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Location on the Chromosome of *Escherichia coli* of Genes Governing Purine Metabolism

Adenosine Deaminase (*add*), Guanosine Kinase (*gsk*) and Hypoxanthine Phosphoribosyltransferase (*hpt*)

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Summary. Genes coding for enzymes functioning in purine salvage pathways have been located on the chromosome of *Escherichia coli*. The gene *add* encoding adenosine deaminase was located by transduction at 31 min, the gene order was established to be *man-uidA-add-aroD*. A deletion covering *man-uidA-add* was obtained. The gene *gsk* encoding guanosine kinase was cotransducible with *purE* and shown to be located at 13 min. The gene *hpt* encoding hypoxanthine phosphoribosyltransferase was cotransducible with *tonA* indicating a location at 3 min. The location of the gene *gpt* encoding guanine (xanthine) phosphoribosyltransferase in the *proA-proB* region was confirmed.

The *de novo* biosynthesis of adenosine monophosphate (AMP) and guanosine monophosphate (GMP) is catalyzed by a total of 13 enzymes (Gots, 1971). In addition, salvage pathways exist which allow the cell to utilize endogenously formed or exogenously supplied purine bases and purine nucleosides (Fig. 1). Purine salvage pathways have been studied in *Salmonella thyphimurium* and *Escherichia coli* and they are very much alike (Magasanik and Karibian, 1960; Hoffmeyer and Neuhard, 1971; Jochimsen, 1974).

Only two genes encoding enzymes involved in purine salvage pathways have been mapped, namely the gene *pup* encoding purine nucleoside phosphorylase which has been located close to *ser*B in both *E. coli* and *S. thyphimurium*: in *E. coli* at 89.5 min (Ahmad and Pritchard, 1969). The gene encoding guanine (xanthine) phosphoribosyltransferase (*gpt*) has been located in the *pro*A – *pro*B region of the chromosome (Gots *et al.*, 1972; Livshitz, 1973).

In the present work we have established the location on the *E. coli* chromosome of the genes coding for adenosine deaminase (*add*), guanosine kinase (*gsk*), hypoxanthine phosphoribosyltransferase (*hpt*) and confirmed the location of the gene encoding guanine (xanthine) phosphoribosyltransferase (*gpt*). The latter has been named *gxu* (Gots *et al.*, 1972) and *gpp* (Livshitz, 1973) and then *gpt* by Gots who first located it (Benson and Gots, 1975).

Adenosine deaminase catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively, and plays a role in a pathway which converts adenine, adenosine and deoxyadenosine to guanine nucleotides (Fig. 1). With histidine present in the culture medium, the alternative pathway

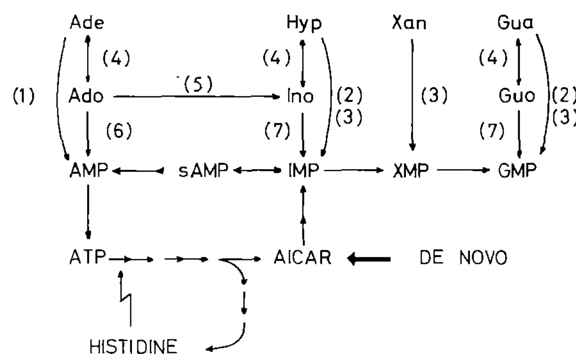

Fig. 1. Salvage pathways for purine bases and nucleosides in *Escherichia coli*. Xanthosine is not included since it cannot be metabolized by *E. coli* K12 (Nygaard unpublished observations). The purine deoxyribonucleosides can only be metabolized via purine nucleoside phosphorylase and adenosine deaminase (Karlström, 1970). (*1*) adenine phosphoribosyltransferase, (*2*) hypoxanthine phosphoribosyltransferase, (*3*) guanine phosphoribosyltransferase, (*4*) purine nucleoside phosphorylase, (*5*) adenosine deaminase, (*6*) adenosine kinase, (*7*) guanosine kinase. ⇥ indicates feedback inhibition by histidine

Abbreviations. sAMP = succinyl-AMP; IMP = inosine monophosphate; XMP = xanthosine monophosphate; Ado = adenosine; Guo = guanosine; Ino = inosine; Ade = adenine; Hyp = hypoxanthine; Gua = guanine; Xan = xanthine; dAdo = deoxyadenosine, and AICAR = 5'phosphoribosyl-5 amino-4 imidazole carboxamide.

via ATP and AICAR is inhibited, and the above pathway via adenosine deaminase is the only one functioning. Addition of adenine, hypoxanthine and their ribonucleosides to cultures of *E. coli* induces the synthesis of adenosine deaminase (Remy and Love, 1968; Nygaard, unpublished data).

Guanosine kinase catalyzes the phosphorylation of guanosine and inosine to GMP and IMP respectively. The enzyme might be involved in the reutilization of endogenously formed nucleosides, since exogenously supplied deoxy- and ribonucleosides are rapidly phosphorylized by purine nucleoside phosphorylase to form free bases and (deoxy)ribose l-phosphate. While the addition of nucleosides to *E. coli* cultures induces the synthesis of purine nucleoside phosphorylase (Hammer-Jespersen *et al.*, 1971), the synthesis of guanosine kinase is not influenced by the addition of nucleosides to the medium, rather it has been found that if purine auxotrophs are starved for purines, a 3 fold increase in guanosine kinase is seen (Vestergaard, unpublished results).

The utilization of exogenously supplied purine bases are facilitated by one of three different purine phosphoribosyltransferases, namely adenine phosphoribosyltransferase, hypoxanthine (guanine) phosphoribosyltransferase and guanine (hypoxanthine, xanthine) phosphoribosyltransferase.

Materials and Methods

Bacterial Strains and Growth Media

The *Escherichia coli* strains employed and their genotypes are listed in Table 1. As basal salt medium the medium of Monod *et al.*, 1961 was used. Carbohydrates were added to 0.2%, amino acid supplements, when used, were added at a final concentration of 50 μg/ml, purines 15 μg/ml, purine nucleosides 30 μg/ml and vitamins 1 μg/ml.

Mutagenesis and Genetic Methods

For mutagenesis UV irradiation and N-methyl-N′-nitro-N-nitrosoguanidine treatments were employed according to the procedure of Adelberg *et al.* (1965). The procedure for penicillin counterselection was as described by Karlström, 1968. Transductions with Plv phages were conducted as described before (Munch-Petersen *et al.*, 1972). Mating, time of entry experiments, and handling of F- primes were performed as described by Miller (1972).

Enzyme Assays

If not otherwise stated, preparation and dialysis of cell extracts were performed as described (Hammer-Jespersen *et al.*, 1971). One unit is defined as the amount of enzyme which converts one nmole of substrate per minute at 37° C. Protein was determined by the method of Lowry *et al.* (1951).

Adenosine Deaminase

Cells were harvested by centrifugation, resuspended in 1 ml 0.1 M Tris-Cl, pH 7.6, 2 mM, EDTA 20% ethylene glycol, and then disrupted by sonic treatment for 1 min. After centrifugation for 5 min at 6000 × *g* the supernatant was dialyzed overnight against 10 mM Tris-Cl, pH 8.0, 50 μM EDTA, 2 mM $CaCl_2$, 20% ethylene glycol. Activity with adenosine as substrate was determined spectrophotometrically by following the decrease in absorbancy at 265 nm (Karlström, 1968). Assay mixtures contained in a volume of 0.4 ml: 25 mM Tris-Succinate buffer pH 7.6, 150 μM adenosine and 1–10 units of enzyme.

Assay of Adenosine Deaminase Activity in Transductants

Transductants were spotted on L-broth plates containing 150 μg per ml of adenine, which induces the synthesis of adenosine deaminase, and incubated overnight at 42° C. Each colony was removed with a loop and resuspended in 100 μl 0.1 M Tris-Cl, pH 7.6; 10 μl of toluene was added and the suspension whirlimixed for 1 min and allowed to stand at 42° C for 30 min. Then 10 μl of 1 mM 8-^{14}C adenosine (2 μCi per μmole) were added. After 5 min of incubation, a 10 μl sample was removed, mixed with 5 μl (5 mM adenosine and 5 mM inosine), and the mixture applied on PEI-plates. After drying, the chromatograms were developped in water which allowed separation of inosine from adenosine; quantitative determinations were as described below.

Guanosine Kinase

Cells were homogenized as previously described (Hammer-Jespersen *et al.*, 1971). After centrifugation, the supernatant was dialyzed overnight against 20 mM Tris-Cl, pH 7.8. Assay mixture contained in a final volume of 100 μl: 70 mM Tris-Cl, pH 7.8, 30 mM KCl, 30 mM $MgCl_2$, 2 mM ATP, 0.5 mM U-^{14}C guanosine (5 μCi per μmole). At 4 min intervals, 15 μl samples were removed and mixed with 5 μl (5 mM guanosine and 5 mM guanosine monophosphate). The mixture was applied to PEI-plates; chromatography and quantitative determinations were as described below for purine phosphoribosyltransferases.

Purine Phosphoribosyltransferase

Assay mixture contained in a final volume of 100 μl: 0.1 mM 8-^{14}C labelled purine (adenine, guanine, hypoxanthine or xanthine), 6 μCi/μmole, 1.0 mM 5-phosphoribosyl-l-pyrophosphate, 4.0 mM magnesium chloride, 75 mM Tris-Cl buffer pH 7.6 and 0.2–1 units of enzyme. At 1 min intervals, 15 μl samples were withdrawn, mixed with 10 μl (5 mM purine nucleoside monophosphate, 5 mM of the free purine and 75 mM EDTA), and chromatographed on polyethylenimine impregnated cellulose plates (PEI-plates). The chromatograms were developed in methanol to the application line and then in water. This allowed separation of the purine from its mononucleotide. Quantitative determinations were performed as previously described (Jensen *et al.*, 1973).

Procedure for the Distinction between gsk^+ and gsk^- Transductants

The Procedure was based on the measurement of the incorporation of U-^{14}C guanosine into *pup* strains. Transductants were spotted

Table 1. Strains used

Sex	Strain No.	Relevant Genotype	Preparation and/or source[a]
F^-	SØ 003	*met*B, *str*A	K12 58–161, Univ. Inst. Microbiology, Copenhagen
F^-	SØ 197	*met*B, *str*A, *pur*B[b]	isolated from SØ 003, NG, Pen.
F^-	SØ 333	*met*B, *str*A, *pur*B, *add*[c]	isolated from SØ 197 (Hoffmeyer and Neuhard, 1971)
F^-	SØ 200	*met*B, *str*A, *pur*B, *Δ add-uid-man*	isolated from SØ 197 (Hoffmeyer and Neuhard, 1971)
F^-	SØ 405	*met*B, *str*A, *pur*B, *Δadd-uid-man, pup*	isolated from SØ 200 (Munch-Petersen *et al.*, 1972)
F^-	S 407	*man, uid*Al	Novel and Novel, 1971
F^+	AB 2848	*aro*D352, *sup*E42	Pittard and Wallace, 1966
F^-	SØ 199	*met*B, *str*A, *pur*E[d]	isolated from SØ 003, NG, Pen.
F^-	SØ 312	*met*B, *str*A, *pur*E, *pup*	isolated from SØ 199 (Munch-Petersen, 1972)
F^-	SØ 445	*met*B, *str*A, *pur*E, *pup, gsk*[c]	isolated from SØ 312, UV., Pen. (see text, Table 4)
F^-	SØ 446	*met*B, *str*A, *pur*E, *pup, apt*[c]	isolated from SØ 312, UV., Pen. unable to use adenine as purine source, Table 6
F^-	CSH 26	*ara, Δpro-lac, thi*	CSH
F^-	SØ 606	*ara, Δpro-gpt-lac, thi, hpt*	isolated from CSH 26, resistance to 6-mercaptopurine (1 mM), Table 6
F^-	SØ 609	*ara, Δpro-gpt-lac, thi, hpt, pup, pur*H,J, *str*A	isolated from SØ 606[e]
F^-	C 600	*leu*	Univ. Inst. Microbiology, Copenhagen
Hfr	P10	*ton*A	from O'Donnovan (Texas A & M)
Hfr	P4X	*met*B, *arg*E, *thi*	from CSH
Hfr	KL 16	*thi*	from CSH
Hfr	KL 14	*thi*	from CSH
Hfr	KL 96	*thi*	Univ. Inst. Microbiology, Copenhagen
Hfr	AB 257 (Cavalli)	*met*B, *rel* 1	Univ. Inst. Microbiology, Copenhagen
F′	W3747/F13	F13: *tsx*$^+$, *pur*E, *arg*F$^+$, *lac*$^+$	CGSC 5218
F′	AB 2463/KLF4	F104: *thr*$^+$, *leu*6$^+$, *proA*2$^+$	CGSC 4251
F′	E5014/Fpro-lac	F128: *pro*B$^+$, *pro*A$^+$, *lac*$^+$	CGSC 4288
F′	KL 251/ORF4	F254: *lac*$^+$, *pur*E$^+$	CGSC 4282

[a] Abbreviations used: NG = mutagenesis by nitrosoguanidine, UV = ultraviolet irradiation. CSH = Cold spring Harbor Laboratory, New York. CGSC = E. Coli Gen. Stock Ctr. Yale Univ., New Haven, Conn. USA. Pen = penicillin counterselection.
[b] The *pur*B mutation was identified by interrupted matings with KL 16 and with KL 96 and from the growth requirement (Table 2).
[c] New gene designations used here for the first time.
[d] The *pur*E mutation was identified by interrupted mating with Hfr Cavalli and by the excretion of aminoimidazol riboside (Lutton and Flaks, 1963).
[e] Strain SØ 606 was made *pup* as described before, Munch-Petersen *et al.* (1972), and then *pur* by UV irridiation and penicillin counterselection (the *pur* mutation was identified as *pur*H or *pur*J) (Lutton and Flaks, 1963). Finally, colonies resistant to streptomycin were isolated, *str*A.

on millipore filters placed on solid glucose minimal medium, containing 50 µM U-^{14}C guanosine (0.2 µCi per µmole). The plates were incubated for 48 hours at 37° C after which time 3 mm big colonies were obtained on the filters. The filters were transferred to plates containing ^{12}C guanosine and further incubated for 4 hours in order to dilute out the radioactivity associated with the membranes. Subsequently, the individual colonies were cut out and radioactivity was counted as described above. Colonies unable to incorporate appreciable amounts of U-^{14}C guanosine, *gsk*$^-$ mutants, could easily be distinguished from *gsk*$^+$ colonies.

Table 2. Enzyme levels[a] and growth response of *E. coli purB* mutants

Strain	Relevant genotype	Purine nucleoside phosphorylase[b] (units/mg protein)	Adenosine deaminase (units/mg protein)	Growth response to purines and purinenucleosides[c]: Ade	dAdo[d] + Hyp	Ade + Ado	Ade + Hyp
SØ 197	*pur*B	127	23	+	−	+	+
SØ 200	*pur*B, *add*	144	<0.1	−	+	−	+
SØ 333	*pur*B, *add*	130	<0.1	−	+	−	+
SØ 405	*pur*B, *add, pup*	<0.5	<0.1	−	−	−	+
SØ 405	*pur*B, *pup* (*add*$^+$)[e]	<0.5	27	−	−	+	+

[a] The cells were grown on glycerol as carbon source, and the activity of adenosine deaminase was determined as described in Materials and Methods.
[b] Purine nucleoside phosphorylase activity with inosine as substrate was determined as described before (Hammer-Jespersen *et al.*, 1971).
[c] Growth was scored on minimal agar plates after 24 hours of incubation at 37° C, in the presence of histidine. The addition of histidine inhibits the conversion of adenine nucleotides to guanine nucleotides via the histidine pathway (Fig. 1). + indicates growth; − indicates lack of growth.
[d] Deoxyadenosine concentration was 4 µg/ml (Hoffmeyer and Neuhard, 1971).
[e] *add*$^+$ transductant.

Results and Discussion

Mapping of the Gene for Adenosine Deaminase (add)

Adenosine deaminase catalyzes the deamination of adenosine (deoxyadenosine) yielding inosine (deoxyinosine). Strains carrying mutations in the gene coding for this enzyme were selected in a *pur*B mutant (SØ197). This strain requires adenine for growth and the requirement will not be satisfied by low amounts of deoxyadenosine due to a rapid deamination (Hoffmeyer and Neuhard, 1971). Thus, from *pur*B mutants able to grow on deoxyadenosine plus hypoxanthine as purine sources, *add* mutants were isolated as described by Hoffmeyer and Neuhard, 1971 (Table 2).

The approximate location of the *add* gene was determined by interrupted matings between Hfr strains KL 16, KL 96 and F$^-$ SØ 405. These experiments placed the *add* gene at about 30 min on the *E. coli* map (Taylor and Trotter, 1972); *add*$^+$ recombinants were selected on plates on which adenosine served as purine source for GMP synthesis and adenine as purine source for AMP synthesis, Table 2.

The location of *add* was established by cotransduction studies, Table 3. These data place *add* between *aro*D and *uid*A (Fig. 2).

The *add* mutation in strain SØ 333 was found to be suppressible when transduced into strain AB2848 which carries a suppressor of amber mutations. However, the enzymatic activity appeared to be thermolabile allowing a distinction between *add*$^+$ and *add*$^-$ at 42° C.

The *add* mutation in strain SØ 200 was found to be due to a deletion, covering *add-uid*A-*man*. Enzymatic analysis on cellfree extracts of SØ 200 revealed

Table 3. Mapping of *add* by transduction[a] and characterization of a deletion mutant (SØ 200)

Transductional donor	Recipient	Selected marker	Number of transductants	Unselected markers	No
SØ 333 (*add*)	S 407 (*man, uid*A)	*man*$^{+}$[b]	108	*uid*$^+$, *add*$^-$	85
				uid$^+$, *add*$^+$	11
				uid$^-$, *add*$^+$	11
				uid$^-$, *add*$^-$	1
SØ 333 (*add*)	AB 2848 (*aro*D)	*aro*$^+$	60	*add*$^+$	59
				add$^-$	1
SØ 200 (*add, uid*) (*man*)	AB 2848 (*aro*D)	*aro*$^+$	244	*man*$^+$, *add*$^+$	237
				man$^-$, *add*$^-$	7
				man$^-$, *add*$^+$	0
S 407 (*man, uid*A)	Ab 2848 (*aro*D)	*aro*$^+$	343	*man*$^+$	341
				man$^-$	2

[a] Transductions were carried out as described in Materials and Methods.
[b] Transductants were tested for a functional *add* gene by assaying for adenosine deaminase activity on toluenized cells at 42° C. Where indicated, transductants were tested for growth on D-mannose or methyl-β-D-glucuronide as carbon source.

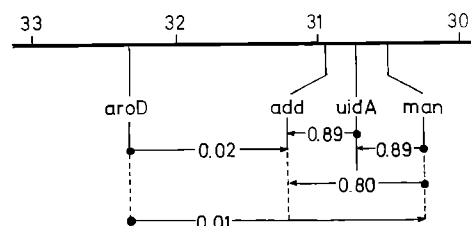

Fig. 2. Detailed genetic map of the *add* region of the E. *coli* map. The order of the markers follow from the results presented in Table 3. The arrows point in the direction from the selected donor markers

Table 4. Purine nucleoside kinase levels in *E. coli* mutants[a] and growth response to purines and purine nucleosides[b]

Strain	Relevant genotype	Purine nucleoside kinase activity			Growth response to purines and purine nucleosides			
		Guanosine (units/mg protein)	Inosine	Adenosine	Hyp	Ade + Guo[d]	Ade	Guo[d]
SØ 312	*pur*E, *pup*	0.70	0.05	0.11	+	+	−	−[c]
SØ 445	*pur*E, *pup* *gsk*	<0.01	0.02	0.11	+	−	−	−

[a] Enzyme assays were performed as described in Materials and Methods. Inosine kinase and adenosine kinase activity was determined essentially as described for guanosine kinase using ^{14}C-labelled inosine and adenosine as substrate (Jochimsen, 1974).
[b] Growth was scored as described in Table 2. All plates contained histidine.
[c] Growth was seen after 48 hours of incubation.
[d] Guanosine could be substituted by inosine.

no adenosine deaminase activity (Table 2) and less than 5% of wild type (SØ 197) phosphomannoisomerase activity when assayed as described by Kang and Markovitz, 1967. Thus strain SØ 200 had lost the ability to use D-mannose and also methyl-*β*-D-glucuronide as carbon source.

The deletion nature of the mutant appears from the following: (1) All three mutations were isolated in a single step (Table 1). (2) The frequency of cotransduction between *aro*D and *man* was found to be higher in SØ200 (2.9%) than in SØ407 (0.6%). (3) No revertants of SØ 200 on mannose or methyl-*β*-D-glucuronide as carbon source or adenosine as nitrogen source were obtained when 10^8 or 10^9 cells were spread on agar plates and incubated for 4 days at 37 °C. Addition of a few crystals of nitrosoguanidine to the plates induced no revertants.

Mapping of the Gene for Guanosine Kinase (gsk)

There are two pathways by which *E. coli* converts guanosine to GMP, namely (1) Guanosine $\xrightarrow{gsk}$ GMP and (2) Guanosine + P_i $\xrightarrow{pup}$ Guanine and ribose-phosphate; Guanine + phosphoribosylpyrophosphate $\xrightarrow{gpt, hpt}$ GMP (Fig. 1). Thus selection and phenotypic recognition of mutations in the *gsk* gene were done in a *pup* background. A *gsk* mutant, SØ 445, was isolated from SØ 312, as a mutant unable to grow on a combination of adenine, histidine and guanosine. In the presence of histidine SØ 312 cannot satisfy its purine requirement by adenine alone. However, addition of guanosine or inosine restores normal growth (generation time around 50 min). Guanosine or inosine alone will serve only poorly as purine source (generation time 380 min) (Table 4). Enzymatic analysis on extracts of SØ 312 and SØ 445 indicate that guanosine kinase has activity for inosine and not adenosine (Table 4).

Table 5. Mapping of *gsk* by transduction[a]

Transductional donor	Recipient	Selected marker	Number of transductants	Unselected markers	No
SØ 003	SØ 445 *pur*E, *pup* *gsk*	*pur*E[+ b]	172	*gsk*$^+$	13
				gsk$^-$	159

[a] Transductions were carried out as described in Materials and Methods.
[b] Transductants were tested for a functional *gsk* gene by analyzing for ^{14}C-guanosine incorporation as described in Materials and Methods.

Preliminary mapping data in *Salmonella typhimurium* revealed a location of the *gsk* gene (encoding guanosine kinase) close to *pur*E (Jochimsen unpublished results).

Matings performed between Hfr Cavalli and SØ 445 transferred *gsk* as an early marker (data not given).

By introduction of F-primes: 13, 128 and 254 (Low, 1972) covering the area around *pur*E, only merodiploids of F′254 was found to contain guanosine kinase activity (3.9 units/mg protein). Transduction experiments (Table 5) revealed 8% cotransduction with *pur*E, indicating a location of *gsk* at 13 min.

Mapping of the Gene for Hypoxanthine Phosphoribosyltransferase (hpt)

Purine phosphoribosyltransferases catalyze the conversion of purine bases to their respective nucleoside monophosphates (Fig. 1), and it is now generally accepted that in enterobacteriaceae there are three genes controlling the purine phosphoribosyltransferases (Chou and Martin, 1972; Krenitsky *et al.*, 1970; Gots *et al.*, 1972; Hochstadt-Ozer, 1971; Martin and

Table 6. Purine phosphoribosyltransferase activities of various *E. coli* mutants[a]

Strain	Phosphoribosyltransferase activity units/mg protein			
	Hypo-xanthine	Guanine	Xanthine	Adenine
SØ 312	86	25	21	90
SØ 446 *apt*[b]	83	24	23	<1
CSH 26 *gpt*	69	7	<1	93
SØ 606 *gpt, hpt*	<1	<1	<1	80

[a] Enzymatic activity was determined as described in Materials and Methods.
[b] New gene designation.

Table 7. Mapping of *hpt* by cotransduction[a]

Trans-ductional donor	Recipient	Selected marker	Number of trans-ductants[b]	Un-selected markers	No
P 10 (*ton*A)	SØ 609[c] (*pur*H, J) (*pup, hpt*) (*gpt*)	*hpt*$^+$	300	*ton*A	180
C 600 (*leu*)	SØ 609	*hpt*$^+$	300	*leu*$^-$	91

[a] Transductions were carried out as described in Materials and Methods.
[b] Recombinants were selected on plates containing glucose, proline, thiamine, threonine, leucine, and hypoxanthine. 300 such recombinants still having a purine requirement (*pur*H, J) and unable to grow on lactose (Δ*pro, gpt, lac*) or guanosine (*pup*) as carbon sources were tested for resistance to T_1-bacteriophages or for a leucine requirement.
[c] The purine requirement can be satisfied only by adenine in the presence of guanosine or inosine. Adenine alone will not support growth while guanosine or inosine are poor purine sources.

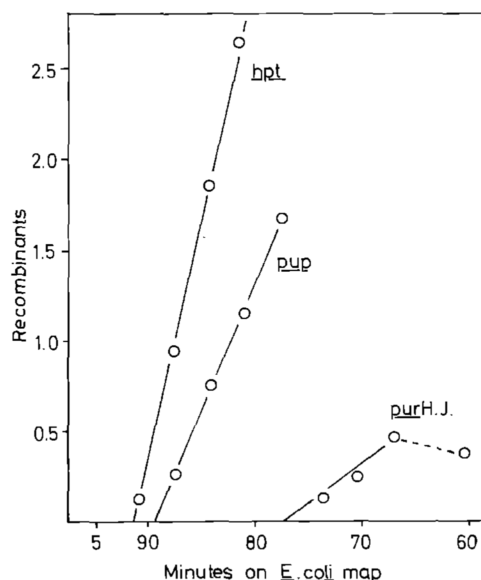

Fig. 3. Mapping of *hpt* by conjugation. The time of enty of *hpt, pup* and *pur*H, J markers were determined by interrupted mating experiments between Hfr P4X and SØ 609. The mating conditions are given under Materials and Methods. *hpt*$^+$ recombinants were selected for their ability to grow on hypoxanthine as purine source. *pup*$^+$ recombinants were selected for their ability to grow on adenine as purine source. (Adenine can only serve as purine source if the purine nucleoside phosphorylase adenosine deaminase pathway is operating, since *pur*H,J mutants are unable to convert AICAR to IMP, Fig. 1.) *pur*H,J$^+$ recombinants were selected fro their ability to grow without added purines. The abscissa represents a fragment of the *Escherichia coli* standard map. The ordinate is the number of recombinants per ml $\times 10^{-4}$

Yang, 1972). Namely: (1) one gene encoding adenine phosphoribosyltransferase (*apt*), (2) one gene controlling xanthine phosphoribosyltransferase (*gpt*), and (3) two genes controlling guanine and hypoxanthine phosphoribosyltransferases (*gpt* and *hpt*) (Table 6). This double specificity has led to some trouble in the location of the two genes on the chromosome. However, the findings of Gots *et al.*, 1972 that certain *pro*AB deletion mutants of *Salmonella typhimurium* simultaneously were deleted in the gene *gpt* makes it possible to locate the *hpt* gene. We therefore investigated xanthine phosphoribosyltransferase activity in *E. coli pro-lac* deletion mutants. One such mutant, CSH 26, appeared to be devoid of activity towards xanthine and had reduced activity for guanine, Table 6. If the episome F′104 (Low, 1972) was transferred into strain CSH 26 by selecting for *pro*$^+$ colonies, the merodiploid had restored xanthine phosphoribosyltransferase activity (32 units/mg protein) in agreement with the location of the *gpt* gene in the *pro*A-*pro*B region on the *E. coli* map (Livshitz, 1973).

From CSH 26, a mutant resistant to 6-mercaptopurine was isolated. This mutant SØ 606 appeared to have lost activity for hypoxanthine and guanine, Table 6. By introduction of additional mutations *pup, purH,* J and *str*A SØ 609 a genetic background was established for the location of the gene encoding hypoxanthine phosphoribosyltransferase, *hpt*.

The approximate location of the *hpt* gene was determined by interrupted matings of Hfr strains KL 14 and P4X with SØ 609. The results with Hfr P4X indicate a location of the *hpt* gene at about 2 min (Fig. 3). Transduction studies showed 60% cotransduction of *hpt* with *ton*A and 3% cotransduction with *leu*, Table 7. These results are consistent with a location of *hpt* between 2 and 3 min on the *E. coli* map.

Thus, all genes involved in "purine salvage" pathways which have been studied are unlinked. Recently mutants have been isolated which are unable to take up guanosine and inosine from the medium. The gene encoding this uptake *gru* has been found to be cotrans-

ducible with *met*C at 57 min on the *E. coli* map (Mygind unpublished observations).

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