinase of *E. coli* is subject to efficient feedback inhibition by GTP and that this regulation was essentially eliminated by the gsk-3 mutation. Because the gsk-3 mutation had no effect on bacterial growth or the sizes of the nucleotide pools in the absence of exogenous guanosine, it appears that the primary role of this regulation is to prevent excessive salvage of guanosine from the external environment.

The strong inhibition of guanosine kinase by GTP is probably...
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FIG. 8. Changes in the nucleotide pools of CN1995 (gsk-3 ΔrelA251:kan) after the addition of guanosine to 30 μg/ml at time zero.

the main reason why exogenous guanosine in wild type cells is predominantly catabolized by purine phosphorylase to ribose-1-phosphate and guanine, which can be converted to GMP on demand by the phosphoribosyltransferases encoded by the gpt and hpt genes (reviewed in Ref. 18). This two-step pathway is energetically expensive; however, a decrease of the GTP pool caused by severe energy limitation would be expected to release feedback inhibition and favor salvage of guanosine via the more energy-efficient guanosine kinase reaction. During energy sufficiency the teleological advantage of predominantly catabolizing guanosine via the purine phosphorylase reaction is probably that the liberated ribose-1 phosphate can be used as a carbon source even if the demand for purine synthesis is saturated. This arrangement has the additional benefit that the accumulating guanine together with the PurR repressor will repress the genes of the purine de novo synthesis pathway (23).

Regulation of the PRPP Pool—1 I have tried to trace how the “shock waves” from the guanosine-induced swelling of the guanine nucleotide pools propagated through the metabolic network of the gsk-3 mutant to eventually inhibit cellular growth. The results strongly suggest that a crucial step in this chain of events involves a severe restriction of PRPP synthesis, initially caused by the increase of the guanine nucleotide pools and later on consolidated by the subsequent swelling of the adenine nucleotide pools. This scenario is supported by the observed pool kinetics, together with the finding that the guanosine sensitivity of the gsk-3 mutant could be alleviated by supplementation with PRPP-derived metabolites. Furthermore, the relief caused by the guaC mutations or by the prs-1 mutation suggests that the most important restriction of PRPP synthesis was caused by an adenine nucleotide, presumably ADP.

The inhibition of PRPP synthesis by purine nucleotides normally serves as a useful feedback control of the de novo synthesis of purines, the major pathway consuming PRPP (3). However, the “gsk-3 syndrome” was in essence caused by the fact that PRPP synthesis is regulated mainly by the purine nucleotide pools, even though PRPP is required for the synthesis of histidine, tryptophan, pyrimidines, and NAD as well. To avoid unnecessary starvation for these other metabolites, it is mandatory that excessive salvage of purines from the external environment is prevented by a tight feedback control, as shown here for guanosine kinase. Indeed, the phosphoribosyltransferases encoded by the apt and gpt genes are also found to be “feedback-regulated” in vitro by their products AMP and GMP, respectively (24–26).

All strains used in the present work contain a deletion of the gpt gene encoding one of the two phosphoribosyltransferases capable of converting guanine to GMP (Fig. 1). However, it should be emphasized that the guanosine sensitivity caused by the gsk-3 mutation was not in any way dependent on or accentuated by the gpt deletion. On the contrary, I found that a gpt+ derivative of CN1932 was even more sensitive to guanosine than the parent strain (data not shown). This might have been expected, because strains with mutations in the gpt or hpt genes are known to have an increased PRPP pool (23).

The large pool of PRPP in such strains is probably the outcome of a complex interplay between the dual levels of feedback control of the purine de novo synthesis pathway by the purine bases and the purine nucleotides. When guanine or hypoxanthine accumulates, the pathway is repressed at the genetic level by the PurR repressor. However, the resulting initial decrease of the purine nucleotide pools presumably leads to reduced feedback inhibition of PRPP synthesis at the enzymatic level. The higher PRPP pool in turn relieves feedback inhibition of the PurF enzyme by GMP and AMP (21), thus allowing establishment of a new steady state with sufficient flux to purine nucleotides despite the repressed level of the enzymes of the pathway.

The function of such a homeostatic mechanism adjusting the PRPP pool to accommodate a sufficient de novo synthesis of purines, may explain the curious swelling of the PRPP pool in the gsk+ strain CN1524 after the addition of guanosine (Fig. 4), which upon conversion to guanine, probably repressed the genes of the de novo pathway. It is also supported by the observation that the unusually large PRPP pool of a gpt hpt mutant is reduced back to normal by introduction of a mutant purR allele.

The Role of ppGpp in Guanosine Sensitivity—The guanosine resistance conferred upon the gsk-3 mutant by the relA mutation remains somewhat of a mystery. Apparently, the accumulation of ppGpp somehow fatally aggravated the consequences of the PRPP restriction imposed by guanosine addition, even though the results provide no definitive clue as to how this was accomplished. However, one notable feature distinguished the
guanosine-sensitive strain CN1932 from the relA derivative and the other guanosine-resistant derivatives, namely its inability to maintain a normal UTP pool when challenged with guanosine. This might hint that ppGpp somehow restricted the synthesis or salvage of pyrimidines already compromised by the limitation for PRPP.

The genes of the pyrimidine synthesis pathway are known to be derepressed in response to PRPP limitation (reviewed in Ref. 14). It is conceivable that such a compensatory derepression might have been blocked by ppGpp, which is known to inhibit expression of the carAB and pyrBI operons encoding the first two enzymes of the pyrimidine de novo synthesis pathway (27–29). ppGpp is also a potent inhibitor of pyrimidine salvage, although the mechanisms responsible for this effect are not fully understood (30). Even though these findings are suggestive, further studies are clearly required to establish if inhibition of pyrimidine metabolism by ppGpp can account for the detrimental effect of the relA allele in the gsk-3 mutant. In any event it was probably the presence of the relA mutation that made possible the isolation of the original gsk-3 mutant on medium containing guanosine (2).

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