JOURNAL OF BACTERIOLOGY, May 1976, p. 814-822
Vol. 126, No. 2

Printed in U.S.A.

Thymidine Uptake and Utilization in *Escherichia coli*: a New Gene Controlling Nucleoside Transport

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Received for publication 30 December 1975

A commonly used strain of *Escherichia coli* K-12 was shown to be deficient in the transport of a number of nucleosides, including thymidine. Thymine incorporation was unaffected. Strain AB2497 exhibited a strikingly lower thymidine pulse-label incorporation at low (<1 μg/ml) thymidine concentrations than do many other strains. The deficiency appeared to be due to mutation in a single gene. This gene, which we designated *nup* (for *n*ucleoside *up*take), is located at 10 to 13 min on the *E. coli* linkage map. In *nup*$^+$ strains, the transport of a given nucleoside was relatively insensitive to large excesses of other nucleosides but was competitively inhibited by the same nucleoside. Mutants deficient in thymidine kinase are deficient in thymidine uptake but normal in deoxyadenosine uptake. A two-step model for nucleoside transport is presented in which the first step, utilizing the *nup* gene product, is a nonspecific translocation of nucleoside to the interior of the cell. In the second step, the individual nucleosides are modified by cellular enzymes (e.g., nucleoside kinases) to facilitate accumulation.

In recent years, radioactive labeling procedures have become central to the study of nucleic acid synthesis and its regulation. The most commonly used precursors for labeling deoxyribonucleic acid (DNA) in *Escherichia coli* have been thymine and thymidine, because they are specifically incorporated into DNA. Mutants of *E. coli* unable to synthesize thymidylate are easily obtained (16, 19). In principle, the use of such mutants makes it possible to control the specific activity of newly synthesized DNA by simply adjusting the specific activity of exogenously added thymidine. It is important to realize, however, that in experiments which measure short-term incorporation of labeled precursors other factors may be important—the pool sizes of endogenous bases, nucleosides, and nucleotides, the rates of exchange between these pools and the rates of uptake, as well as the points of entry of the exogenous labels.

The pathways for thymidine metabolism in *E. coli* and the chromosomal locations of genes for the known enzymes involved are shown in Fig. 1 and 2, respectively. In wild-type *E. coli*, thymine is not readily incorporated into DNA because of the low levels of deoxyribose-1-phosphate. In Thy$^-$ mutants, the level of deoxyribose-1-phosphate is increased due to the breakdown of otherwise unmetabolized deoxyuridine monophosphate (15). Consequently these mutants will grow on high concentrations (e.g., 50 μg/ml) of thymine. Additional mutations, blocking the breakdown of deoxyribose-1-phosphate (Dra$^-$ or Drm$^-$), raise the levels of deoxyribose-1-phosphate further and increase the efficiency of thymine incorporation. Such mutations allow Thy$^-$ bacteria to grow normally on concentrations as low as 2 μg of thymine per ml. Mutations blocking thymidine phosphorylase (EC 2.4.2.4) render cells unable to incorporate thymine or to degrade thymidine. The genes for thymidine catabolism, along with the gene for purine nucleoside phosphorylase, are grouped in a single operon, the *deo* operon (1, 20) located between 89 and 90 min on the *E. coli* linkage map (1). This operon is catabolite repressible (6, 20) and under the control of at least two other genes, *nucR* (2, 6, 20) and *cytR* (6).

An important factor that may result in differences between strains in the incorporation of labeled precursors into DNA is the transport of nucleosides and bases from the medium into cells. This may be evident only when low concentrations of precursors are used, and it may give the appearance of altered levels of DNA synthesis. In this paper, we report a mutation in a commonly used strain of *E. coli* K-12 that affects the transport of a number of nucleosides, including thymidine. Since this strain, AB2497, and its Thy$^+$ parent, AB1157, have been used as the parental strains for a number of derivatives

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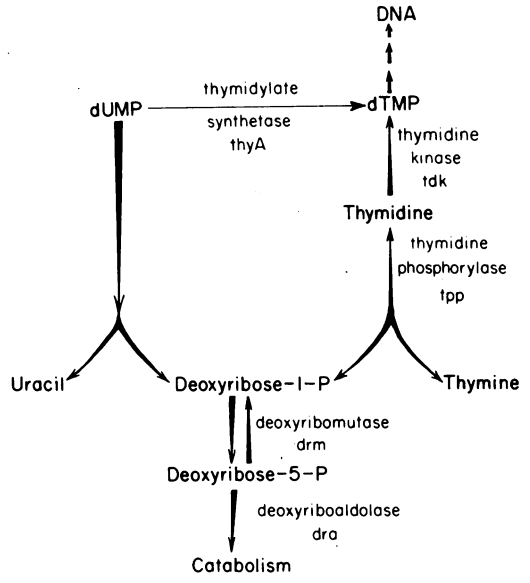

FIG. 1. *Thymidine metabolism pathways in E. coli. Shown are the pathways of thymidine metabolism in E. coli along with the enzymes involved and the genetic loci for the enzymes (15). dUMP, Deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate.*

carrying mutations affecting DNA metabolism (3), we believe that it is important that researchers working with these strains be aware of the consequences of this mutation. We also report studies on specificity and competition in nucleoside transport and we present a simple, two-component model for nucleoside transport in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this study are derivatives of *E. coli* K-12 and are listed in Table 1.

Reagents. The following radioactive chemicals were obtained from New England Nuclear Corp.: [^{14}C]thymine and [^{14}C]thymidine (40 to 60 mCi/mmol), [^{3}H]thymine (18.3 Ci/mmol), [^{3}H]thymidine (40 to 60 Ci/mmol), [^{3}H]deoxyadenosine (25 Ci/mmol), [^{3}H]uridine (39.3 Ci/mmol), [^{3}H]adenosine (40.8 Ci/mmol), and 0.02 N $H_3{}^{32}PO_4$. In addition, [^{3}H]deoxyadenosine (14.5 Ci/mmol) was obtained from Amersham/Searle.

Unlabeled thiamine-hydrochloride, thymine, deoxyadenosine, deoxyuridine, and deoxycytidine were obtained from Calbiochem. Unlabeled thymidine and uridine were obtained from Sigma. Casamino Acids (vitamin free) were from Difco. Nalidixic acid (NAL) was a kind gift of Thomas Simon.

Growth conditions. Cells were grown in Tris-(hydroxymethyl)aminomethane (Tris)-salts buffer (7) supplemented with 1% glucose, 0.5% Casamino Acids, and 2 μg of thiamine-hydrochloride per ml. In experiments with Thy$^-$ bacteria, either thymine (2 μg/ml) (1.58 $\times$ 10^{-2} mM) or thymidine (3.84 μg/ml)(1.58 $\times$ 10^{-2} mM) was added to the growth media. Prelabeling of Thy$^-$ cells was done with [^{14}C]thymine or [^{14}C]thymidine at a final radioactivity of 0.2 μCi/ml. To prelabel with $^{32}PO_4$, Thy$^+$ cells were grown in Tris-salts buffer containing only 5% of the usual amount of phosphate and prelabeled with $^{32}PO_4{}^{2-}$ at approximately 0.5 μCi/ml.

Unlabeled 2-ml cultures were routinely grown overnight with shaking and diluted 1:50 into fresh medium containing prelabel. These cultures were incubated with shaking for 2 h at 37 C and further incubated at 25 C with shaking for at least 90 min. Doubling times at these temperatures are 40 to 45 min and 85 to 90 min, respectively.

Measurements of uptake and incorporation. To measure uptake or incorporation in the absence of thymine or thymidine, the cells were harvested by centrifugation and resuspended in identical medium lacking thymine or thymidine. They were then incubated for a short time (less than 2 min unless otherwise indicated) at 25 C before pulse labeling. Pulse labeling was initiated by the addition of tritium-labeled compound at the concentration given in the respective figure legends. To measure incorporation of labeled material into DNA, aliquots (0.1 to 0.5 ml) were taken and mixed with equal volumes of a "stop mix" containing 0.4 NaOH, 2% Sarkosyl, 0.02 M ethylenediaminetetraacetic acid, and 0.02 M NaN_3 (14), and DNA was precipitated with 5% trichloroacetic acid and filtered on glass-fiber filters (Whatman GFA). To measure uptake into whole cells,

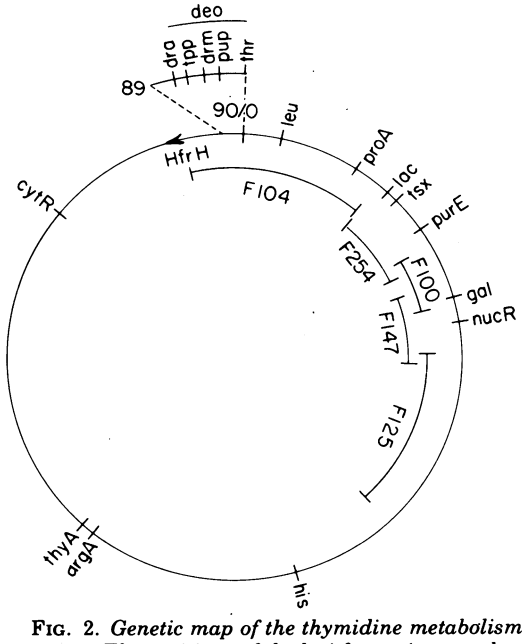

FIG. 2. *Genetic map of the thymidine metabolism enzymes. The positions of the loci for various nucleoside metabolism enzymes. Genetic symbols are as in Taylor and Trotter (21). Also shown are the F' factors used in this study (12).*

TABLE 1. *Major bacterial strains used in this study*

Strain	Genotype and comments	Source	Reference
W3110 Thy⁻	*thyA tlr*	A. K. Ganesan	3
AB1157	*thr, leu, proA, arg, his lacY, gal, ara, xyl, mtl str, tsx*	A. K. Ganesan	3
AB2497	AB1157, *thyA, drm*	A. K. Ganesan	3
K12-SH	Wild type	W. Fangman	5
PCH 37	K12-SH, *thyA, tlr*	Trimethoprim selection	This work
AB259	HfrH, *thi*		3
JC4251	Carries F104	A. J. Clark	12
ORF4/KL251	Carries F254	B. Low	12
PA2005^R	Carries F100	B. Low	12
KLF47/KL262	Carries F147	B. Low	12
KLF25/KL181	Carries F125	B. Low	12
KY895	*tdk, ilv*	B. Bachmann	9

aliquots (0.1 or 0.5 ml) were filtered on Whatman GFA filters or Reeve-Angel glass-fiber filters, and immediately washed with 5 ml of Tris-salts buffer. The filtering and washing process was accomplished in less than 5 s. Filters were dried under infrared lamps and counted in a Packard Tri-Carb scintillation counter using a toluene-based scintillation fluid. Background and counting channel overlap were subtracted, and the ratio of pulse label to prelabel was calculated as a measure of uptake. In all samples, the prelabel radioactivity exceeded 2,000 counts/min.

Uptake of thymidine into whole cells was generally studied in the presence of 100 μg of NAL per ml. Control experiments indicated that this treatment inhibits DNA synthesis by over 90%. Initial rates of uptake into cells were shown to be the same in the presence or absence of NAL. When competing nucleosides were used, they were added 10 s before the initiation of pulse labeling. Pulse labeling was carried out at 25 C to slow metabolism and to facilitate temperature control by working closer to the ambient temperature. Control experiments not utilizing the shift to 25 C indicated that the conclusions drawn at 25 C were valid for 37 C.

Mapping experiments. Mapping experiments were performed at 37 C following the procedure of Miller (13). Bacterial matings were stopped by vortexing at high speed for 45 to 60 s. Matings with strains carrying F′ factors were carried out in LB broth containing 0.2% glucose. Such matings were allowed to continue for 1 h before vortexing and plating.

RESULTS

Incorporation of thymidine into DNA. While studying intermediates in DNA replication in *E. coli*, we observed a large difference in the amount of [^{3}H]thymidine incorporated into acid-precipitable material by various thymine-requiring strains after short periods of labeling. This effect was observed at thymidine concentrations of less than 1 μg/ml (Fig. 3). W3110 and most other strains examined reached maximal levels of incorporation at thymidine concentrations as low as 0.2 to 0.3 μg/ml, whereas strain AB2497 and closely related strains did not reach that level of incorporation until the thymidine concentration was nearly 1 μg/ml. At such concentrations, both W3110 and AB2497 exhibited similar levels of incorporation, as expected from their similar growth rates at these higher thymidine concentrations. The same results were obtained when thymine was present in the medium at 2 μg/ml during pulse labeling.

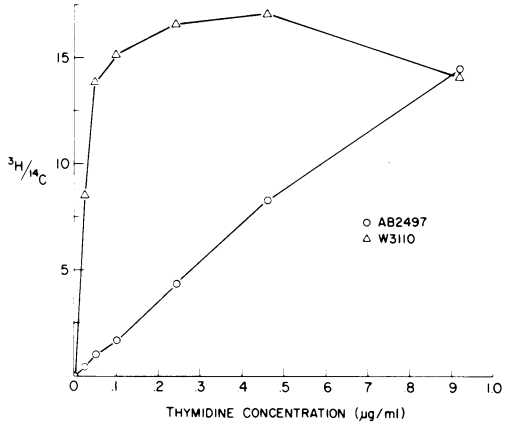

FIG. 3. *Incorporation of thymidine into DNA. The incorporation of [^{3}H]thymidine into acid-precipitable material was measured at various thymidine concentrations. Cells were grown as in Materials and Methods, harvested, suspended in media lacking thymine or thymidine, and pulse-labeled for 2 min at 25 C with the concentrations of labeled thymidine shown.*

We were interested in determining whether this difference in incorporation was specific for thymidine or if it also occurred with low thymine concentrations. From Fig. 4, it is clear that thymine is incorporated with equal efficiency in both strains at all concentrations examined. Thus, the observed difference in incor-

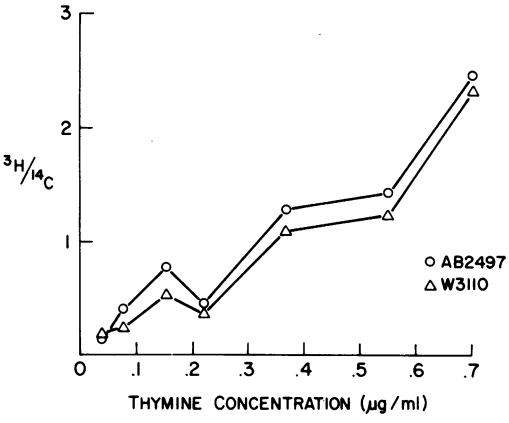

FIG. 4. *Incorporation of thymine into DNA. The incorporation of [^{3}H]thymine into acid-precipitable material was measured at various thymine concentrations. Procedures were as in Fig. 3, except that cells were pulse-labeled for 3 min with the concentrations of thymine shown.*

poration appears to affect nucleosides rather than bases, and is not simply the result of some difference between these strains in the control of DNA synthesis rates at low levels of nucleic acid precursors. This conclusion is also supported by our observation of this phenomenon in the presence of thymine concentrations that are adequate for maximal growth rates.

We considered the possibility that the lower levels of thymidine pulse incorporation in AB2497 might be due to larger internal pools of thymidine nucleosides. In an attempt to raise pulse incorporation levels in AB2497 by depleting internal pools, we examined the effect of extended periods of thymine deprivation prior to pulse labeling. In Fig. 5 the incorporation after various times of thymine starvation is expressed relative to that with no starvation. As can be seen, the amount of incorporation of thymidine into DNA actually decreased with length of thymine starvation. The percent decrease was nearly identical in W3110 and AB2497, although the level of incorporation in W3110 was always at least 5 to 10 times greater than in AB2497. This decrease occurred in spite of the expected initiation of new growing points upon the readdition of thymidine (18). The reason for this apparent discrepancy is unclear, but the slower growth rates at 25 C and the low level of exogenously added thymidine may prevent reinitiation during the 3-min period of pulse labeling.

The effects of mutations in various genes of the *deo* region of the chromosome have been examined (M. McKeown, Senior Honors Thesis, Stanford Univ., Stanford, Calif., 1975). A

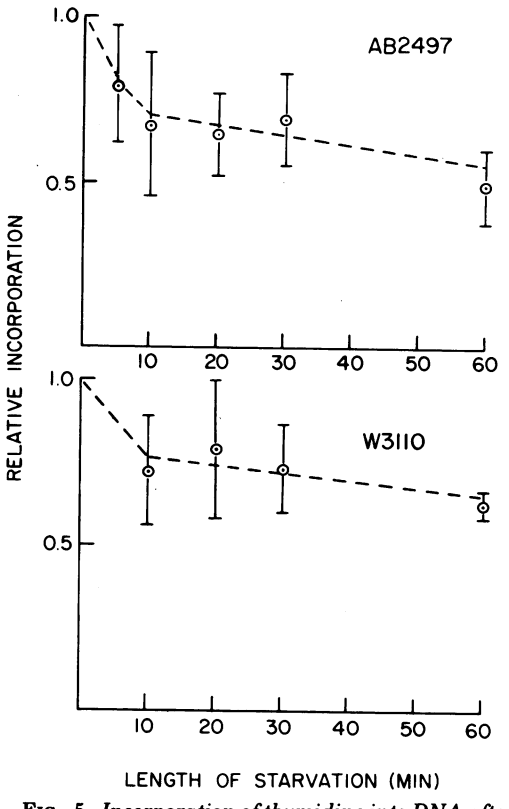

FIG. 5. *Incorporation of thymidine into DNA after thymine starvation. Cells were grown as in Materials and Methods, harvested by centrifugation, and suspended in medium lacking thymine or thymidine. After various periods of incubation at 25 C, they were pulse-labeled for 3 min with 0.046 μg of thymidine per ml, and incorporation into acid-precipitable material was measured. Incorporation is expressed as the fraction of incorporation after zero time of starvation.*

difference in incorporation was observed between isogenic Dra$^-$ and Drm$^-$ strains, with the Drm$^-$ strains showing about twice as much incorporation of thymidine as the Dra$^-$ strains. Mutants lacking thymidine phosphorylase showed reduced levels of incorporation unless prestarved for thymidine, possibly because of higher internal thymidine pools. In examining one Tpp$^-$, four independent Drm$^-$, and three independent Dra$^-$ derivatives of AB1157, we did not observe any *deo* mutation that increased incorporation of thymidine to the extent obtained with W3110. We conclude that although internal pools are of importance, intermediary metabolism is not responsible for the observed strain differences between W3110 and AB2497.

Transport of thymidine. One possible explanation for the observed differences in incorpora-

tion is that different strains have different efficiencies in the transport of exogenously added thymidine. AB2497 and W3110 were tested for transport at various thymidine concentrations. It is clear (Fig. 6) that W3110 is indeed much more efficient in thymidine transport than is AB2497. This difference is large enough to entirely account for the differences in incorporation of thymidine into acid-precipitable material.

Initial rates of uptake were the same when DNA synthesis was proceeding normally or when it was suppressed over 90% by NAL. We take this to indicate that we are looking at a phenomenon not closely coupled to DNA synthesis. Based upon the following calculation, we conclude that the cells are able to accumulate thymidine against a substantial concentration gradient. Strain PCH37 was pulse-labeled for 180 s in the presence of NAL with [^{3}H]thymidine (10 μCi/ml, 0.046 μg/ml). The concentration of cells was roughly 2×10^8/ml, giving (for 1 ml of medium) a total cell volume of 4×10^{-4} ml. The amount of radioactivity transported into the cells in a 1-ml sample was 5.86×10^5 counts/min, to give a concentration of [^{3}H]thymidine in cells of 1.46×10^9 counts/min per ml. Our counting efficiency was 20%, so there were 7.3×10^9 dpm/ml. The total radioactivity in the medium was 10 μCi/ml or 2.22×10^7 dpm/ml (10 μCi/ml $\times$ 2.22×10^6 dpm/μCi). This indicates that the concentration of thymidine within the cells is of the order of 300 times the concentration of thymidine in the medium after 3 min of labeling.

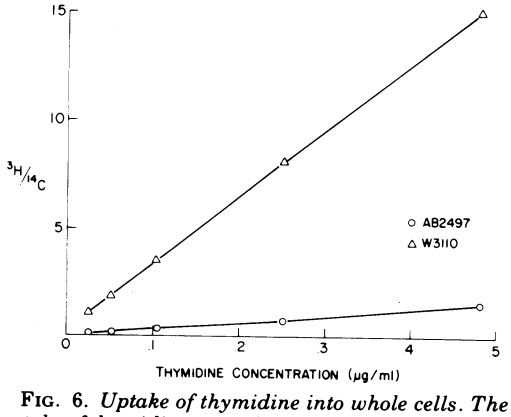

FIG. 6. *Uptake of thymidine into whole cells. The uptake of thymidine into whole cells was measured as in Materials and Methods. The cells were grown as in Materials and Methods, harvested, suspended in medium lacking thymine or thymidine, and NAL (100 μg/ml) was added. They were then pulse-labeled for 20 s with the concentrations of thymidine shown.*

Experiments were performed to determine whether cellular energy is involved in thymidine transport and, if so, how this energy might be coupled to transport (M. McKeown, Senior Honors Thesis, Stanford Univ., Stanford, Calif., 1975). Cyanide was found to reduce uptake and accumulation of thymidine to 10% of normal levels, indicating the involvement of metabolic energy. Experiments designed to test whether the transport is driven by adenosine 5′-triphosphate or by an activated membrane state gave inconclusive results; both 2,4-dinitrophenol (an uncoupler of the activated membrane state) and arsenate (an inhibitor of phosphate transfer reactions) gave about 50% inhibition of uptake.

Differences in the ability to transport exogenously added thymidine are also observed in Thy$^+$ strains (Fig. 7). In this experiment, the cultures were grown and pulse-labeled in medium containing either glucose or glycerol as carbon source, to test the possibility that the thymidine transport genes might be catabolite repressed. In addition, thymidine was present in the growth media at 50 μg/ml in case it was needed for induction of any thymidine transport genes. The cells were removed from this medium and placed in medium lacking thymidine before initiation of pulse labeling. Experi-

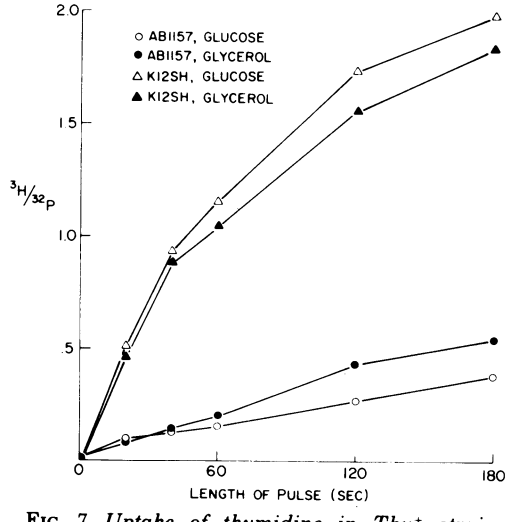

FIG. 7. *Uptake of thymidine in Thy$^+$ strains. Cells were grown as in Materials and Methods, except that glycerol replaced glucose in two cultures, and thymidine (50 μg/ml) was present. Cells were harvested by centrifugation and suspended in identical medium lacking thymidine. They were then pulse-labeled for the times shown with 0.046 μg of [^{3}H]thymidine per ml. Uptake into whole cells was determined as in Materials and Methods. NAL was not present during pulse labeling.*

ments in which thymidine was absent from growth medium were also performed and gave similar results. Thus the cell constitutents responsible for thymidine transport are normally present in wild-type as well as in Thy⁻ bacteria and they do not require induction or relief from catabolite repression.

The nup (*n*ucleoside *up*take) locus. We developed a simple method to select for Thy⁻ cells that are capable of efficient thymidine transport. The selection is based upon the fact that strains competent for thymidine transport are able to incorporate thymidine into DNA at a maximal rate at thymidine concentrations as low as 0.1 to 0.4 μg/ml, whereas strains deficient in transport are not (Fig. 3). As a result, Thy⁻ transport-competent cells are capable of forming small colonies on agar plates containing 0.2 μg/ml of thymidine. This selection has been useful in preliminary genetic studies.

W3110-derived P1 transductants of AB2497 selected for the ability to grow on low-thymidine plates show increased levels of thymidine transport (19 of 19 tested). This indicates that a relatively small region of the genome (<2 min of the chromosome), possibly only one gene, is responsible for the observed difference between transport-competent and -deficient strains. Since this mutation also appears to affect the transport of a number of nucleosides (Table 2), we suggest that this locus be designated *nup* (*n*ucleoside *up*take) and that transport-proficient strains designated *nup*⁺.

Mating with HfrH and various F's. To determine the approximate position of the *nup* locus on the *E. coli* linkage map, AB2497 was mated with a *thy*⁻ *drm*⁻ derivative of strain AB259 (HfrH). In such a mating, the *nup* locus is transferred approximately 5 min after ProA (7.5 min), placing it at about 10 to 13 min on the *E. coli* linkage map. This conclusion is supported by crosses between AB2497 and strains carrying F′ factors covering the region from 87 to 30 min on the *E. coli* chromosome (12) (Fig. 2). F254, covering the region from 9 to 15 min, gave large numbers of recombinants able to grow on low-thymidine plates. This selection with limiting thymidine concentrations resulted in the loss of the F factor and in many cases loss of unselected markers. Ten out of ten recombinants tested showed similarly high levels of thymidine transport. Mating to F′ factors on either side of F254 gave levels of growth no higher than background. One other F′, F147, did give recombinants able to grow on low-thymidine plates, but the levels of transport in these recombinants were quite variable from isolate to isolate. No further work has been done on these. This phenomenon could be the result of the *nucR* gene carried by F147 (2).

TABLE 2. *Uptake of various nucleosides in nup⁺ and nup⁻ cells*[a]

Strain	Percentage of AB2497			
	Thymidine	Deoxyadenosine	Adenosine	Uridine
AB2497	100	100	100	100
W3110	1,276	1,903	1,555	1,301
AB2497-F254	2,055	1,769		

[a] The concentrations of nucleosides during pulse labeling were 0.046 μg of thymidine per ml, 0.017 μg of deoxyadenosine per ml, 0.066 μg of adenosine per ml and 0.062 μg of uridine per ml. Twenty-second pulses were used here, although 40-s labeling gave essentially the same result.

Uptake of other nucleosides in *nup*⁺ and *nup*⁻ cells. It seemed possible that the *nup* gene product might be involved in the transport of a number of nucleosides, and incorporation studies have shown that this is the case (Table 2). We conclude from this that the *nup* mutation affects the transport of ribo- and deoxyribonucleosides with either purine or pyrimidine bases. When *nup*⁻ cells are converted to *nup*⁺ they regain the ability to transport thymidine and other nucleosides (Table 2).

Effect of competing nucleosides on transport. The possibility that some portion of the thymidine transport system might show specificity for thymidine was examined (Fig. 8). In this experiment, various heterologous nucleosides were added in a 20-fold mass excess 10 s before the initiation of pulse labeling. None of the nucleosides gave an inhibition of uptake of greater than about 50%, and those nucleosides that acted as inhibitors gave essentially equal amounts of inhibition. Deoxycytidine gave little or no inhibition of uptake, possibly due to the ability of deoxycytidine nucleotides to act as strong activators of thymidine kinase (see below) (10). In separate experiments testing inhibition of labeled thymidine uptake by various excesses of unlabeled thymidine, excesses of unlabeled thymidine from 5- to 80-fold gave a reduction in the amount of labeled material incorporated in direct proportion to the amount of the excess.

Experiments to test the effects of competing nucleosides on deoxyadenosine uptake gave comparable results (Table 3). Deoxycytidine again had little or no effect as an inhibitor of uptake, whereas thymidine gave an inhibition of deoxyadenosine uptake nearly equal to that of deoxyadenosine on thymidine. We take these results to indicate that although the *nup* mutation affects the transport of a number of nucleosides, there is some portion of the transport

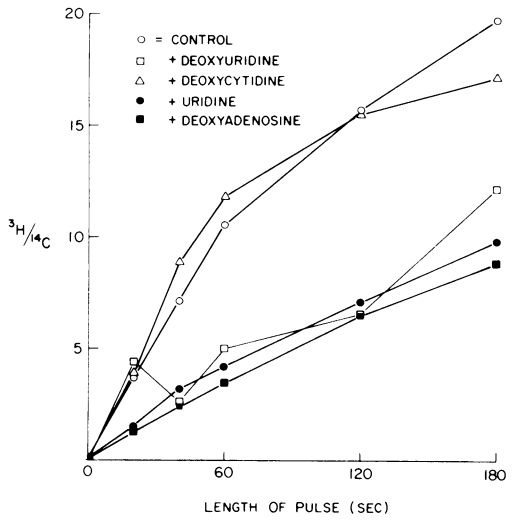

FIG. 8. *Effect of competing nucleosides on thymidine uptake. Strain W3110 was grown as in Materials and Methods. Cells were harvested and suspended in medium lacking thymine or thymidine but containing 100 μg of NAL per ml. Pulse labeling was done with 0.046 μg of [^{3}H]thymidine per ml. Competing nucleosides were added in a 20-fold mass excess 10 s before the initiation of pulse labeling. The amount of uptake by whole cells was measured as in Materials and Methods after the pulse lengths shown.*

TABLE 3. *Deoxyadenosine uptake in the presence of a 20-fold excess of other nucleosides*[a]

Strain	Addition	Ratio (^{3}H/^{14}C)	% Control
W3110			
	None	16.08	100
	Deoxycytidine	15.36	95.5
	Thymidine	11.03	68.6
PCH37			
	None	16.30	100
	Deoxycytidine	15.94	97.3
	Thymidine	12.39	76.0

[a] Deoxyadenosine concentration during the 1-min pulse was 0.1 μg/ml.

system that is specific for the transport and accumulation of specific nucleosides.

One class of enzymes known to be nucleoside specific is the nucleoside kinases (11). A mutant of *E. coli* K-12 lacking thymine kinase has been isolated (8). Using such a mutation (in a W3110-related background) (9), we measured the incorporation of thymidine and deoxyadenosine. It is clear from Table 4 that the thymidine kinase mutation has a drastic effect on thymidine accumulation, but little if any effect on deoxyadenosine incorporation.

TABLE 4. *Uptake of various nucleosides in thymidine kinase-deficient cells*[a]

Strain	Uptake (% AB1157)	
	Thymidine	Deoxyadenosine
AB1157 *nup*$^-$ *tdk*$^+$	100	100
K12-SH *nup*$^+$ *tdk*$^+$	628	796
KY895 *nup*$^+$ *tdk*$^-$	37	473

[a] Cells were pulse-labeled in the growth medium for 2 min with thymidine (0.046 μg/ml) or deoxyadenosine (0.17 μg/ml). Uptake was measured as in Materials and Methods.

DISCUSSION

In the course of this study, we learned the following about nucleoside transport: (i) a mutation in the *nup* locus reduces transport of a number of ribo- and deoxyribonucleosides; (ii) transport of a given nucleoside is relatively insensitive to large excesses of other nucleosides; (iii) transport and accumulation of a given nucleoside are very sensitive to excesses of the same nucleoside; (iv) although only a slight amount of inhibition by heterologous nucleosides is observed, the amount of inhibition is relatively constant no matter which nucleosides are used; and (v) mutants lacking thymidine kinase are very deficient in thymidine accumulation but are normal in deoxyadenosine uptake.

We conclude from this that the nucleoside transport pathway must consist of at least two parts. One part, which contains the *nup* product, is involved in the transport of a number of nucleosides. The other portion is responsible for nucleoside specificity. Any model of nucleoside transport must take into account the dual nature of the transport system.

We would like to present a preliminary model of nucleoside transport based upon the data presented in this paper. Our model (Fig. 9) hypothesizes that the *nup* gene product is part of a relatively nonspecific nucleoside "permease" that is involved in the translocation of nucleosides from the exterior of cells to the interior. This permease has relatively equal affinities for all nucleosides that it transports and is not easily saturated. This would account for the low but equal amounts of inhibition observed by various competing nucleosides. Coupled to this permease are the nucleoside kinases or other nucleoside metabolism genes. These would account for the nucleoside specificity observed in incorporation and also for the accumulation itself.

There are several factors that might complicate this simple model. One difficulty is that

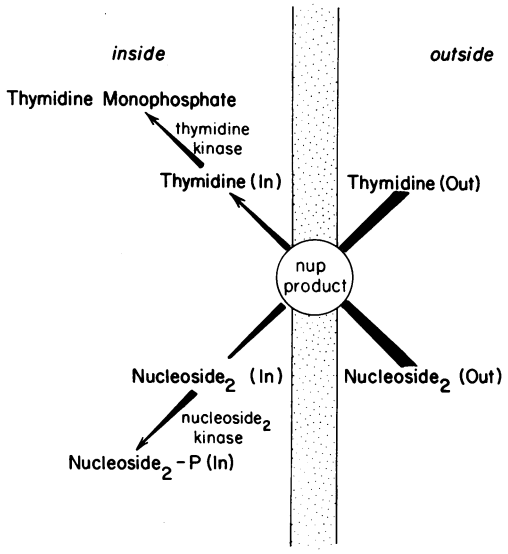

FIG. 9. *Preliminary model of nucleoside transport in E. coli K-12.*

our assay for transport cannot distinguish between transport and accumulation. If back flow of nucleosides out of cells is relatively rapid, the uptake step through the membrane might supply significant energy and still be unable to maintain high internal concentrations, except as nucleosides are metabolized. This could account for the effects of the thymidine kinase mutation. We have suggested that the nucleoside kinases are involved in the accumulation step, but we have only been able to examine a mutant lacking thymidine kinase. There may be other nucleoside-specific proteins in the transport system not observed in our system. Finally, since our measurements of uptake and accumulation of thymidine are dependent on thymidine kinase, precise quantitation of competition with other nucleosides may be difficult to obtain and interpret because deoxycytidine and deoxyadenosine nucleotides are known to be activators of thymidine kinase, deoxycytidine being a much stronger activator than deoxyadenosine (10).

Our results emphasize some difficulties in the interpretation of pulse-labeling experiments. The most notable problem is that different strains may show drastically different levels of transport of exogenously added nucleosides. It is especially important to note that strain AB1157 has been used as the parental strain for a large number of commonly used recombination- and repair-deficient strains (3). The results presented here also indicate that exogenously added nucleosides and bases are present at different concentrations within cells than the medium, and that the intracellular concentrations will change during the early periods of pulse labeling. These concentration differences may explain some of the differences observed between thymine and thymidine during pulse labeling of nascent DNA chains (4, 17, 22). Since in most strains thymidine is efficiently incorporated into cells, an internal concentration of thymidine sufficient to permit normal DNA synthesis rates will be obtained quite rapidly. Thymine, on the other hand, is not accumulated rapidly enough to allow normal levels of DNA synthesis at low concentrations (Fig. 4), and is not metabolized for incorporation into DNA nearly as efficiently as thymidine. It seems likely that pulses of thymine give a slower approach to concentrations of deoxythymidine triphosphate sufficient to support normal DNA synthesis. This may affect the relative rates of different steps of DNA synthesis, and lead to the accumulation of a different set of intermediates.

ACKNOWLEDGMENTS

We wish to thank Debra Miyajima for technical assistance and Chris Spraker for manuscript preparation. We appreciate the helpful comments and suggestions of John Little, James Loehr, Tom Simon, and Robert Simoni.

This work was supported by a Public Health Service grant GM 09901 from the National Institute of General Medical Sciences and by a predoctoral traineeship to M.K. from the National Institutes of Health.

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