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Variations in UDP-*N*-Acetylglucosamine and UDP-*N*-Acetylmuramyl-Pentapeptide Pools in *Escherichia coli* after Inhibition of Protein Synthesis

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The pool levels of the nucleotide precursors of peptidoglycan were analyzed after inhibition of protein synthesis in various *Escherichia coli* strains. In all cases UDP-*N*-acetylglucosamine (UDP-GlcNAc) and UDP-*N*-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) cell pools increased upon treatment with chloramphenicol or tetracycline. Similar results were observed after the treatment of K-12 strains with valine. Since the intermediate nucleotide precursors did not accumulate after the arrest of protein synthesis and since a feedback mechanism was unlikely, the increases of the UDP-MurNAc-pentapeptide pool appeared as a consequence of that of the UDP-GlcNAc pool by the unrestricted functioning of the intermediate steps of the pathway. The highest increase (sixfold) of UDP-GlcNAc was observed with strain K-12 HfrH growing in minimal medium and treated with chloramphenicol. When a pair of isogenic Rel$^+$ and Rel$^-$ strains were considered, both the UDP-GlcNAc and UDP-MurNAc-pentapeptide pools increased upon treatment with chloramphenicol or valine. However, the UDP-GlcNAc pool of the Rel$^+$ strain was at a high natural level, which increased only moderately (20%) after the addition of valine. The increase of the UDP-GlcNAc pool after the various treatments could be due to an effect on some upstream step by an unknown mechanism. The possible correlations of the variations of the precursor pools with the rate of synthesis and extent of cross-linking of peptidoglycan were also considered.

Although the syntheses of peptidoglycan (6) and protein (7) have both been extensively studied in *Escherichia coli*, the possible correlations between these two essential systems have not been investigated in detail. The approach has been to inhibit protein synthesis by amino acid deprivation or by chloramphenicol and to examine the consequences on the structure of peptidoglycan (4, 5, 15) or on the level of its main cytoplasmic precursor, UDP-*N*-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) (8). In the latter case, Ishiguro and Ramey (8) have shown that the response depends on the *relA* gene. In a Rel$^-$ strain auxotrophic for lysine, UDP-MurNAc-pentapeptide accumulates upon lysine deprivation, whereas no accumulation was observed with the corresponding Rel$^+$ strain. However, UDP-MurNAc-pentapeptide accumulates when the Rel$^+$ strain is treated with chloramphenicol in the presence or absence of lysine. From these experiments it was concluded that the *relA* gene product plays in some way a role in regulating the synthesis of the nucleotide precursor.

The cytoplasmic pathway of peptidoglycan synthesis in *E. coli* is characterized by a sequence of six enzymatic reactions which leads from UDP-*N*-acetylglucosamine (UDP-GlcNAc) to UDP-MurNAc-pentapeptide. The cell contents of the nucleotide precursors involved in these steps have been determined for various growth conditions (9, 11, 12). UDP-GlcNAc and UDP-MurNAc-pentapeptide were always present at the highest concentrations. Speculating that there is apparently a free flux with no limiting step between UDP-GlcNAc and UDP-MurNAc-pentapeptide, the variations of the UDP-MurNAc-pentapeptide pool, observed by Ishiguro and Ramey (8) upon inhibition of protein synthesis, could therefore well be due to variations in the UDP-GlcNAc pool. In the present paper a detailed analysis of the pools of the various peptidoglycan nucleotide precursors after either amino acid deprivation or chloramphenicol or tetracycline treatment was undertaken in various *E. coli* strains and in particular in two isogenic Rel$^+$ and Rel$^-$ strains. Furthermore, the possible correlations between these pool variations and the rate of synthesis and degree of cross-linking of peptidoglycan were examined.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains used in this study are listed in Table 1. Cells were grown as previously described (9) in LB medium or minimal medium M63 supplemented with glucose (0.2%). When required, thiamine (0.5 mg liter^{-1}), L-lysine (100 μg ml^{-1}), and kanamycin (25 μg ml^{-1}) were added. In all cases, 2-liter flasks containing 500 ml of medium were inoculated with 0.5 ml of overnight precultures and growth was monitored by measuring the optical density at 600 nm in a spectrophotometer (model 240; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). General genetic and cloning techniques were carried out by the methods of Miller (13). For the Rel phenotype, the quick test described by Uzan and Danchin (18) was used; replica patches of Rel$^+$ clones, previously grown in broth, grow in minimal medium supplemented with serine, glycine, and methionine, each at 100 μg ml^{-1}, whereas Rel$^-$ clones do not. A more thorough identification was made by the study of incorporation of [^{14}C]uracil into stable RNA after valine addition by the method of Stent and Brenner (14).

Chemicals and analytical procedures. All the reagents used were of commercial analytical grade. *meso*-3,4,5-[^{3}H]diaminopimelic acid (DAP; 888 GBq mmol^{-1}) was purchased from the Commissariat à l'Energie Atomique (Saclay, France), and [2-^{14}C]uracil (2 GBq mmol^{-1}) was from Amersham (Les

TABLE 1. Bacterial strains used in this study

Strain	Relevant genotype	Source or reference
E. coli K-12		
RM4102 *lysA*	*araD139* Δ(*lac*)U169 *strA thi lysA*::Km[r]	Printz[a]
MC4100 *lysA*		20
FB8 Rel[+]	F^{−} proťotrophic	18[b]
FB8r Rel[−]	*relA*, from FB8	18[b]
FB8 *lysA*	FB8 *lysA*::Km[r]	This study[c]
FB8r *lysA*	FB8r *lysA*::Km[r]	This study[c]
HfrH	*thi-1 rel-1*	Patte[d]
HfrH *lysA*	*thi-1 rel-1 lysA*::Km[r]	This study
E. coli B		ATCC[e]
E. coli K235		ATCC[f]

[a] Obtained from C. Printz (Institut de Microbiologie, Orsay, France).
[b] Obtained from A. Danchin (Institut Pasteur, Paris, France).
[c] These strains were constructed by P1 grown on RM4102 *lysA*.
[d] Obtained from J. C. Patte (Institut de Microbiologie, Orsay, France).
[e] ATCC 11303.
[f] ATCC 13027.

Ulis, France). Chloramphenicol was bought from Labosi (Paris, France), and kanamycin and tetracycline were from Serva (Heidelberg, Federal Republic of Germany). High-pressure liquid chromatographies (HPLC) were carried out with already described devices (9, 11). Amino acid and amino sugar compositions were determined with an amino acid analyzer (model LC2000; Biotronik, Frankfurt/Main, Federal Republic of Germany) after hydrolysis of samples in 6 M HCl at 95°C for 16 h and by using *o*-phthalaldehyde and 2-mercaptoethanol as reagents.

Pools of peptidoglycan precursors. Cultures (1 liter) of mid-exponential-phase cells (180 mg of bacteria [dry weight] liter^{-1} of culture) were rapidly chilled to 0°C, and cells were harvested in the cold. The extraction of free amino acids and peptidoglycan nucleotide precursors and the analytical procedure used for their quantification were previously described (9, 11).

Isolation of sacculi and quantification of peptidoglycan. Cells from 1-liter cultures were rapidly chilled to 0°C and harvested in the cold. Pellets were washed with a cold aqueous 0.85% NaCl solution and centrifuged again. Bacteria were then rapidly suspended under vigorous stirring in 40 ml of a hot (95 to 100°C) aqueous 4% sodium dodecyl sulfate solution for 30 min. After standing overnight at room temperature, suspensions were centrifuged for 90 min at 100,000 × *g*, and the pellets were washed several times with water. Final suspensions made in 5 ml of water were homogenized by brief sonications. The peptidoglycan content of sacculi was expressed in terms of DAP content (12).

Uptake of *meso*-[^{3}H]DAP and its incorporation into peptidoglycan. To measure the kinetics of DAP incorporation into peptidoglycan, exponentially growing cultures of the *lysA* strains were diluted into 50 ml of fresh prewarmed minimal medium containing *meso*-[^{3}H]DAP (18.5 kBq ml^{-1}). After designated time intervals, 500-μl samples were removed and centrifuged for 3 min with an Eppendorf centrifuge. The supernatants were analyzed for radioactivity in order to estimate the rate of DAP uptake. In parallel, other 500-μl samples were added to 9.5 ml of ice-cold 5.5% trichloroacetic acid (TCA). Suspensions were kept at 0°C for 60 min and then filtered over glass fiber filters (GF/C; Whatman, Inc., Clifton, N.J.). The filters were washed with cold 5% TCA, dried, and immersed in 2 ml of 0.1 N NaOH in counting vials. Finally, 16 ml of Aqualyte mixture (J. T. Baker Chemical Co., Deventer, The Netherlands) was added, and counts were made in a spectrophotometer (model SL30; Intertechnique, Plaisir, France).

RESULTS

Effects of inhibition of protein synthesis on peptidoglycan precursor pool levels. The effects of chloramphenicol on the pool levels of the nucleotide precursors of peptidoglycan were examined in different wild-type *E. coli* strains grown in glucose minimum medium (Table 2). In all cases, the UDP-GlcNAc and UDP-MurNAc-pentapeptide contents increased after drug treatment. The effect was greatest with strain K-12 HfrH, in which a 6-fold increase of UDP-GlcNAc and a 13-fold increase of UDP-MurNAc-pentapeptide were observed. Considering the latter strain only, similar results were obtained when the carbon source was changed from glucose to GlcNAc. In glucose minimum medium, the effects of tetracycline were the same as those with chloramphenicol. In rich medium, the tetracycline treatment led only to twofold increases (Table 2). In *E. coli* K-12 strains, protein synthesis can also be easily and efficiently inhibited by the addition of valine to cultures (17). This is due to the fact that the valine-sensitive acetohydroxybutyric acid synthetase also catalyzes the formation of acetolactic acid, which is a precursor of isoleucine. Under these conditions a 3- to 4-fold increase in UDP-GlcNAc and a 10-fold increase in UDP-MurNAc-pentapeptide were observed (Table 2).

Strain K-12 HfrH used here was described as *relA* (Table 1). It behaved as such when the uracil uptake into stable RNA was examined by the method of Stent and Brenner (14) after the addition of valine. To examine possible relationships between the Rel phenotype and peptidoglycan precursor pool levels, it was necessary to consider two isogenic strains with well-defined Rel$^+$ and Rel$^-$ phenotypes. For this purpose, Rel$^+$ strain FB8 and Rel$^-$ strain FB8r (Table 1) were used. Their phenotypes were verified by the Stent and Brenner test (14) and by the Uzan and Danchin test (18). With Rel$^-$ strain FB8r, the pool levels of UDP-GlcNAc and UDP-MurNAc-pentapeptide increased after inhibition of protein synthesis by chloramphenicol or valine (Table 2), although to a lesser extent than with strain HfrH. With Rel$^+$ strain FB8, similar results were observed except that during normal growth it possessed a high UDP-GlcNAc pool level, which increased only by 20% after the addition of valine. In both strains, the effect of the combined treatment with chloramphenicol and valine was that of chloramphenicol alone.

Effect of inhibition of protein synthesis on the rate of peptidoglycan synthesis. Since UDP-GlcNAc and UDP-MurNAc-pentapeptide are the nucleotide precursors used as substrates in the first two membrane steps of peptidoglycan biosynthesis (6), the sharp increase of their pools upon inhibition of protein synthesis could have an effect on the rate and amounts of material synthesized. To investigate such possible correlations, a first approach was to inhibit protein synthesis by chloramphenicol, tetracycline, or valine, to isolate after 30 min (one-half a generation time) crude sacculi, and to quantify peptidoglycan material by determination of DAP contents. Results (Table 3) were expressed as total amounts present per unit volume of cultures. With strain K-12 HfrH, only after chloramphenicol

TABLE 2. Effects of chloramphenicol, tetracycline, and valine on the pool levels of the peptidoglycan nucleotide precursors of *E. coli*[a]

Strain and growth medium	Pool level (nmol/g of bacteria [dry wt]) of:				
	UDP-GlcNAc	UDP-MurNAc	UDP-MurNAc-dipeptide	UDP-MurNAc-tripeptide	UDP-MurNAc-pentapeptide
K-12 HfrH in M63 + glucose					
Control	925	280	5	45	230
Chloramphenicol	5,650	515	30	12	3,050
Tetracycline	3,980	440	15	11	2,120
Valine	3,130	335	17	85	2,300
K-12 HfrH in M63 + GlcNAc					
Control	1,390	300	5	40	340
Chloramphenicol	6,040	527	10	65	2,600
K-12 HfrH in LB					
Control	1,200	190	20	30	1,300
Tetracycline	2,600	265	100	65	2,350
B in M63 + glucose					
Control	940	230	5	215	1,750
Chloramphenicol	1,815	290	6	125	4,150
K235 in M63 + glucose					
Control	365	215	2	80	1,250
Chloramphenicol	1,010	210	3	390	3,330
FB8 *rel*$^+$ + glucose					
Control	805	265	24	106	930
Chloramphenicol	1,884	385	18	201	2,950
Valine	961	312	9	67	1,850
Chloramphenicol + valine	2,055	380	19	189	3,070
FB8r *relA* + glucose					
Control	356	210	19	95	693
Chloramphenicol	1,342	360	29	260	2,401
Valine	987	340	15	215	2,008
Chloramphenicol + valine	1,205	360	38	300	2,470

[a] Experiments were performed with exponential-phase cultures (1 liter) of the various *E. coli* strains grown in minimal or LB medium. At an optical density at 600 nm of 0.5, corresponding to ca. 180 mg of bacteria (dry weight) per liter of culture, chloramphenicol (200 μg ml^{-1}), tetracycline (2 μg ml^{-1}), or L-valine (500 μg ml^{-1}) were respectively added at the indicated final concentrations. At 30 min later, cells were rapidly chilled to 0°C and harvested in the cold, and their peptidoglycan nucleotide precursors were extracted and quantified as detailed in Materials and Methods.

treatment was the increase in peptidoglycan content lower (16%) than in the control, whereas with Rel$^+$ strain FB8 and Rel$^-$ strain FB8r, 20 to 40% lower increments were observed whatever the treatment.

The determination of peptidoglycan contents over a 30-min period gives only a very rough idea of the variations of the rate of synthesis. It would be necessary to carry out measurements at various time intervals. Although in principle possible, this is not readily feasible considering the amount of work required. A more convenient approach was to follow the incorporation of exogenous radioactive DAP into TCA-insoluble material. To avoid interference with labeling of cell proteins, conversion of DAP to lysine must be blocked. This was achieved by introducing the *lysA* mutation, affecting DAP decarboxylase, into the Rel$^+$ FB8 and Rel$^-$ FB8r strains. The Rel$^+$ or Rel$^-$ phenotype of the resulting FB8 *lysA* and FB8r *lysA* strains was verified by the Stent and Brenner test (14) and by the Uzan and Danchin test (18). When [^{3}H]DAP of high specific activity was added (1.11 MBq ml^{-1}) to early-exponential-phase cultures (10^8 cells ml^{-1}) of both strains, a rapid uptake of most of the radioactivity was observed (Fig. 1) within 5 min and was thus equivalent to a short pulse. In control cultures, the incorporation into TCA-insoluble material was linear over a 3-h period (Fig. 1). The observed linear kinetics were presumably the result of the exponential synthesis of peptidoglycan and of the steady dilution of the radioactive DAP pool by newly made endogenous unlabeled DAP. To obtain the true kinetics of peptidoglycan synthesis, a mathematical treatment of the data would be required. However, for the comparative studies developed here, this was unnecessary. After the addition of chloramphenicol or valine, the rate of incorporation into TCA-insoluble material first remained constant for a certain time and then steadily decreased with similar kinetics in both the Rel$^+$ and Rel$^-$ strains (Fig. 1). However, the decrease was initiated somewhat earlier in the Rel$^+$ strain (ca. 10 min) than in the Rel$^-$ strain (ca. 30 min). It was noteworthy that the initial uptake of the added radioactive DAP was followed by a steady, slow release of radioactive material (Fig. 1). This excretion, which increased greatly with chloramphenicol-treated cells of both strains and decreased with the valine-treated cells of the Rel$^+$ strain, was not further analyzed.

Extent of cross-linking of peptidoglycan in the absence of protein synthesis. Previous reports (4, 5, 15) indicated that change in the extent of cross-linking is an important feature of the structural modifications undergone by peptidoglycan after inhibition of protein synthesis. Since the slowdown in peptidoglycan synthesis varied with the Rel phenotype, it was essential to examine the extent of cross-linking of peptidoglycan in the two isogenic strains FB8 and FB8r before and after the arrest of protein synthesis. This was performed in two different ways. The first approach was to analyze by HPLC the fragments resulting from *N*-acetylmuramidase digestion of sacculi isolated after chloramphenicol, tetracycline, or valine treatments of both strains. The degrees of cross-linking were determined by considering only the relative amounts of the main monomer and dimer frag-

TABLE 3. Effects of chloramphenicol, tetracycline, and valine on the rate of peptidoglycan synthesis in *E. coli*[a]

Strain and growth conditions	Amt (nmol/liter of culture) of isolated peptidoglycan
HfrH	
Control at:	
Time zero	1,550
30 min	2,380
30 min after:	
Chloramphenicol	2,250
Tetracycline	2,340
Valine	2,400
FB8 *rel*$^+$	
Control at:	
Time zero	2,580
30 min	4,160
30 min after:	
Chloramphenicol	3,490
Valine	3,830
Chloramphenicol + valine	3,580
FB8r *relA*	
Control at:	
Time zero	2,210
30 min	3,570
30 min after:	
Chloramphenicol	3,170
Valine	3,175
Chloramphenicol + valine	2,860

[a] Cells (1-liter cultures) were grown exponentially in minimal medium supplemented with glucose. At an optical density at 600 nm of 0.5, corresponding to ca. 180 mg of bacteria (dry weight) per liter of culture, chloramphenicol (200 μg of ml^{-1}), tetracycline (2 μg ml^{-1}) or L-valine (500 μg ml^{-1}) was added at the indicated final concentrations. At 30 min later, cells were rapidly chilled to 0°C and harvested in the cold, and their peptidoglycan was isolated and analyzed as detailed in Materials and Methods.

ments designated as tetra and tetra-tetra by Glauner (3). No significant differences were observed in the HPLC patterns (data not shown), except that the extent of cross-linking in valine-treated cells was ca. 15% lower than in the control.

The second approach was to analyze newly synthesized peptidoglycan material. For this purpose valine-treated cells of strains FB8 *lysA* and FB8r *lysA* were labeled with [^{3}H]DAP for 5 or 30 min and the labeled fragments resulting from *N*-acetylmuramidase digestion of the isolated sacculi were analyzed by HPLC. It was verified that the valine treatment had no effect on the uptake of DAP. Both *lysA* and *relA lysA* strains were grown in the presence of methionine and threonine to enhance the specific radioactivity of DAP incorporated into peptidoglycan (Fig. 2) as recently shown by Wientjes et al. (20). This appeared as a consequence of the sharp decrease of the unusually high endogenous DAP pool levels of *lysA*-deficient strains (Table 4). When controls were compared (Table 5), newly made 5-min-labeled peptidoglycan appeared 21 to 24% less cross-linked than 30-min-labeled material. The extent of cross-linking at 30 min (21.1 and 20.7%) was close to that (20.5%) determined by HPLC analysis for unlabeled peptidoglycan (data not shown). The lower cross-linking of newly made peptidoglycan material was in agreement with previous investigations (1, 2) and observed in both strains. When the valine-treated cells were considered, the extent of cross-linking was 6 to 20% lower than in the controls after 5 or 30 min of labeling. This was also true with both strains (Table 5).

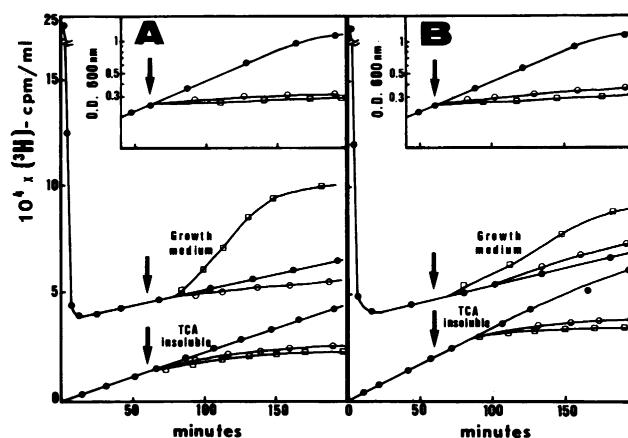

FIG. 1. Effect of valine and chloramphenicol on the rate of peptidoglycan synthesis in isogenic stringent (A) and relaxed (B) strains of *E. coli*. FB8 *rel*$^+$ *lysA* (A) and FB8r *relA lysA* (B) were grown exponentially in 50-ml cultures of minimal medium supplemented with lysine (100 μg/ml). When the optical density (O.D.) at 600 nm reached 0.1 (ca. 36 mg [dry weight] l^{-1}), *meso*-[^{3}H]DAP (18.5 kBq ml^{-1}) was added to the culture, and the incubation was continued for 60 min before the addition of L-valine (500 μg/ml^{-1}) or chloramphenicol (200 μg/ml^{-1}). As described in Material and Methods, samples were removed at different times and analyzed for radioactivity present either in the supernatant growth medium or in the TCA-precipitable material (peptidoglycan). Symbols: ●, control cells; ○, valine; □, chloramphenicol.

DISCUSSION

The pool levels of both UDP-GlcNAc and UDP-MurNAc-pentapeptide increased upon inhibition of protein synthesis in *E. coli*. The variations of these pools appeared roughly correlated: the higher the UDP-GlcNAc content, the higher the UDP-MurNAc-pentapeptide content. Considering that the intermediate precursors do not accumulate to a great extent under these conditions, most likely the increase in UDP-MurNAc pentapeptide is merely a consequence of that of UDP-GlcNAc, owing to the unrestricted functioning of the intermediate steps of the pathway. This is in agreement with the fact that under conditions of normal growth, UDP-GlcNAc and UDP-MurNAc-pentapeptide pool levels are higher than those of the other intermediate peptidoglycan nucleotide precursors (11). Although an indirect effect of UDP-MurNAc-pentapeptide on the UDP-GlcNAc pool through the intermediate steps was thus excluded, a direct in vitro effect of UDP-MurNAc-pentapeptide on UDP-GlcNAc-enolpyruvyl transferase with a 40% inhibition at 10^{-3} M has been reported (11). Since the normal concentration in the cell of this nucleotide is ca. 10^{-4} M (9), a feedback mechanism could perhaps function in vivo for high UDP-MurNAc-pentapeptide pool levels. However, under two circumstances, we have observed significant increases in the UDP-MurNAc-pentapeptide pool without any effect on the UDP-GlcNAc pool. A two- to threefold increase was found upon treatment with moenomycin or vancomycin at sublytic concentrations, and a sevenfold increase was found with cells grown in minimum medium with L-alanine as the sole carbon source (unpublished data). Therefore, the accumulation of UDP-GlcNAc upon the arrest of protein synthesis can be considered as an effect on some upstream step by a yet unknown mechanism. It will be of importance to determine which step between fructose-6-phosphate and UDP-GlcNAc is involved in this control (19). It is noteworthy that

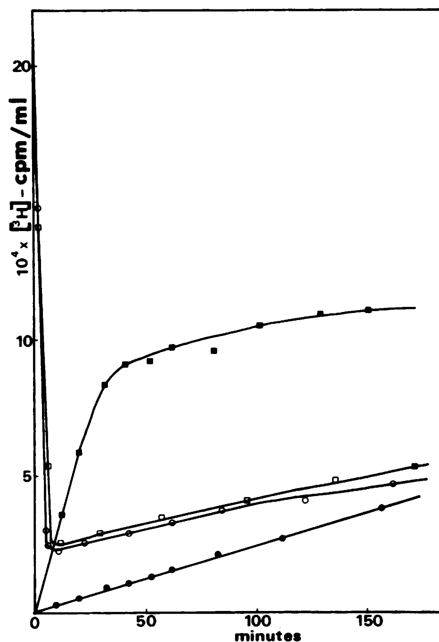

FIG. 2. Uptake of DAP and its incorporation into peptidoglycan of strain FB8 *lysA*. FB8 *lysA* was grown exponentially in 50-ml cultures of minimal medium supplemented with lysine or lysine, threonine, and methionine (each at 100 μg ml^{-1}). When the optical density at 600 nm reached 0.1 (ca. 36 mg [dry weight] $liter^{-1}$), *meso*-[^{3}H]DAP (18.5 kBq ml^{-1}) was added to the cultures. As described in Material and Methods, samples were removed at different time intervals and analyzed for radioactivity present either in the supernatant growth medium (open symbols) or in the TCA-precipitable material (peptidoglycan) (closed symbols). Symbols: ○ and ●, with lysine; □ and ■, with a mixture of lysine, threonine, and methionine.

UDP-GlcNAc is also a precursor for the syntheses of lipopolysaccharide and enterobacterial common antigen. Any regulation prior to this branching point would thus enable a coordinate control of the syntheses of these two outer membrane macromolecules and peptidoglycan.

The present data confirm the initial observation of Ishiguro and Ramey (8), who described the accumulation of UDP-MurNAc-pentapeptide upon the arrest of protein synthesis in a Rel$^-$ strain or in a Rel$^+$ strain treated with

TABLE 4. DAP pools of various *lysA* strains

Strain and growth conditions	Pool of DAP	
	Amt (nmol/g of bacteria [dry wt])[a]	Molar concn[b]
FB8 *rel*$^+$ *lysA* + lysine	66,400	2.9×10^{-2}
FB8 *rel*$^+$ *lysA* + lysine + threonine + methionine	3,120	1.4×10^{-3}
FB8r *relA lysA* + lysine	60,900	2.7×10^{-2}
MC4100 *lysA* + lysine	73,000	3.2×10^{-2}
HfrH *lysA* + lysine	55,100	2.4×10^{-2}

[a] Cells were grown exponentially in minimal medium supplemented with glucose and 100 μg ml^{-1} of the listed amino acids. At an optical density at 600 nm of 0.7 (250 mg of bacteria [dry weight] per liter of culture), cells were harvested in the cold, and DAP pool values were determined as described in Materials and Methods. In all cases, the DAP pool contained 65 and 35% of the *meso*-isomer and LL-isomer, respectively, as determined by a recently described HPLC procedure (10).

[b] Molar concentrations were calculated assuming a 70% cell water content.

TABLE 5. Extent of cross-linking of newly synthesized peptidoglycan before and after isoleucine deprivation in isogenic relaxed and stringent *E. coli* strains

Strain and labeling conditions	Cross-linkage[a]
FB8 *rel*$^+$ *lysA*	
5-min labeling	
Without valine	13.2
With valine	12.4
30-min labeling	
Without valine	17.5
With valine	14.0
FB8r *relA lysA*	
5-min labeling	
Without valine	13.7
With valine	12.9
30-min labeling	
Without valine	17.3
With valine	13.9

[a] The extent of cross-linkage was determined as the radioactivity ratio of [(dimer tetra-tetra)/2]/[(monomer tetra) + (dimer tetra-tetra)]. FB8 *rel*$^+$ *lysA* and FB8r *relA lysA* were grown exponentially in 50-ml minimal medium cultures supplemented with 0.2% glucose, and lysine, threonine, and methionine (100 μg/ml). When the optical density of cultures at 600 nm reached 0.3 (ca. 108 mg l^{-1}), isoleucine deprivation was induced by adding L-valine to the medium (500 μg/ml). At 10 min later, *meso*-[^{3}H]DAP (7.4 kBq ml^{-1}) was added, and cells were harvested after further incubation for 5 or 30 min. As previously described (10), sacculi were prepared and digested with *N*-acetylmuramidase, and the resulting peptidoglycan fragments were analyzed by HPLC. The radioactivity of the main monomer and dimer fragments was measured.

chloramphenicol. It was suggested that both the *relA* gene product and a feedback inhibition are involved in the regulation of its synthesis. From the present results, the control must now be looked for at the level of UDP-GlcNAc or upstream. Ishiguro and Ramey (8) also observed that the accumulation of UDP-MurNAc-pentapeptide does not occur in Rel$^+$ cells submitted to an amino acid deprivation, whereas in our case the same accumulation after valine treatment was observed irrespective of the Rel phenotype. Furthermore, Ishiguro and Ramey (8) have found that peptidoglycan synthesis is under stringent control, since 15 min after amino acid deprivation, the rate of synthesis is greatly reduced in a Rel$^+$ strain, whereas in the isogenic Rel$^-$ strain, no decrease was observed. In our case, the regulation by the stringent control mechanism was likewise suggested by the fact that the slowdown in peptidoglycan synthesis after the addition of valine occurred earlier in the Rel$^+$ strain than in the Rel$^-$ strain. However, to ascertain this point, more accurate kinetics of the incorporation of DAP into peptidoglycan during the first 30 min after inhibition of protein synthesis will be necessary.

It can be estimated that the UDP-MurNAc-pentapeptide pool determined after 30 min of inhibition of protein synthesis represents only 30 to 60% (Table 2) of the amount of peptidoglycan synthesized over that period (Table 3). This implies a continuous supply of both UDP-GlcNAc and UDP-MurNAc-pentapeptide but in no way accounts for the need for higher pool levels. A possible explanation could be that higher pool levels of these cytoplasmic precursors are required to insure a higher pool of the membrane precursor directly used in the polymerization reactions of peptidoglycan synthesis (6). The increased membrane pool could perhaps help to sustain peptidoglycan synthesis by compensating for the arrest of synthesis of new enzymes involved in this metabolism. A better understanding of the possible

correlations between the cytoplasmic precursor pools and peptidoglycan synthesis would imply analyzing the membrane precursor pool and comparing the variations of the different pools with the kinetics of peptidoglycan synthesis.

Two additional features of the present work should be stressed. The first one is the much lower DAP pool of cells of strain FB8 *rel*$^+$ *lysA* grown in the presence of methionine and threonine. Its value (1.4×10^{-3} M) is close to that (0.6×10^{-3} M) encountered in a wild-type K-12 strain (9). Considering this fact and the fast uptake of exogenous radioactive DAP, the *lysA* strains used under the conditions described here could be useful for various studies of peptidoglycan metabolism, as recently suggested by Wientjes et al. (20). The second feature concerns the cross-linking of newly made peptidoglycan material after inhibition of protein synthesis. Recently, HPLC analysis revealed various structural modifications 15 min after lysine deprivation (16), the most striking being the threefold decrease of the main dimer tetra-tetra and thus a very low degree of cross-linking. In our case, the inhibition by valine led to a lesser decrease in cross-linking, which was due to a decrease (ca. 20%) in dimer tetra-tetra content but also to a small increase in the main monomer tetra content. Further detailed HPLC analyses will be necessary to evaluate all the structural modifications involved in this type of inhibition.

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