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GENETIC AND BIOCHEMICAL STUDIES OF THE GUANOSINE 5'-MONOPHOSPHATE PATHWAY IN *ESCHERICHIA COLI*

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SUMMARY

Three genes which are involved in the guanosine 5'-monophosphate cycle of *Escherichia coli* were mapped on the chromosome. The genes *guaA* and *guaB* were located by transduction with phage P1kc, the *guaC* gene by conjugation. The *guaA* and *guaB* loci have been mapped between the genes for tyrosine and histidine biosynthesis; they are clustered and their order is from the tyrosine locus, *guaA–guaB*. The *guaC* locus was mapped on quite another part of the chromosome, in the *thr–leu* region.

Enzyme levels of $guaA^-$ and $guaB^-$ strains, grown under various conditions of repression and derepression, were determined. The results showed a coordinate control of the synthesis of inosine-5'-monophosphate dehydrogenase (EC 1.2.1.14) and xanthosine-5'-monophosphate aminase (EC 6.3.4.1). Guanosine-5'-monophosphate reductase (EC 1.6.6.9), however, is not coordinately regulated with these two enzymes. It is suggested that *guaA* and *guaB* are structural genes of one operon.

INTRODUCTION

Biochemically purine biosynthesis is a well known pathway in the metabolism of avian, mammalian and microbial cells (reviewed by BUCHANAN AND HARTMAN[1], and SCHULMAN[2]). In *Escherichia coli*, genetic research on the purine pathway has confined itself to the isolation of a number of mutants with known biochemical blocks[3–7] and to the mapping of purine markers on the *E. coli* K12 chromosome[7,8].

From the control mechanisms in the biosynthesis of purine nucleotides, the feedback inhibition has been thoroughly investigated (reviewed by ATKINSON[9], and STADTMAN[10]), but little is known about the control of enzyme synthesis in the purine pathway.

The attempt of this work was to make a genetic and biochemical study of the mutants *guaB* and *guaA*, which are blocked in the syntheses of the repressible enzymes inosine-5'-monophosphate dehydrogenase[11] (IMP:NAD^+ oxidoreductase, EC 1.2.1.14) and xanthosine-5'-monophosphate aminase[12] (XMP:ammonia ligase (ADP), EC 6.3.4.1) respectively, and of *guaC*, a mutant which lacks guanosine-5'-monophosphate reductase (NADPH:GMP oxidoreductase, EC 1.6.6.9).

In this paper the mapping of *guaC* by conjugation, and the determination of the order of the clustered genes *guaB* and *guaA* by transduction, are described. We found in the present study a coordinate control of the synthesis of IMP dehydrogenase and XMP aminase. GMP reductase is shown not to be coordinately regulated with IMP dehydrogenase and XMP aminase.

MATERIALS AND METHODS

Bacterial strains and bacteriophages

All experiments were done with derivatives of *E. coli* K12. The properties and origin of the strains are presented in Table I. The steps and biochemical deficiencies of purine biosynthesis, relevant for this paper are given in Fig. 1. Phage P1kc (ref. 13) was employed for transduction experiments.

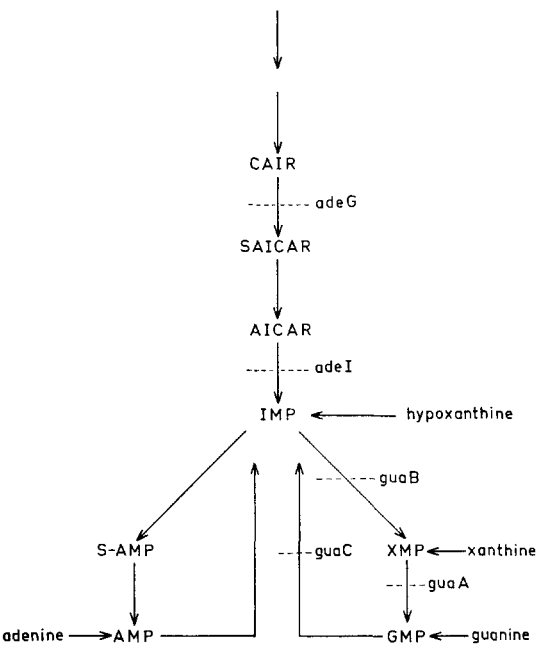

Fig. 1. Pathway of purine biosynthesis. CAIR, 5-amino-4-imidazole-carboxylic acid ribonucleotide; SAICAR, 5-amino-4-imidazole-*N*-succinocarboxamide ribonucleotide; AICAR, 5-amino-4-imidazolecarboxamide ribonucleotide; IMP, inosine 5-monophosphate; XMP, xanthosine 5-monophosphate; GMP, guanosine 5-monophosphate; S-AMP, succinyl adenosine 5-monophosphate; AMP, adenosine 5-monophosphate.

Media

The following media were used: Minimal medium[14]: K_2HPO_4, 0.75 %; KH_2PO_4, 0.45 %; NH_4Cl, 0.2 %; $MgSO_4 \cdot 7H_2O$, 0.005 %; $FeSO_4$, 0.0005 %; glucose, 0.2 %. This medium was supplemented with the appropriate growth factors in the following concentrations: histidine, 50 μg/ml; tryptophan, 10 μg/ml; tyrosine, 10 μg/ml; thiamine, 10 μg/ml; purines, when not otherwise mentioned 15 μg/ml; streptomycin was

TABLE I

CHARACTERISTICS AND ORIGIN OF BACTERIAL STRAINS*

Strain	*Mating type*	*Purine markers*					*Other markers*	*Origin*
		Genotype		*Phenotype*		*Deficiency*		
		ade	*gua*	*ade*	*gua*			
H 865	F⁻	+	+	+	+			STOUTHAMER
H 713	F⁻	+	B_3	+	−	IMP dehydrogenase		*et al.*[34]
H 719	F⁻	+	A_1	+	−	XMP aminase		
H 675	F⁻	I_1	+	−	−	AICAR transformylase	*thi⁻, his⁻,*	STOUTHAMER
H 677	F⁻	G_1	+	−	−	Conversion of CAIR to SAICAR	*tyr⁻, try⁻,*	*et al.*[7]
H 883	F⁻	U_1	+	−	−	Early step before AIR	*lac⁻, gal⁻,*	
H 1167	F⁻	I_1	C_2	−	−	AICAR transformylase GMP reductase	*str^r*	H 675
H 724	F⁻	G_1	B_7	−	−	Conversion of CAIR to SAICAR IMP dehydrogenase		H 677
H 725	F⁻	G_1	A_{26}	−	−	Conversion of CAIR to SAICAR XMP aminase		H 677
R 4	Hfr	+	+	+	+			REEVES[35]
R 4	Hfr	I_2	+	−	−	AICAR transformylase	*str^s*	R 4
R 1	Hfr	+	+	+	+			REEVES[35]
H	Hfr	+	+	+	+			HAYES[36]

Abbreviations: *ade⁻*, *gua⁻*, *thi⁻*, *his⁻*, *tyr⁻* and *try⁻* refer to inability to synthesize adenine, guanine, thiamine, histidine, tyrosine and tryptophan, *lac⁻* and *gal⁻* to the inability to ferment lactose or galactose, while *str^r* and *str^s* means resistant or sensitive against streptomycine. Other abbreviations see legend of Fig. 1.

* Nomenclature rules as proposed by DEMEREC *et al.*[37] are generally followed. As a consequence of one of these rules the symbols for IMP dehydrogenase and XMP aminase synthesis in *E. coli* are *guaB* and *guaA* respectively, instead of *guaA* and *guaB* as we used previously[7].

used at a final concentration of 100 μg/ml. Medium for phage experiments[13]: L. Broth: Bactotryptone, 1 %; yeast extract, 0.5 %, NaCl 0.5 %; L. Broth agar: Bactopeptone, 1 %; meat extract, 0.5 %; NaCl, 1 %; agar, 1.5 %. Soft agar: Bactotryptone, 1 %; yeast extract, 0.5 %; NaCl, 0.5 %; agar, 0.6 %. To these media $CaCl_2$ was added at a final concentration of 2.5 mM. Culture media for the preparation of cell-free extracts were described by MAGASANIK, MOYED AND GEHRING[11].

Isolation of auxotrophic mutants

Auxotrophic mutants were isolated by replica-plating[15], after treatment with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine[7], followed by penicillin enrichment[16].

No selective techniques are available for the isolation of strains with *guaC*$^-$ as the only purine marker, as such strains are able to grow on a medium without added purine. The *guaC*$^-$ mutant was therefore isolated from a strain with a block before IMP. An *adeI*$^-$ mutant was selected for this purpose and *adeI-guaC*$^-$ mutants were obtained by penicillin enrichment in minimal medium supplemented with guanine only.

Procedures for transduction and conjugation

For transduction experiments, transducing phage suspensions of high titre were prepared by the confluent lysis method on agar[17]. This technique yielded suspensions with titres between 1×10^9 and 1×10^{10} plaque-forming units per ml. Transduction experiments were performed as described by LENNOX[13]; the phage–bacteria mixtures however were incubated for 15 min to allow phage adsorption, rather than for 20 min. The multiplicity ranged from 1 to 3. The infected cells were plated on appropriate medium supplemented with sodium citrate to a final concentration of 0.05 %.

Bacterial mating was performed as described by WOLLMAN AND JACOB[18]. After appropriate dilution (in minimal medium) the mating mixtures were plated on various selective media for scoring of recombinants.

Preparation of cell-free extracts

The bacteria were grown overnight, and then diluted 1 in 10 in pre-warmed fresh medium. When exponential growth was obtained, fresh medium was inoculated with 1/10 vol. of the log phase culture. The cultures were grown aerobically in a shaking machine at 37°. The extinction of the cultures was measured in a Unicam SP 600 at 660 mμ. Preparation of extracts was carried out by methods described previously[11]. The amount of protein in the cell-free extracts was determined with the Folin reagent[19].

Enzyme assays

IMP dehydrogenase was assayed according to the method of MAGASANIK, MOYED AND GEHRING[11]; instead of 3 μmoles IMP and 2.5 μmoles NAD^+, 1.5 μmoles IMP and 1.25 μmoles NAD^+ were used in the reaction mixtures. XMP aminase was assayed by the method of MOYED AND MAGASANIK[20]; later on in this work a modified

assay was used[21]. GMP reductase assay was described by MAGER AND MAGASANIK[22]. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed by the method of GLAZER AND BROWN[23].

In all enzymatic assays the change in absorbance of the reaction mixtures was measured in a Beckman DU spectrophotometer. Specific activities are expressed as μmoles substrate utilized or μmoles product formed per min per mg protein.

Special chemicals

Special chemicals were obtained as follows: IMP from Sigma Chemical Company, St. Louis, Mo.; XMP and GMP from Nutritional Biochemicals Corporation, Cleveland, Ohio; NAD^+, $NADP^+$, NADPH, GSH, ATP and glucose 6-phosphate from Boehringer, Mannheim. Psicofuranine was obtained by a generous gift from Dr. M. A. LASSANCE.

RESULTS

Genetic studies

The transfer of the guaC marker. Preliminary experiments have shown that the *guaC* marker was transferred by Hfr R4, Hfr R1 and by Hfr H when the transfer period was limited to 40 min. In a Hfr R4 *adeI*$^-$ × F$^-$ *adeI*$^-$*guaC*$^-$ cross, *guaC*$^+$ recombinants were selected on guanine supplemented medium. The *adeI*$^-$ mutation in the donor prevents the formation of *adeI*$^+$ recombinants which would grow on the selective medium. The *guaC* marker was transferred by Hfr R4 after about 10 min. The entrance time of the *guaC* marker suggests that it is located between the purine marker *adeK* (adenylosuccinate synthetase), which was mapped previously by STOUTHAMER, DE HAAN AND NIJKAMP[7], and the threonine marker.

Mapping of guaA and guaB by transduction. From conjugation experiments described previously[7], it is known that the markers *guaA* and *guaB* are located between the markers *tyr* and *adeG* and that both *gua* markers are closely linked. The close linkage between *guaA* and *guaB* was confirmed by determining the percentage cotransduction. Phage P1kc propagated on a *guaA*$^+$*guaB*$^-$ strain was used as transducing phage for the acceptor strain *guaA*$^-$*guaB*$^+$; *guaA*$^+$ transductants were isolated on xanthine supplemented medium, and scored for the *guaB* marker. The result, 93 % cotransduction of *guaB* with *guaA*, shows that the two genes are very closely linked.

The conjugation experiments suggested the order *tyr–guaB–guaA–adeG*. This conclusion was doubtful due to the low resolution power of the conjugation experiments (*cf.* VERHOEF AND DE HAAN[24]). The exact order was therefore determined by transduction with phage P1kc. Preliminary experiments had shown that contrary to the *tyr* marker, *adeG* was cotransducible with the *gua* markers ($\pm$ 25 %). The cotransduction of *guaA* and *guaB* with *adeG* was studied in a transduction experiment with wild type K12 as donor and *adeG*$^-$*guaA*$^-$ and *adeG*$^-$*guaB*$^-$ as recipients. *Gua*$^+$

TABLE II

COTRANSDUCTION BETWEEN *gua* MARKERS AND *adeG*

The donor in these transduction experiments was always *guaA*$^+$*guaB*$^+$*adeG*$^+$.

Acceptor strain	*Selection for*	*adeG*$^+$/*gua*$^+$ *ratio*	*Cotransduction* (%)
guaA$^-$*guaB*$^+$*adeG*$^-$	*guaA*$^+$	174/723	24.1
guaA$^+$*guaB*$^-$*adeG*$^-$	*guaB*$^+$	278/1079	25.3

transductants were selected on hypoxanthine supplemented medium and scored for cotransduction with *adeG*. The results given in Table II suggest that the order of the markers is *guaA–guaB–adeG*; however the cotransduction frequencies are not significantly different (X^2 test). The exact order was therefore determined in a reciprocal three-factor cross. The strains *guaA*$^+$*guaB*$^-$*adeG*$^+$ and *guaA*$^-$*guaB*$^+$*adeG*$^-$ were used as recipients in a series of experiments in which the complementary strains *guaA*$^-$*guaB*$^+$*adeG*$^-$ and *guaA*$^+$*guaB*$^-$*adeG*$^+$ were used as donors. In both transduction experiments selection was made for *pur*$^+$ transductants on a medium without added purines. The transduction efficiency of the phage suspensions was checked by studying the transduction of a single marker. The characteristics of the crosses are given in Fig. 2 in which the order *guaA–guaB–adeG* was assumed. It may be seen

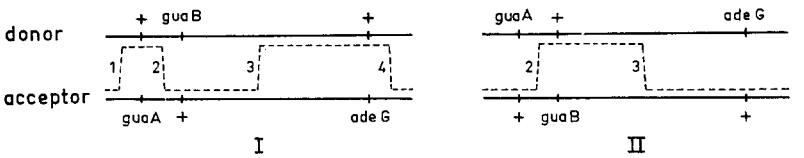

Fig. 2. Cross I and II are reciprocal crosses with marker order *guaA–guaB–adeG*. Broken lines represent crossing-overs necessary for the formation of *pur*$^+$ recombinants.

from this figure that four crossing-overs are required for a *pur*$^+$ recombinant in cross I against two crossing-overs in cross II. Thus cross I is expected to yield a lower number of transductants than cross II. When the order of loci is *guaB–guaA–adeG*, two crossing-overs are required for a *pur*$^+$ recombinant in both cases and equal

TABLE III

DETERMINATION OF THE ORDER OF *guaA* AND *guaB* BY THREE-POINT TRANSDUCTION

Reciprocal crosses were carried out under standard conditions, by using bacterial suspensions of equal *A* and phage suspensions of identical titres. The adsorption of the phages to the acceptor bacteria was in both cases > 98 %.

Donor	*Acceptor*	*Crossing-over regions**	*pur*$^+$ *recombinants*
guaA$^+$*guaB*$^-$*adeG*$^+$ ×	*guaA*$^-$*guaB*$^+$*adeG*$^-$	1–2–3–4	50
guaA$^-$*guaB*$^+$*adeG*$^-$ ×	*guaA*$^+$*guaB*$^-$*adeG*$^+$	2–3	633

* Crossing-over regions if order is *guaA–guaB–adeG* (*cf.* Fig. 2).

numbers of transductants are expected. The outcome of the transduction experiments, given in Table III, shows that a lower number of transductants is obtained from cross I, indicating that the true order is indeed *guaA–guaB–adeG*. This result is in agreement with the order of the loci in *Salmonella typhimurium* (J. S. GOTS, personal communication see ref. 25).

Biochemical studies

The close linkage between the *guaA* and *guaB* marker suggests that the two markers might be part of one operon; the synthesis of both enzymes, XMP aminase and IMP dehydrogenase, is thus expected to be coordinated. The coordinated enzyme synthesis was studied by growing *pur*$^-$ strains in media with various concentrations of guanine. Table IV shows the repressive effect of guanine on the synthesis of IMP dehydrogenase in a *guaA*$^-$ strain and the effect on XMP aminase synthesis in a *guaB*$^-$ strain. The specific activity of GMP reductase was also studied. It may be seen from this table that high concentrations of guanine repress the synthesis of IMP dehydrogenase and XMP aminase. No detectable activity of these enzymes was observed at 20 μg/ml guanine. The enzyme-level of the GMP reductase increased with increasing concentrations of guanine. The level of an arbitrary enzyme, glucose-6-phosphate dehydrogenase, was found to be independent of guanine concentrations from 1.5–20 μg/ml. At very low concentrations of guanine (0.5 μg/ml) the specific activity of glucose-6-phosphate dehydrogenase is decreased. This decrease is also observed for IMP dehydrogenase and XMP aminase. It is probably the consequence of a general effect on RNA and protein synthesis, due to very low intracellular concentrations of guanine nucleotides.

TABLE IV

THE EFFECT OF GUANINE ON ENZMYE ACTIVITY IN GUANINE-REQUIRING MUTANTS OF *E. coli* K12

Specific activities of IMP dehydrogenase and XMP aminase were determined in cell-free extracts of 16-h cultures of *guaA*$^-$ and *guaB*$^-$ strains respectively, grown with various concentrations of guanine. GMP reductase and an arbitrarily chosen control enzyme, glucose-6-phosphate dehydrogenase, were determined in a *guaB*$^-$ strain.

μg guanine per ml medium	*Enzyme activity (μmoles/mg protein per min) × 10³*			
	IMP dehydrogenase	*XMP aminase*	*GMP reductase*	*Glc-6-P dehydrogenase*
0.5	47.4	14.3	0.0	13.5
1.5	94.6	36.0	0.0	48.8
2.5	74.4	24.7	0.0	48.3
7.5	43.1	15.7	0.07	46.9
10.0	30.3	11.7	—	47.8
20.0	0.0	0.0	0.20	47.3
40.0	0.0	0.0	0.20	52.7

The derepression of the synthesis of IMP dehydrogenase and XMP aminase was further studied by guanine deprivation. *Pur*$^-$ mutants were first grown under repressive conditions (15 μg guanine per ml) and then resuspended in fresh medium supplemented with a suboptimal guanine concentration (4 μg/ml). After the cells had

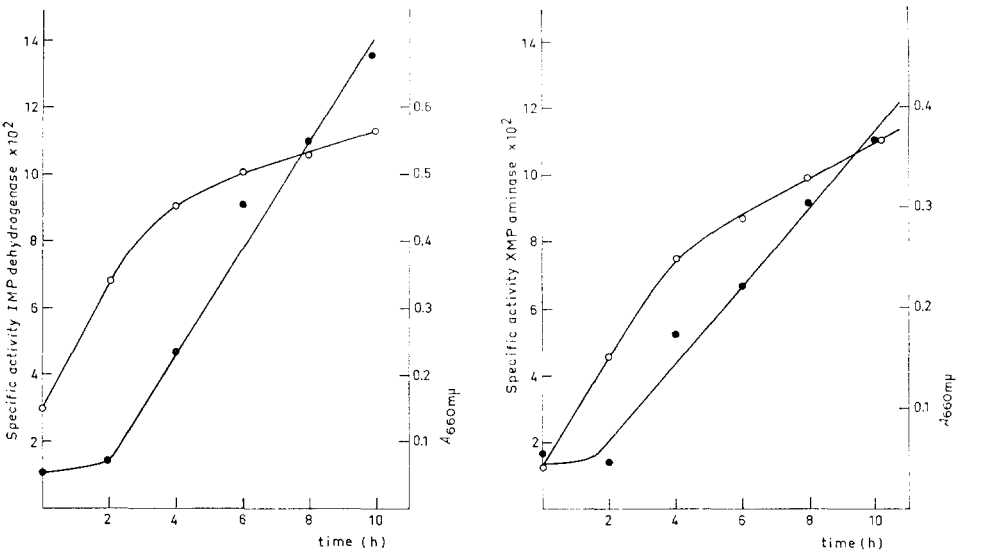

Fig. 3. Kinetics of IMP dehydrogenase synthesis of a *guaA*⁻ strain (a) and XMP aminase of a *guaB*⁻ strain (b), under conditions of derepression (experimental procedure described in the text). ○–○, bacterial growth; ●–●, specific activity (μmoles/mg protein per min).

exhausted their guanine, there was a constant linear increase of the activity of IMP dehydrogenase and XMP aminase during 8–15 h. During this period, the specific activity of the enzymes was determined at various intervals (see Figs. 3a and 3b).

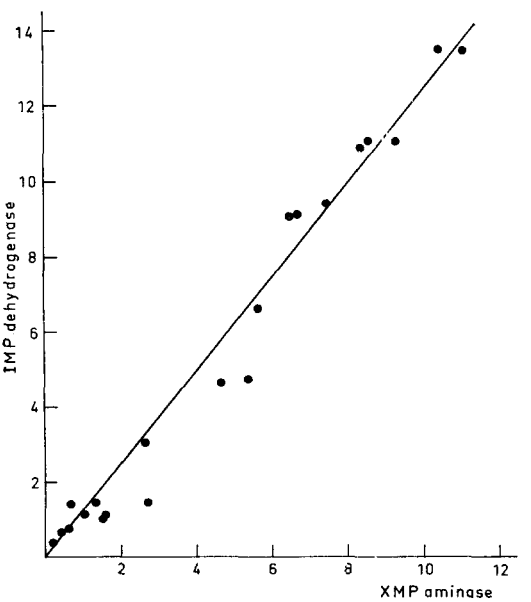

Fig. 4. Dispersion diagram of the specific activities of IMP dehydrogenase and XMP aminase determined at various intervals of the derepression phase in guanine-starving cultures of *guaA*⁻ and *guaB*⁻ mutants.

GMP reductase was not detectable at the low concentrations of guanine used. The specific activity of IMP dehydrogenase was plotted against that of XMP aminase. In the case of coordinately controlled enzymes one obtains a straight line[26]. Fig. 4 gives the specific activities of IMP dehydrogenase and XMP aminase from several experiments. From the straight line obtained coordinate control of these enzymes is concluded.

The purine mutants with a biochemical block before IMP as *adeI*$^-$, *adeG*$^-$, *adeG*$^-$*guaA*$^-$ and *adeG*$^-$*guaB*$^-$, had under conditions of derepression a shorter increase of enzyme activity of IMP dehydrogenase and XMP aminase, followed by a sharp decrease to a low level, which is contrary to the derepression of these enzymes in strains with a block between IMP and GMP. The differences in control of IMP dehydrogenase and XMP aminase in *guaA*$^-$ and *guaB*$^-$ strains on one hand, and the strains blocked before IMP on the other hand are dealt with in a following paper.

DISCUSSION

The results of the genetic experiments show that the two genes, *guaA* and *guaB* are clustered and that the order between *tyr* and *adeG* is *tyr–guaA–guaB–adeG*. This order is in agreement with the sequence in *S. typhimurium*[25]. The third gene of the GMP cycle, *guaC*, is located on a different part of the chromosome.

The clustering of *guaA* and *guaB* leads to the hypothesis that they constitute an operon, and thus behave as an unit of coordinate expression[27]. In agreement with this hypothesis are the results of the biochemical study, which shows a coordinate control of IMP dehydrogenase and XMP aminase, the products of the *guaB* and the *guaA* genes. This result is comparable with the results of MOYED[12] who found a derepression of both IMP dehydrogenase and XMP aminase in cells in which XMP aminase activity was inhibited by addition of the antibiotic psicofuranine, which causes a lack of GMP. The derepression of these enzymes in mutants, blocked in the conversion from 5-amino-4-imidazolecarboxamide ribonucleotide to IMP, growing in adenine supplemented medium, also supports the idea of coordinated control[28]. In these strains the accumulation product, ribosyl-4-amino-5-imidazolecarboxamide, inhibits adenosine deaminase, an enzyme catalysing one of the necessary steps in the conversion of adenine to GMP. When adenine is the only purine source, this accumulation product prevents the synthesis of GMP, which consequently derepresses the synthesis of IMP dehydrogenase and XMP aminase.

All results mentioned do not exclude the possibility of two very closely linked operons, which are under the control of one repressor. Evidence to decide between the two possibilities may be obtained from studying constitutive strains and from studying strains with polar mutations. Different attempts were made to isolate constitutive strains either by alternated growth in a medium with guanine and a medium with hypoxanthine, or with the antibiotic psicofuranine. This antibiotic was expected to give strains which could overcome psicofuranine inhibition through a very high level of the enzymes, due to a constitutive synthesis. However, all attempts were unsuccessful. Suitable purine analogues, which are not metabolised on the one hand, and which on the other hand compete with the end product for the apo-repressor, as for instance 5-methyltryptophan in tryptophan biosynthesis[29] and

canavanine in arginine biosynthesis[30], are not known. This is a serious draw-back for the isolation of constitutive strains.

Further evidence for one *gua* operon may obtained by the study of polarity. From the work with the *his* operon[31], the *lac* operon[32] and the *try* operon[33] it is known that the enzymes of an operon are all coded on one large messenger RNA molecule, starting from the operator. From recent experiments with strains with polar *gua* mutations it is concluded that the *guaA* and the *guaB* gene form one operon with the operator at the *guaA* site (NIJKAMP AND OSKAMP, manuscript in preparation).

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