Molec. Gen. Genetics 111, 303—316 (1971)

Location on the Chromosome of *Salmonella typhimurium* of Genes Governing Pyrimidine Metabolism

CHRISTOPH F. BECK and JOHN L. INGRAHAM

Department of Bacteriology, University of California, Davis, California 95616

Received May 4, 1971

Summary. Genes encoding the enzymes cytidine deaminase (*cdd*), uridine monophosphate pyrophosphorylase (*upp*), cytidine triphosphate synthetase (*pyrG*), and uridine phosphorylase (*udp*) were located on the *Salmonella typhimurium* chromosome at 68, 77, 90 and 122 min, respectively. Strains carrying mutations in *pyrG* must also carry mutations in *cdd* in order for cytidine to be sufficiently stable metabolically to supply the cell's requirement for CTP[1].

The *de novo* synthesis of uridine monophosphate (UMP) which serves as the total source of pyrimidine nucleoside triphosphates is catalyzed by six enzymes. The biochemistry and genetics of this pathway in enteric bacteria has been studied in some detail and it has been established that the genes encoding these enzymes do not form an operon, rather they are all unlinked.

If uracil is provided in the medium the *de novo* pathway ceases to function and an auxillary pathway is used. UMP is then synthesized from the exogenous uracil by a single enzymatic reaction catalyzed by UMP-pyrophosphorylase (Fig. 1).

Uridine (UR) is also capable of serving as a total source of pyrimidine either by first being converted to uracil by the enzyme uridine phosphorylase or by being converted to UMP by uridine kinase. Although cytosine cannot enter the nucleotide pools without first being converted to uracil [*Salmonella typhimurium* lacks a cytidine monophosphate (CMP) pyrophosphorylase] enzymes exist which can convert cytidine (CR) to cytidine triphosphate (CTP). However CR is metabolically unstable due to the presence of enzymes which degrade it. In strains carrying proper genetic blocks which render CR metabolically stable, CR can serve as source of CTP and, in such strains, mutants with specific requirements for CR may be isolated. These mutants are blocked in CTP-synthetase, the enzyme which converts UTP to CTP.

In this paper we report the location of the gene for CTP synthetase (*pyrG*) as well as the location of genes encoding certain enzymes of the auxillary pathway [CR deaminase (*cdd*), UR phosphorylase (*udp*), and UMP-pyrophosphorylase

1 Abbreviations: C = cytosine; CMP = cytidine monophosphate; CR = cytidine; CTP = cytidine triphosphate; FCdR = 5′fluorodeoxycytidine; FU = 5′fluorouracil; NG = N-methyl-N-nitroso-N′-nitroguanidine; U = uracil; UMP = uridine monophosphate; UR = uridine; UTP = uridine triphosphate.

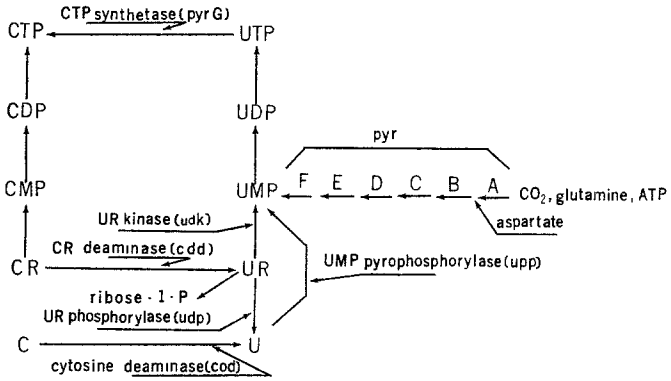


Fig. 1. *De novo* and auxillary pathway of pyrimidine biosynthesis

(*upp*)] and show that they too, are all unlinked. Also we show which mutations conferring CR stability are essential in order that a mutation in *pyrG* can be tolerated.

Materials and Methods

Media. The 007 medium (Clark and Maaløe, 1967) was used as a basal salts medium. Glucose was added to 0.1%. Amino acid supplements, when used, were added at a final concentration of 50 μg/ml; pyrimidine and purine supplements when used, were added at the following final concentrations: uracil, 30 μg/ml; uridine, 80 μg/ml; cytidine, 80 μg/ml; hypoxanthine, 21 μg/ml; guanine, 10 μg/ml; adenine, 10 μg/ml; and guanosine, 20 μg/ml.

The vitamins, thiamine and nicotinic acid, were added, when required, at a final concentration of 1 μg/ml. Plates in which cytidine was the total source of nitrogen contained the normal minimal salt medium without inorganic nitrogen but with cytidine added to a final concentration of 200 μg/ml. When uridine served as a carbon source, glucose was replaced by 500 μg uridine/ml.

Difco nutrient broth was used as an enriched medium. When strains requiring a pyrimidine nucleoside, thiamine or nicotinic acid, were grown in nutrient broth, the medium was supplemented with the corresponding compound at the same concentration used in minimal medium. The nutrient agar contained 1.5% agar; supplements were the same as those mentioned for nutrient broth.

Bacterial Strains. Salmonella typhimurium, strain LT2, was the original parent of all strains used in these experiments. Table 1 lists the strains used, their genotype and certain pertinent characteristics. Strains without an indicated source are from our own culture collection, or were constructed during the course of the experiments. Strain JL1055 (formerly designated DP55) the parent of many strains used in this study was isolated and described by Neuhard and Ingraham (1968). This strain had been further characterized by measurements of the pertinent enzyme activities by Neuhard (1968).

Mutagenesis. Mutants were induced by treatment with N-methyl-N-nitroso-N′-nitroguanidine (NG) according to the procedure of Adelberg *et al.* (1965). Some of the analogue-resistant mutants were selected without prior mutagenesis: *upp* mutants were isolated as being resistant to FU, *cdd* mutants as being resistant to FCdR and *thyA* mutants as being resistant to aminopterin (Okada *et al.*, 1962).

Mating Procedures. Time of Entry Experiments. The donor cultures were grown overnight without shaking at room temperature in nutrient broth. They were then incubated at 37° C in a slowly shaking water bath, following a 20 fold dilution with fresh broth. They were grown until the culture reached a density of approximately 10^8 cells per ml. The recipient strain

was grown with aeration at 37° C in nutrient broth; full-grown overnight cultures were normally used for mating. Approximately 3×10^8 donor cells and 1.4×10^9 recipient cells were mixed on a filter membrane (Millipore filter, diameter 47 mm). Excess liquid was removed, care being taken to leave the membrane surface still somewhat moist. This membrane was then placed on a nutrient agar plate at 37° C, which had been overlayered with about 10 ml of nutrient soft agar. The plate with the filter was incubated at 37° C for 5 min after which time the membrane was placed into 10 ml of nutrient broth at 37° C in a 125 ml Erlenmeyer flask. After 3 min incubation in a slowly shaking water bath at 37° C, the membrane was removed and the incubation continued. During long matings, the mating mixture was diluted 1:1 with fresh, prewarmed broth, when the cell density increased above about 4×10^8 cells per ml. As 0 time we took the moment when the membrane was placed on the nutrient agar plate. Samples (0.4 ml) were taken and added to 0.6 ml basal salts. After vigorous shaking for 30 sec, 0.1 ml of the suspension or an appropriate dilution was added to minimal medium soft agar tubes and plated immediately.

Uninterrupted Matings. Matings, performed to determine the co-inheritance frequency between different markers were carried out in the same fashion as described above except that the cells on the membrane were not resuspended until 20 min after the last marker of interest was expected to have entered the recipient. Serial dilutions of the resuspended mating mixture were plated onto appropriate plates.

Sex Factor Transfer. The same method as described for uninterrupted matings was used for the transfer of the sex factor in the construction of strains JL689 and JL378. The incubation time at 37° C was extended to 180 min. Fresh, prewarmed nutrient broth was added from time to time on and around the filter membrane.

Transduction. L4, a non-lysogenizing derivative of *Salmonella* phage P22 (Smith and Levine, 1967) was used for all transductions. Lysates were prepared by infection of the recipient culture, growing in nutrient broth, with L4 phage at a MOI of 0.02. After about 7 hours incubation, the lysate was concentrated by means of centrifugation at 35000 g for 2 hours, resuspended in T2 buffer (Hershey and Chase, 1952), and treated with chloroform.

Transductions were carried out by dispersing the phage directly onto plates, spread beforehand with 0.15 ml of full-grown cultures in nutrient broth (2×10^9 cells/ml) (or an equal concentration of cells in T2 buffer) of the recipient strain; 0.1 ml of three serial dilutions of the phage stock, ranging from about 5×10^{11} to 5×10^9 phage per ml were spread on different plates. Transductants were picked from the plates with the lowest multiplicity of infection which showed a significant number of transductants.

Selection Procedures. udp. udp^+ recombinants were selected on plates in which uridine was the total carbon source. Recipients for transductions were always resuspended in T2 buffer before spreading onto these plates. The soft agar, used for mating experiments also contained uridine instead of glucose.

cdd. cdd^+ recombinants were selected for their ability to grow on cytidine as the exclusive nitrogen source. In transductions as well as matings, care was taken to minimize the carry over of inorganic nitrogen.

pyrG: $pyrG^+$ recombinants were selected for cytidine independence.

upp: upp^+ recombinants can grow on uracil as a pyrimidine source, upp^- strains cannot.

Results and Discussion

Mapping of the Gene for Uridine Phosphorylase: udp. Uridine phosphorylase catalyzes the phosphorolytic cleavage of uridine yielding uracil and ribose-1-phosphate. The enzyme of *Salmonella typhimurium* is specific; cytidine is not cleaved (unpublished results). Strains carrying mutations in genes encoding this enzyme were selected for their inability to use uridine as sole source of carbon (Neuhard and Ingraham, 1968). This enzyme seems to function only catabolically

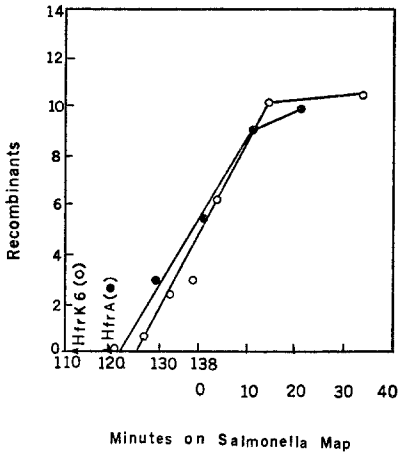


Fig. 2. Mapping of *udp* by interrupted matings. Mating conditions and selection procedures are given under Materials and Methods. Two independent mating experiments with two different Hfrs are shown. *udp*$^+$ recombinants were selected for from a cross of Hfr A with JL1039 (•) and from a cross of Hfr K6 with JL673 (○). The abscissa represents a fragment of the *Salmonella typhimurium* standard map. The ordinate is the number of recombinants per ml $\times 10^{-6}$ with Hfr A and $\times 5 \times 10^{-4}$ with Hfr K6

in vivo; strains which produce this enzyme but lack UMP-pyrophosphorylase are unable to grow on uracil as a pyrimidine source when normal concentrations of uracil (~30 μg/ml) are added to the growth medium (unpublished results). Consistent with the exclusive catabolic role of uridine phosphorylase is the finding that cytidine and uridine induce its biosynthesis (Neuhard, 1968).

The approximate location of the *udp* gene was determined by interrupted matings of Hfrs A and K6 with JL673 and JL1039. The results (Fig. 2) indicate a location of the *udp* gene at about 122 min on the *Salmonella* map.

Transduction experiments showed 39% cotransduction of *udp* with *metE* and 0.5% cotransduction with *hisR*. The frequency of cotransduction between *hisR* and *metE* in this cross was 6.5% which approximates the data reported by Roth and Hartman (1965). The data of Table 2 are consistent with the gene order: *ilvC-hisR-metE-udp*. No cotransduction of *udp* with *metB* nor with a rhamnose marker could be observed.

Mapping of the Gene for UMP Pyrophosphorylase: upp. UMP pyrophosphorylase catalyzes the reaction between uracil and PRPP to form uridine monophosphate, which is the only route by which *Salmonella typhimurium* is able to use exogenous uracil as pyrimidine source. Mutants in *upp* were selected as being FU resistant (Brockman *et al.*, 1960). Strains with negative mutations in *upp* are unable to use uracil, but can use uridine as a pyrimidine source. Most of the genetic work was carried out with strain JL662 and derivatives thereof, isolated and biochemically characterized by Jan Neuhard. This strain also harbors a temperature-sensitive lesion in the *pyrC* gene; it can grow without

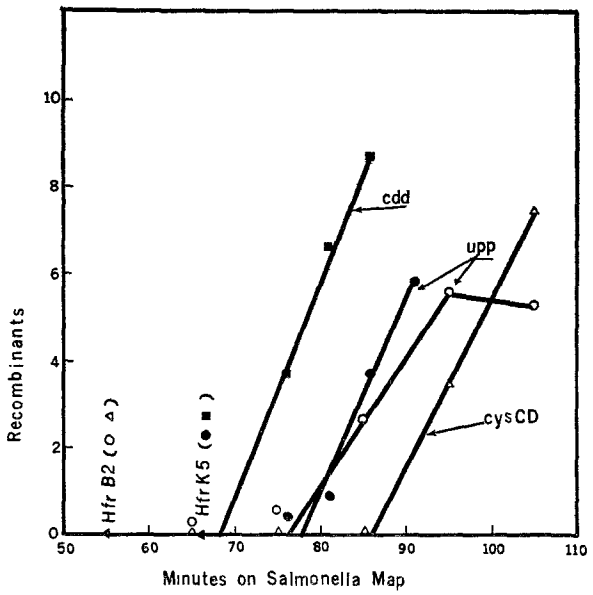


Fig. 3. Interrupted mating experiments to locate the *upp* gene. Mating conditions and selection procedures are given under Materials and Methods. Two different mating experiments using two different Hfrs and different recipients are summarized in this figure. Hfr B2 (JL626) was crossed with JL688 and *upp*$^+$ (○) and *cys*519$^+$ (△) recombinants were selected. Hfr K5 (JL689) was mated with JL662 and *cdd*$^+$ (■) and *upp*$^+$ (●) recombinants were selected. The ordinate represents the number of recombinants per ml. The number of *cdd*$^+$ and *upp*$^+$ recombinants from the cross with Hfr K5 has to be multiplied by 10^3; the *upp*$^+$ recombinants from the cross with Hfr B2 by a factor of 10^2 and the *cys*$^+$ recombinants by a factor of 10. The abscissa depicts the segment from 60 to 110 min of the *Salmonella typhimurium* map

pyrimidine source at 30° C but requires uridine for growth at 42° C. This temperature-sensitive block proved valuable for: (a) isolation of FU-resistant mutants at 30° C since FU is toxic only in the absence of exogenous uracil and (b) scoring the presence of the *upp* marker by determining if uracil satisfies the pyrimidine requirement at 42° C.

The location of *upp* gene was determined by interrupted mating experiments with various Hfrs. The results (Fig. 3) place *upp* approximately midway between *cdd* and the *cysC-J* gene cluster at about 76 to 78 min on the *Salmonella typhimurium* map. Uninterrupted matings with an Hfr K5, carrying a *cysA* marker yielded 80% coinheritance between *upp* and *cysA*. An analogous experiment, using Hfr A, harboring a *purC* marker yielded 100% coinheritance between *purC* and *upp*.

Subsequently, cotransduction was shown between *upp* and *purC*, *purI*, and *guaA*. The data of Table 3, summarized in Fig. 4 support the gene order: *purC-upp-purI-guaA*. Cotransduction frequencies between *purC*, *purI*, and *guaA* (first 3 lines of Table 3) agree generally with those reported earlier by Roth *et al.* (1966).

Table 1. *Bacterial strains*[a]

Sex	Strain No.	Genotype	Parental strain	Derived by	Source
Hfr A	JL84	*metB*406 *hisD*23 *gal*-50			
Hfr B2	JL626	*pro*$^-$, *aro*$^-$			SA[b]
Hfr K5	JL629	*serA*13			SA
Hfr K5	JL689	*ara*-9, *metG*319 *trp*$^-$	JL384	C[f]	
Hfr A	JL627	*purC*7			
Hfr K6	JL630	*serA*13			SA
Hfr K5	JL378	*cysA*1348 *thyA*1262	JL610	C	
F'32	JL657	*his*-2461 *aroD*5 *purF*145/F'32*dsd*			J. Roth[c]
F$^-$	JL673	*cdd*-7, *udp*-2	JL1039	T[g]	
F$^-$	JL373	*cdd*-7, *udp*-2 *dsd*	JL673	NG[e]	
F$^-$	JL374[h]	*cdd*-101, *hisW*3333	JL682	NG	
F$^-$	JL685[h]	*cdd*-52 *udp*-51 *hisW*3333	JL682	NG	
F$^-$	JL1039	*pyrA*81 *cdd*-7 *udp*-2	JL1055	T	
F$^-$	JL646	*ara*-9 *hisR*1223 *metE*339 *strA*			J. Roth
F$^-$	JL375[i]	*pyrA*81, *cdd*-7, *udp*-2, *pyrG*1606, *thyA*1261 *strB*	JL674		
F$^-$	JL376	*cys*-519[k]			J. Roth
F$^-$	JL672	*pyrG*1606 *udp*-2 *cdd*-7	JL1055	T	
F$^-$	JL509	*pheA*			
F$^-$	JL51	*tyrA*			
F$^-$	JL595	*argB*69			
F$^-$	JL683	*cys*-519 *cdd*-7 *udp*-2	JL672	T	
F$^-$	JL686	*pyrC*1502[j] *cdd*-9 *udp*-8 *cod*-8 *pyrG*1501 *upp*-17	JL662	NG	
F$^-$	JL688	*pyrC*1502 *cdd*-9 *udp*-8 *cod*-8 *upp*-17 *cys*-519	JL686	T	
F$^-$	JL662	*pyrC*1502 *cdd*-9 *udp*-8 *cod*-8 *upp*-17			Neuhard[d]
F$^-$	JL647	*purC*7 *proA*46 *iM*-10 *fla*-56 *strA* *ilv*-405 *rha*-461 *fim*			J. Roth
F$^-$	JL377	*purI*305			J. Roth
F$^-$	JL379	*pyrC*1502 *purC*7	JL385	C	
F$^-$	JL663	*pyrC*1502 *cdd*-9 *udp*-8 *cod*-8 *upp*-17 *udk*-6			Neuhard
F$^-$	JL380	*guaA*1			J. Roth
F$^-$	JL381	*pyrC*1502 *udp*-8 *cod*-8 *upp*-17 *cys*-519	JL688	T	
F$^-$	JL382	*pyrC*1502 *upp*-17 *purC*7	JL688	C	
F$^-$	JL383	*cys*-519 *cdd*-7	JL683	T	
F$^-$	JL602	*metE*338			

Reverse transductions of *upp* with the 3 purine markers were made; the direction of transduction has considerable influence on the relevant cotransduction frequencies (Table 3, Fig. 4). The discrepancies between "forward" and "reverse" crosses seems to be marker specific and may be explained by differences in DNA at the molecular level (Norkin, 1970). To confirm the gene order *purC-upp-purI*, we performed a three factor cross, using a *purC, upp* strain as transductional

Table 2. *Mapping of udp by transduction*[a]

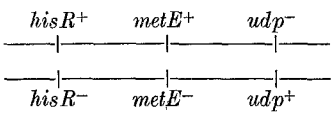
Transductional donor	Recipient	Selected marker	Number of transductants	Unselected markers	No.
JL673[d]	JL646	*metE*$^+$	411	*hisR*$^+$ *udp*$^+$[b]	27
				hisR$^+$ *udp*$^-$	2
				hisR$^-$ *udp*$^+$	225
				hisR$^-$ *udp*$^-$	157
JL673	JL602	*metE*$^+$	328	*udp*$^-$	132
JL673	JL84	*metB*$^+$	252	*udp*$^-$	0
JL673	JL647	*rha*$^+$	120	*udp*$^-$[c]	0

[a] Transductions were carried out as described in Materials and Methods.

[b] The presence of the *hisR* marker in transductants was determined by the appearance of wrinkles on 2% glucose plates (Roth and Hartman, 1965).

[c] The *rha*$^+$ transductants were restreaked for single colonies on rhamnose minimal plates prior to a test of the presence of a functional *udp* gene.

[d] Indicated gene order:



[a] New gene designations, used here for the first time, are explained in Fig. 1.

[b] Kenneth Sanderson, University of Calgary, Department of Biology, Canada.

[c] John Roth, University of California, Berkeley, Department of Molecular Biology, California.

[d] Jan Neuhard, University of Copenhagen, University Institute of Biological Chemistry, 83, Sølvgade, Copenhagen, Denmark.

[e] Nitrosoguanidine mutagenesis.

[f] Conjugation.

[g] Transduction.

[h] Selected for as FCdR resistant.

[i] Strain selected for spontaneous low level streptomycin resistance, which simultaneously created a requirement for thiamine and nicotinic acid in this strain.

[j] *pyrC*1502 is a heat sensitive lesion.

[k] *cys*519 represents a deletion mutation of the C and D genes in the *cys* gene cluster.

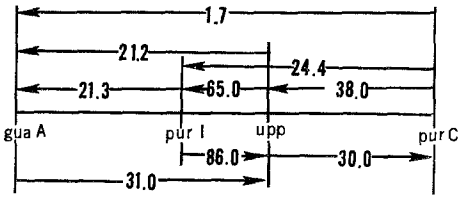


Fig. 4. Detailed genetic map of the *upp* region. The map corresponds to the region around 77 min on the *Salmonella typhimurium* map of Sanderson (1970). The distances between the markers are arbitrary. The order of the markers follows from the results presented in Tables 3 and 4. The values correspond to the cotransduction frequencies as given in tables. The arrows point in the direction of the selected donor marker

Table 3. *Mapping of the upp gene within the purC, purI, guaA gene cluster by transduction*[a]

Transductional donor	Recipient	Selected marker	Number of transductants	Unselected marker	% cotransduction
JL377	JL380	*guaA*$^+$	240	*purI*$^-$[b]	21.3
JL379	JL380	*guaA*$^+$	239	*purC*$^-$[b]	1.7
JL379	JL377	*purI*$^+$	160	*purC*$^-$[c]	24.4
JL662	JL379	*purC*$^+$	398	*upp*$^-$[d]	30.0
JL379	JL662	*upp*$^+$	134	*purC*$^-$	38.0
JL663	JL377	*purI*$^+$	240	*upp*$^-$[e]	65.0
JL377	JL662	*upp*$^+$	73	*purI*$^-$	86.0
JL663	JL380	*guaA*$^+$	288	*upp*$^-$	21.2
JL380	JL662	*upp*$^+$	81	*guaA*$^-$	31.0

[a] Transductions were carried out as described in Materials and Methods.

[b] The *guaA*$^+$ *purI*$^-$ resp. *purC*$^-$ transductants were selected on glucose minimal plates, containing hypoxanthine and thiamine.

[c] The *purI*$^+$ *purC*$^-$ transductants were selected on glucose minimal + hypoxanthine plates—*purI*$^-$ strains have an absolute requirement for thiamine (Newell and Tucker, 1968).

[d] *purC*$^+$ transductants were scored for their ability to grow on glucose minimal + uracil resp. uridine at 42° C. Those which grew on the uridine but not on the uracil-containing plates were presumed to be *upp*$^-$.

[e] The transductants of these crosses were scored for the absence of a functional UMP pyrophosphorylase by spotting onto glucose minimal plates onto which 1 mg FU had been spread. The incubation temperature was 37° C. Those strains which grew on the FU plate were presumed to have a *upp*$^-$ genotype.

donor and selected *purI*$^+$ transductants. All *purI*$^+$, *purC*$^-$ transductants (Table 4) also inherited the *upp*$^-$ allele from the donor. If *upp* were on the left side of *purI*, a high number of *upp*$^+$, *purI*$^+$, *purC*$^-$ transductants would have been recovered. As the *upp* gene is located in a cluster of genes involved in purine biosynthesis, the possibility of *upp* being essential for the pyrophosphorylation of purine nucleotides arises.

This possibility was ruled out by the construction of a strain harboring *purC* and *upp* simultaneously. This strain was able to utilize all purines tested and we

Table 4. *Localization of the upp gene by three factor crosses*[x]

Transductional donor	Recipient	Selected marker	Unselected markers	Number of transductants
JL382[y]	JL377	*purI*$^+$	*purC*$^-$[c]	81
			purC$^-$ *upp*$^+$[e]	0
			purC$^-$ *upp*$^-$[e]	81

[x] For footnotes see the corresponding letters of Table 3.

[y] Indicated gene order

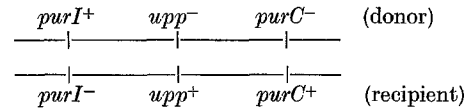


Table 5. *Mapping of the cdd gene relative to hisW and dsd by crosses with Hfrs*[a]

Pertinent cross	Selected marker	Number of recombinants	Unselected marker	% coinheritance
HfrB2 × JL373	*cdd*$^+$	118	*dsd*$^+$[b]	7.6
	dsd$^+$	48	*cdd*$^+$	79
HfrK5 × JL374	*cdd*$^+$	162	*hisW*$^+$[c]	47.5
	hisW$^+$	187	*cdd*$^+$	87
HfrB2 × JL685	*cdd*$^+$	171	*hisW*$^+$	43.3
	hisW$^+$	208	*cdd*$^+$	94

[a] Matings and selections were carried out as described in Materials and Methods.

[b] *dsd*$^+$ recombinants were selected on plates containing D-serine as nitrogen source.

[c] The cold sensitive *hisW* marker was isolated by Jean Brenchley (Thesis, U.C. Davis, 1970). Strains harboring this mutation are unable to grow at 20° C.

conclude from these results that *upp* does not participate in the pyrophosphorylation of purine nucleotides.

Mapping of the Gene for Cytidine-Deaminase: cdd. The isolation and characterization of mutants in cytidine-deaminase has been described by Neuhard and Ingraham (1968). A nonfunctional cytidine-deaminase is prerequisite to maintaining a viable *pyrG* mutant, as will be established later. Mutants in the *cdd* gene are unable to use cytidine as sole nitrogen source, a characteristic which has been used for the selection of *cdd*$^+$ recombinants.

Interrupted matings of JL673 with Hfrs B2 and K5 established the approximate location of the *cdd* gene (Fig. 5) at about 68 min on the *Salmonella typhimurium* standard map. These results were supported by the finding that the *Escherichia coli* episome F'32 covers the *cdd* gene (Fink and Roth, 1968). No cotransduction was found between *cdd* and *aroD*, *purF*, *dsd*, *hisW*, or *metG*, we attempted to order the *cdd* gene relative to those other genes by crosses with Hfrs. These results (Table 5) indicate that *cdd* must be located before the *dsd* and *hisW* genes in clockwise direction. Next we determined the relative gene order of *cdd*, *metG*, and *hisW*. For this purpose we constructed a Hfr K5, harboring

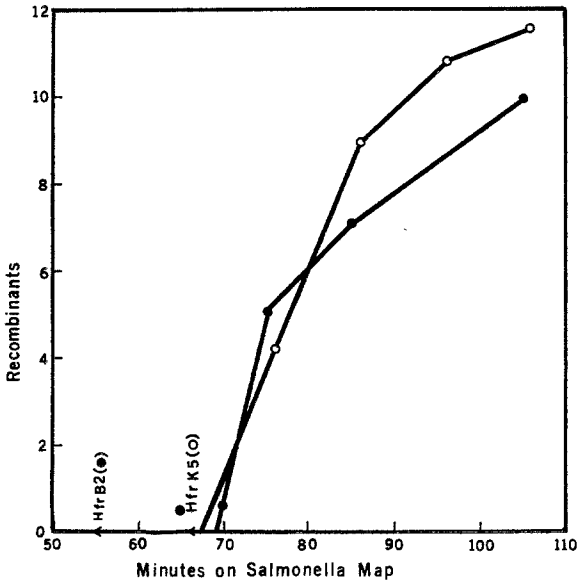


Fig. 5. Mapping of the *cdd* gene by interrupted matings. Mating conditions and selection procedures are given under Materials and Methods. The ordinate represents the number of *cdd*$^+$ recombinants per ml. In the cross Hfr B2 × JL673 (•) a factor of 10^4 and in the cross Hfr K5 × JL673 (○) one of 10^2 has to be used to determine the true number of recombinants. The abscissa represents the segment from 50 to 110 min of the *Salmonella typhimurium* chromosome

a *metG* marker and mated it with a *cdd, hisW* female strain. The results of several three factor crosses are given in Table 6. We attribute the low frequency with which the *metG* gene, carried by the Hfr K5, is inherited by the recombinants, to the earlier observation (Pittard and Walker, 1967) which showed a low frequency of inheritance for very early donor markers in Hfr mediated crosses. The presence of a few *hisW*$^+$ *cdd*$^-$ *metG*$^-$ recombinants and the absence of *hisW*$^+$ *cdd*$^+$ *metG*$^-$ recombinants seems to be inconsistent with the proposed gene order (Table 6). But as the numbers involved are very small and in one out of two experiments no *hisW*$^+$ *cdd*$^-$ *metG*$^-$ recombinants were detected, we attach little significance to these numbers. The combined data of Tables 5 and 6 are in agreement with a gene order: *metG-cdd-hisW-dsd.*

Recently, cotransduction between *cdd* and *glpT*, a gene responsible for the uptake of alpha glycerolphosphate was observed (R. Vinopal, personal communication).

Mapping of the Gene for Cytidine Triphosphate Synthetase: pyrG. A mutant with a nonfunctional cytidine triphosphate synthetase was first isolated by Neuhard and Ingraham (1968) in a strain which also carried lesions in *cdd* and *udp*. These latter mutations were introduced in order to render cytidine metabolically stable. Interrupted mating experiments of Hfr K5 with a multiply-

Table 6. *Mapping of the cdd gene relative to metG by mating of HfrK5 (JL689 × JL685)*[a]

Selected marker	Number of recombinants	Unselected markers			% coinheritance
		hisW	*cdd*	*metG*	
cdd+[c]	164	+[b]		+	22
		+		−	1.8
		−		+	66
		−		−	10
hisW+[c]	279		+	+	96
			−	+	1.75
			+	−	0
			−	−	2.15
cdd+ *hisW*+[c]	276			+	94
				−	6

[a] See Table 5 for footnotes.
[b] "+" represents a wildtype gene.
[c] Indicated gene order:

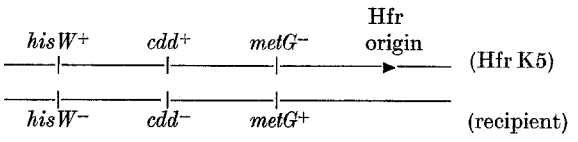


Table 7. *Mapping of pyrG by transduction*[a]

Transductional donor	Recipient	Selected marker	Number of transductants	Unselected marker	% cotransduction
JL376	JL672	*pyrG*+	672	*cys*−	15.5
JL672	JL683	*cys*+[b]	336	*pyrG*−	26.5
JL376	JL686	*pyrG*+[c]	168	*cys*−	6.0
JL672	JL688	*cys*+	246	*pyrG*−	29.3
JL509	JL672	*pyrG*+	160	*pheA*−	0
JL51	JL672	*pyrG*+	160	*tyrA*−	0
JL595	JL672	*pyrG*+	168	*argB*−	0

[a] Transductions were performed as written out under Materials and Methods.
[b] To allow the appearance of the class of *pyrG*− transductants, the recipient has to have a *cdd*− genotype (see text for explanation).
[c] The *pyrG* marker of this strain is nonidentical with that of JL672.

marked recipient show the entry of *pyrG* about 2 min before *thyA* (Fig. 6). These data were confirmed when cotransduction between a *cysCD* marker and *pyrG* was found; depending on the direction of genetic transfer and on the recipient used, cotransduction frequencies between 6 and 30% were observed (Table 7).

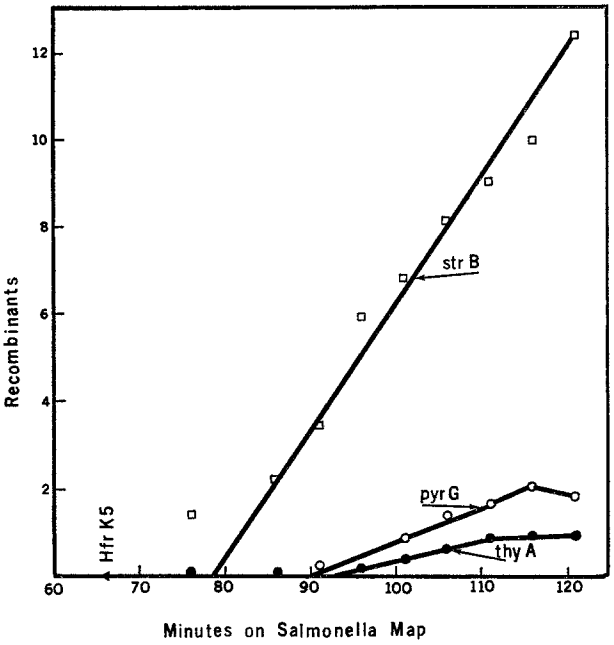


Fig. 6. Mapping of *pyrG* by conjugation. The time of entry of *pyrG*, *thyA*, and *strB* markers was determined by interrupted mating experiments of Hfr K5 × JL375. The mating conditions are given under Materials and Methods. *strB*$^+$ recombinants (□) were selected for their ability to grow without thiamine and nicotinic acid supplement and *thyA*$^+$ recombinants (•) for growth in the absence of thymine. *pyrG*$^+$ recombinants (○) grow in the absence of cytidine. The ordinate represents the number of recombinants per ml × 10^{-3}. The abscissa depicts the segment from 60 to 120 min of the *Salmonella typhimurium* chromosome

The discrepancy in the cotransduction frequencies with JL672 and JL686 as recipients may be a reflection of the heterogeneity of these 2 strains (Norkin, 1970) in this region.

As we did not find any cotransduction of *pyrG* with the neighboring markers *argB*, *pheA* or *tyrA*, we do not know the gene order nor the orientation in this segment of the chromosome.

"Genetic Constellation" Required for Mutations in pyrG. The isolation of mutations in *pyrG* was based on the assumption that cytidine in wild type cells is broken down rapidly thus leaving cells with a block in *pyrG* without an adequate supply of the precursor of CTP. Following this hypothesis, Neuhard and Ingraham (1968) introduced mutations in *cdd* and *udp* respectively and, in another strain, in *cdd* and *cod* in order to make cytidine "metabolically stable", before attempting the isolation of mutations in *pyrG;* such mutations were isolated in both strains.

We have re-evaluated their hypothesis by asking if it is possible to introduce a nonfunctional *pyrG* gene (by means of cotransduction with *cysCD*) into strains which carry mutations in *cdd*, *udp*, or *cod*. A mutation in *cdd* is essential (second experiment, Table 8). Strain JL381 is unable to harbor a mutation in *pyrG*, whereas its parent, JL688 a *cdd*$^-$ strain, can. Thus a mutation in *pyrG* is incompatible with a wild type *cdd* allele.

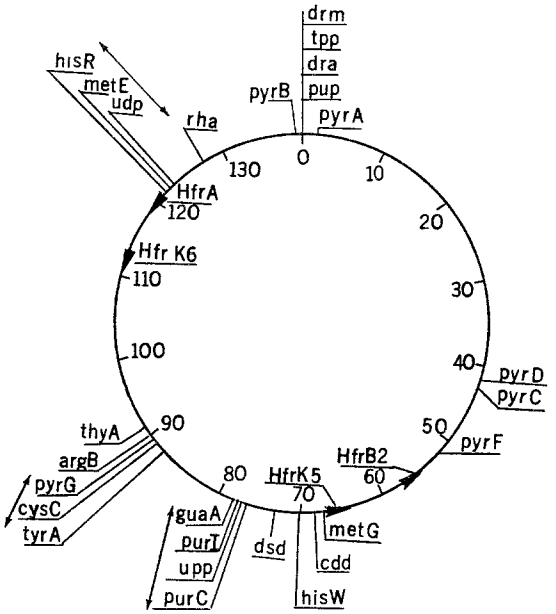


Fig. 7. Chromosome map of *Salmonella typhimurium* showing the approximate location of the newly mapped genes of pyrimidine metabolism. Also shown are the mapped genes of pyrimidine biosynthesis, deoxyribose catabolism, and several other markers, included for purposes of orientation. Further are given the origins of the Hfrs used in the mating experiments. This map is based on that of Sanderson (1970). Double headed arrows (↔) indicate cotransductional fragments

Table 8. *Crosses which establish the essentiality of a mutation in cdd prior to a block in pyrG*[a]

Recipient	Transductional donor	Selected marker	Number of transductants	Unselected marker	Number of transductants with unselected marker
JL688 (*cdd*⁻ *udp*⁻)	JL672	*cysCD*⁺	246	*pyrG*⁻	72
JL681 (*cdd*⁺)	JL672	*cysCD*⁺	243	*pyrG*⁻	0
JL683 (*udp*⁺)	JL672	*cysCD*⁺	240	*pyrG*⁻	92

[a] Transductions were performed as written out under Materials and Methods.

In contrast, the *udp* gene, functional or nonfunctional, probably has no effect on the metabolic stability of cytidine (line 3, Table 8). This observation can be interpreted as genetic evidence that there is no significant phosphorolytic cleavage of cytidine, which is in agreement with the *in vivo* specificity of uridine phosphorylase. As cytidine cannot be converted to cytosine, it is apparent, that a mutation in *cod* will similarly have no effect on the metabolic stability of cytidine.

Thus, all the genes of pyrimidine metabolism which have been studied are unlinked (Fig. 7) and except for *pyrG* mutations in them may be selected for directly in wild type strains. For the selection of mutations in *pyrG* only a block in *cdd* is prerequisite.

Acknowledgements. We thank Dr. John Roth for his helpful suggestions and for providing us with many strains used in these experiments. We also wish to thank Dr. Jan Neuhard for his stimulating interest in this work and Marge Ingraham for drawing the figures. The investigation was supported by Public Health Service grant AI 05526 from the National Institute of Allergy and Infectious Diseases.

References

Adelberg, E. A., Mandel, M., Chen, G. C. C.: Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in *Escherichia coli* K-12. Biochem. biophys. Res. Commun. **18**, 788–795 (1965).

Brockman, R. W., Davis, T. M., Stubbs, P.: Metabolism of uracil and 5-fluorouracil by drug-sensitive and by drug-resistant bacteria. Biochim. biophys. Acta (Amst.) **40**, 22–32 (1969).

Clark, D. J., Maaløe, O.: DNA replication and the division cycle in *Escherichia coli*. J. molec. Biol. **23**, 99–112 (1967).

Fink, G. R., Roth, J. R.: Histidine regulatory mutants in *Salmonella typhimurium*. J. molec. Biol. **33**, 547–557 (1968).

Hershey, A. D., Chan. M.: Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. gen. Physiol. **36**, 39–56 (1952).

Neuhard, J.: Pyrimidine nucleotide metabolism and pathways of thymidine triphosphate biosynthesis in *Salmonella typhimurium*. J. Bact. **96**, 1519–1527 (1968).

— Ingraham, J. L.: Mutants of *Salmonella typhimurium* requiring cytidine for growth. J. Bact. **95**, 2431–2433 (1968).

Newell, P. C., Tucker, R. G.: Biosynthesis of the pyrimidine moiety of thiamine. Biochem. J. **106**, 279–287 (1968).

Norkin, L. C.: Marker specific effects in genetic recombination. J. molec. Biol. **51**, 633–655 (1970).

Okada, T., Homma, F., Sonohara, H.: Improved method for obtaining thymineless mutants of *Escherichia coli* and *Salmonella typhimurium*. J. Bact. **84**, 602–603 (1962).

Pittard, J., Walker, E. M.: Conjugation in *Escherichia coli*. Recombination events in terminal regions of transferred deoxyribonucleic acid. J. Bact. **94**, 1656–1663 (1967).

Roth, J. R., Antón, D. N., Hartman, P. E.: Histidine regulatory mutants in *Salmonella typhimurium*. J. molec. Biol. **22**, 305–323 (1966).

— Hartman, P. E.: Heterogeneity in P22 transducing particles. Virology **27**, 297–307 (1965).

Sanderson, K. E.: Current linkage map of *Salmonella typhimurium*. Bact. Rev. **34**, 176–193 (1970).

Smith, H. O., Levine, M.: A phage P22 gene controlling integration of prophage. Virology **31**, 207–216 (1967).

Communicated by W. Maas

Dr. Christoph F. Beck
Department of Bacteriology, University of California
Davis, Calif. 95616/USA