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Mutations Affecting Uridine Monophosphate Pyrophosphorylase or the *argR* Gene in *Escherichia coli*

Effects on Carbamoyl Phosphate and Pyrimidine Biosynthesis and on Uracil Uptake

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Summary. In the course of experiments directed towards the isolation of mutants of *Escherichia coli* K12 with altered regulation of the synthesis of carbamoylphosphate synthetase, two types of mutations were found to affect the cumulative repression of this enzyme by arginine and uracil. Alteration of the arginine pathway regulatory gene, *argR*, was shown to reduce the repressibility of the enzyme by both end products while mutations affecting uridine monophosphate pyrophosphorylase (*upp*) besides affecting uracil uptake preclude enzyme repression by uracil or cytosine in the biosynthesis of carbamoylphosphate and the pyrimidines. The *upp* mutations were located on the chromosome near the *gua* operon. Mutations previously designated as *uraP* are shown to belong to this class.

The relation that could exist between the loss of uridine monophosphate pyrophosphorylase and the impairment of uracil uptake is discussed.

A new method for isolating *argR* mutants in arginine-less strains is described.

Introduction

In *Escherichia coli*, a single enzyme (Piérard *et al.*, 1965) made up of two kinds of subunits (Trotta *et al.*, 1971) provides carbamoyl phosphate (CP)[1] for both the arginine and pyrimidine pathways. This enzyme, glutamino-carbamoyl phosphate synthetase (GCPSase) is submitted to cumulative repression by arginine and by the pyrimidines (Piérard and Wiame, 1964). Repression of enzyme synthesis also controls the arginine and pyrimidine pathways (Maas, 1961; Gorini, Gundersen and Burger, 1961; Vogel, 1961; Beckwith *et al.*, 1962). The end products responsible for the control of the pyrimidine pathway are probably cytidine triphosphate (CTP) and uridine triphosphate (UTP) as inferred from parallel studies conducted with *Salmonella typhimurium* (Neuhard and Ingraham, 1968; Abd-El-Al and Ingraham, 1969).

1 *Abbreviations:* CP = carbamoyl phosphate; GCPSase = glutaminocarbamoyl phosphate synthetase; OTCase = ornithine carbamoyl transferase; ATCase = aspartate carbamoyl transferase; DHOdehase = dihydroorotate dehydrogenase; PRPP = phosphoribosyl pyrophosphate; upp = uridine monophosphate pyrophosphorylase, uracil phosphoribosyl transferase.

The mutants analyzed in this report were obtained by procedures aimed at the selection of regulatory mutants of carbamoyl phosphate synthesis. Two classes of organisms have been obtained so far: 1. Mutants of the regulatory gene of arginine biosynthesis (*argR*) for which a new selective technique is described, directly applicable to arginine less mutants; 2. mutants lacking uridine monophosphate pyrophosphorylase (uracil phosphoribosyltransferase, *upp*).

Besides calling attention to the role played by the *argR* product in the regulation of CP synthesis, our observations disclose the pleiotropic effect of UMP pyrophosphorylase mutations on uracil uptake and enzyme repression in CP and pyrimidine biosynthesis. By showing that the symbols *uraP* (Taylor and Trotter, 1967; Taylor, 1970; O'Donovan and Neuhard, 1970) and *upp* have been assigned to the same type of mutants, a possible confusion is avoided.

Material and Methods

Bacterial Strains. All the strains described in this study are derived from the strain P4X (Hfr *metB*) with the exception of PA3305, a strain found by chance to carry a *upp* mutation that could not be traced up in its pedigree.

Media, Culture and Genetic Techniques. All the media, the culture conditions and the detailed procedure used in matings and transductions have been described previously (Glansdorff, 1965).

Mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine was performed according to the method of Adelberg *et al.* (1965).

The cells used for the enzyme assays were grown on a rotary shaker at 37° C in minimal medium No. 132 (Glansdorff, 1965) supplemented with 0.5% glucose and, when specified, with arginine (100 μg/ml) or uracil (50 μg/ml). The cells were harvested by centrifugation at mid log phase.

The feeding experiments were performed by streaking heavy suspensions (10^9 cells per ml) of the tested organism and of the pyrimidine-less indicator strain P4XSB22 side by side at a distance of 2 mm on a medium devoid of uracil.

Enzyme Assays. The sonic disruption of the cells and the treatment of the extracts on Sephadex G25 were performed as previously described (Piérard and Wiame, 1964).

Carbamoyl phosphate synthetase was assayed by a modification of the previously described method (Piérard and Wiame, 1964; Piérard, 1966). The modified method involves the conversion into ^{14}C-citrulline in presence of an excess of ornithine carbamoyl transferase of the ^{14}C-carbamoyl phosphate synthesized from ^{14}C-bicarbonate. Incubations were performed at 37° C for 15 min and contained the following in a volume of 1 ml: ATP, 10 μmoles; $MgCl_2$, 10 μmoles; glutamine, 10 μmoles; ^{14}C-$NaHCO_3$ (0.1 to 0.2 μc per μmole), 10 μmoles; ornithine, 6 μmoles; phosphate buffer, pH 7.5, 50 μmoles, partially purified ornithine carbamoyl transferase from *Escherichia coli*, 100 to 200 units. The reaction was stopped by the addition of cold 0.25 M trichloracetic acid and the precipitated proteins removed by centrifugation. After elimination of the excess ^{14}C-$NaHCO_3$, an aliquot of 1 ml was transferred to a polyethylene scintillation vial containing 10 ml of scintillation cocktail (4 parts of Triton X100, 6 parts of a solution containing 0.5% 2.5-diphenyloxazole and 10% naphthalene in reagent grade dioxane. The samples were counted in a model LS-100 Beckman liquid scintillation spectrometer with an efficiency of 55%.

Ornithine carbamoyl transferase was assayed by the method of Jones *et al.* (1955) and citrulline formed was determined colorimetrically by a modification of the method of Archibald (1944).

Aspartate carbamoyl transferase determinations were performed according to Gerhart and Pardee (1962) in a reaction mixture containing: L-aspartate, sodium salt, 20 μmoles; dilithium carbamoyl phosphate, 20 μmoles; potassium phosphate buffer, pH 7.5, 100 μmoles. Incubation was at 37° C for 15 min. The colorimetric estimation of carbamoyl aspartate was performed according to Gerhart and Pardee (1962) or Yashphe (in preparation).

Dihydroorotate dehydrogenase was assayed by the method of Beckwith *et al.* (1962). The method used for the assay of UMP pyrophosphorylase was that of Crawford *et al.* (1957) except that the reaction mixture contained 1 μmole of tetrasodium-PRPP. The radioactivity of the samples was determined as described in the assay of GCPSase (see above).

Measurement of the initial velocity of uracil uptake was performed by an adaptation of the method described for yeast by Grenson (1969). Unless otherwise specified the initial velocity of uptake was followed over a period of 2 min after the addition of ^{14}C-uracil to a culture growing exponentially on minimal medium.

Results

A. Methods of Selection of the Mutants

A first set of mutants has been selected as resistant to growth inhibitory conditions thought to interfere with the synthesis of carbamoyl phosphate synthetase. Three modes of selection have been used.

1. Resistance Towards a Mixture of Toxic Analogs of Pyrimidines and Arginine. Having in mind that the release by mutation of the cumulative control exerted on CP synthesis by arginine and the pyrimidines might bring about a partial resistance to a mixture of toxic analogs of both end products, we tried pairs of the following substances: 6-azauracil, 5-methyl cytosine, 2-aminopyrimidine, 5-aminouracil, 2-thiouracil, 2-thiocytosine, isocytosine, barbituric acid, 5-fluorouracil, canavanine and homoarginine.

Two combinations of analogs were found to slow down growth sufficiently to permit the selection of mutants: canavanine (100 μg/ml) plus azauracil (100 μg/ml) and canavanine (100 μg/ml) plus 2-thiouracil (100 μg/ml). These compounds were added to AUF medium (Piérard *et al.*, 1965) which reduces the cellular pools of arginine and pyrimidines and is thus expected to enhance the toxicity of the analogs.

Canavanine and thiouracil used separately bring about a moderate inhibition of growth, thiouracil being somewhat less effective than canavanine. The combination of the two inhibitors cause a larger delay in the appearance of colonies. Azauracil alone (100 μg/ml) is already a potent inhibitor.

Cells growing in minimal medium supplemented with arginine and uracil were centrifugated, washed, resuspended in minimal medium and spread out on selective plates. Nitrosoguanidine treated cells were also used. Mutants resistant to canavanine plus thiouracil began to appear after 15 hrs. An important yield (60% in some instances) of *argR* mutants was obtained among them. The other mutants were probably affected in the arginine permease. Mutants resistant to a mixture of canavanine plus azauracil appeared after 40 hrs and were, in their large majority, deficient in UMP-pyrophosphorylase. However, a few late appearing colonies (2 out of 45 mutants) were identified as *argR* mutants.

2. Resistance Towards a Mixture of Arginine and 2-Thiouracil. This combination was already known (Ben-Ishai *et al.*, 1964) to drastically inhibit growth at a time when the mechanism of CP biosynthesis and its regulation were still unknown. As thiouracil alone is a moderate inhibitor (see above) and arginine inhibits neither growth nor the activity of GCPSase, a possible target of this combination was the repression of the synthesis of this enzyme. The strain used in this selection was P4X170, an *argC* derivative of HfrP4X, this in order to avoid the selection of arginine permease mutants. Both arginine and thiouracil

were used at the concentration of 100 μg per ml. By this procedure, out of 22 mutants chosen among the late appearing resistant colonies (between 48 and 72 hours), 3 were *argR* mutants, the others were either *upp* or of an unknown type presently under study. The *upp* mutants being easily identified by their resistance to azauracil the present method provides an efficient method for the selection of *argR* mutants and is, in addition, directly applicable to arginine auxotrophs.

3. Resistance Towards 6-Azauracil Alone. Although it was known that *upp* mutants could be obtained by selecting for resistance to 6-azauracil (see Discussion), mutants affected in the control of GCPSase synthesis were looked for by this technique. A large number of resistant mutants were examined, all of them were *upp* mutants. However, it was observed that the *argR* mutations confers some resistance towards this analog, significant growth of such an mutant being observed after several days on plates containing azauracil.

4. Selection from an Arginine-Uracil Bradytrophic Mutant. The last method of selection to be mentioned here made use of a leaky frameshift mutant of GCPSase, P4XMi178. This organism, initially isolated as a uracil sensitive mutant has a GCPase which is deficient in the synthesis of CP from glutamine, the physiological substrate of the enzyme (Mergeay, 1969; Piérard, *et al.*, in preparation). It is able to grow at near wild type rate in a minimal medium supplemented with 0.15 M $(NH_4)_2SO_4$(low affinity nitrogen donor for GCPSase) but it does so only after a lag period of about 2 hrs when it has been precultivated in a minimal medium supplemented with arginine and uracil. In the cultures adapted to the high ammonium concentration medium, the enzymes of the arginine and pyrimidine pathways are fully derepressed. By serial transfers from one medium to the other, we obtained mutants exhibiting no more lag. Mutations affecting the operator of the gene of GCPSase or a repressor controlling the synthesis of the product of this gene, were looked for in these experiments.

Some of the mutants isolated by this method have been first characterized on the basis of their ability to grow in a medium containing uracil, the parental strain being sensitive. Again, both *argR* and *upp* mutants were obtained, in addition to strains carrying intragenic suppressors. Other mutants have been retained as being able to feed a uracil-less indicator mutant. All strains identified by the latter criterium were found to be *upp* mutants.

B. Properties of the Mutants

The mutants were screened for a modification of the control of the synthesis of GCPSase. They were further characterized by the determination of the level of OTCase and ATCase, considered as indicators of the repression conditions in the pathways utilizing CP.

1. Influence of the argR Mutation on the Control of the Synthesis of Carbamoyl Phosphate Synthetase. One class of mutants was found to exhibit fully derepressed level of OTCase in addition to partial derepression of GCPSase. They were identified as *argR* mutants. This class included a large number of the mutants selected as resistant to the combination of thiouracil with arginine or canavanine and also a few organisms isolated by the other selection procedures. The behaviour

of these mutants suggested an influence of the *argR* mutation on the synthesis of GCPSase. Such an effect is indeed apparent if one considers Table 1. GCPSase, in the wild type strain, is submitted to partial repression by either arginine or uracil; the simultaneous presence of both end-products is required to achieve maximum repression of the enzyme. The presence of the *argR* mutation not only reduces the repressibility of GCPSase by arginine but also restricts the effect of uracil so that less than 50% repression is attained in the presence of both metabolites. Resistance of *argR* mutants towards the selection procedures used here seems to imply that this regulatory mutation is able to produce an increase of the pyrimidine nucleotides pool because of the derivation of an excess of CP through the pyrimidine pathway. The slightly lower ATC level of the *argR* mutants (Table 1) may be related to such an effect although a direct involvement of arginine in the control of ATC synthesis can not be excluded. Neither is it known if the 1.5 to 2 fold reduction of ATC synthesis observed when arginine is added to $argR^+$ or $argR^-$ cells is due to a direct effect, or, in spite of the control of carbamoylphosphate synthetase, to the escape of a slight excess of CP through the pyrimidine pathway. This point is under investigation.

Table 1. Influence of the *argR* mutation on the cumulative repression of E. coli carbamoyl phosphatase synthetase [a,b]

Growth medium	Wild type strain (P4X)			*argR* strain (P4XB2)		
	GCPSase	OTCase	ATCase	GCPSase	OTCase	ATCase
Minimal	1.04	80	9.3	1.93	1250	4.2
Minimal + arginine 100 μg/ml	0.52	3	5.5	1.71	1150	2.5
Minimal + uracil 50 μg/ml	0.38	300	0.7	1.65	1350	0.6
Minimal + arginine (100 μg/ml) and uracil (50 μg/ml)	0.05	2.5	1.1	1.10	1100	0.7

[a] The numbers represent specific activities expressed as μmoles of product formed per hour per mg of protein.
[b] The specific activities listed in this table have been established on the basis of several independent experiments.

2. Properties of the upp Mutations. A second class of mutants was characterized by a partial but significant decrease of the repressibility of both GCPSase and ATCase. Ten mutants representative of this class are listed in Table 2 and were submitted to a detailed analysis. P4XEM1 and P4XAZUR21 were selected as resistant to azauracil, P4XA3 and P4XA5 as resistant to arginine plus thiouracil and P4XCAZUR13, 21, and 25 as resistant to canavanine plus azauracil. P4X7531 and P4X8183 were originally obtained by serial transfers from the arginine-uracil bradytrophic mutant P4XMi178 but received a wild type GCPSase by transduction, before being submitted to the present analysis. The origin of the azauracil resistance of PA3305 is undetermined.

As appears in Table 2, these mutants, in addition to exhibiting negligible activity of UMP pyrophosphorylase, differ from the wild type strain in a number

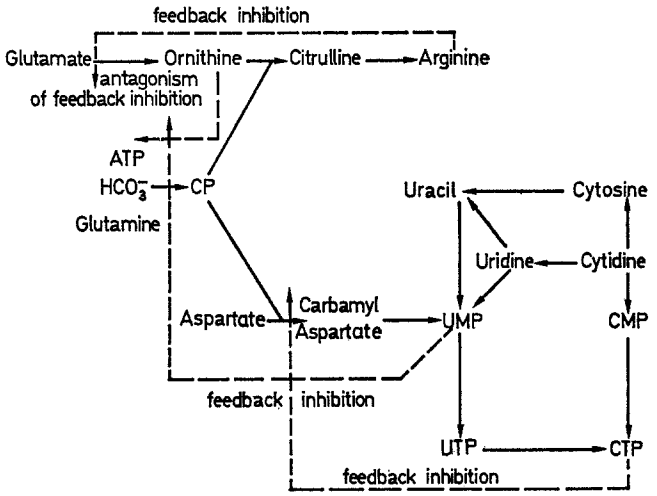

Fig. 1. Pathways of arginine biosynthesis, pyrimidine biosynthesis and pyrimidine interconversion in *Escherichia coli* and *Salmonella typhimurium*. The directions of the arrows correspond to the physiological roles which are currently accepted for the metabolic steps indicated (O'Donovan and Neuhard, 1970)

of other properties. The repressibility pattern of the pyrimidine pathway enzymes is considerably modified. The repression of GCPSase by uracil—and also by cytosine (data not shown) which enters the metabolism through cytosine deaminase and UMP pyrophosphorylase (see Fig. 1)—are markedly reduced. In all mutants, the level of ATCase is no longer repressed and, in most of them, is even significantly increased when the cells are grown in the presence of uracil. Another

Table 2. Properties

Strain	GCPSase activity[a]				ATCase activity[a]			
	min	min + arg	min + ura	min + arg + ura	min	min + arg	min + ura	min + arg + ura
P4X wild type	1.04	0.52	0.38	0.05	9.3	5.5	0.7	1.1
P4XEM1	1.02	0.53	0.77	0.43	11.5	9.3	23.9	28.5
PA3305[c]	—	0.60	—	0.45	—	5.5	—	3.8
PAXA3[c]	—	0.34	—	0.37	—	6.6	—	31.8
P4XA5[c]	—	0.31	—	0.28	—	—	—	—
P4XAZUR21	—	—	—	—	—	—	—	—
P4XCAZUR13	1.43	0.90	1.16	0.60	4.3	2.3	7.5	10.1
P4XCAZUR21	0.97	0.45	0.71	0.33	6.0	4.8	8.9	8.3
P4XCAZUR25	1.10	0.54	0.81	0.44	7.3	4.9	19.7	17.7
P4X7531	1.11	0.71	0.88	0.64	6.3	3.0	11.5	12.1
P4X8183	1.44	0.70	1.06	0.48	7.5	3.9	12.5	14.9

[a] Enzyme activities are expressed as μmoles of product formed per hour per mg of protein
[b] Initial velocities of uracil uptake are expressed as nmoles per min per mg of protein.

enzyme of the pyrimidine pathway, dihydroorotate dehydrogenase seems affected in a similar way even though the variations of this enzyme with respect to growth conditions are considerably less important than those of ATCase. In addition, all ten mutants are resistant to azauracil, are pyrimidine excretors (as shown by their ability to feed uracil-less mutants).

In addition, the ability to take up uracil was found considerably reduced in these mutants (Table 2). Uracil uptake in the wild type strain proceeds linearly for at least 20 min after addition of ^{14}C-uracil to the growth medium. When the cells are shocked with 10 ml of cold distilled water to release the small molecule pool, 25 to 35% of the radioactivity taken up is extracted; according to a rough estimation, this fraction contains free uracil (approximately 50 percent) and pyrimidine nucleotides. In presence of a *upp* mutation the total uracil uptake is reduced to less than 1% of the wild-type value (Table 2), thus implying that impairment of UMP pyrophosphorylase affects both accumulation and utilization of uracil.

A similar observation was made in yeast and was shown to result from feedback inhibition of uracil permease by its products which accumulate in the absence of UMP pyrophosphorylase (Grenson, 1969). Evidence for this mechanism was gained from the observation of enhanced uracil uptake after pyrimidine starvation of uracilless mutants or UMP pyrophosphorylase mutants.

Similar experiments were conducted with *E. coli* strains. After several hours of pyrimidine starvation, the initial velocity of uracil uptake in a pyrimidine-less strain was not increased with respect to the uptake in the wild-type strain. In addition, no detectable restoration of uracil uptake was observed after pyrimidine starvation of a *upp pyr* mutant. Consequently, in *E. coli*, the absence of uracil uptake does not appear to be an indirect consequence of the block in uracil utilization.

of the *upp* mutants

DHOdehase activity[a]				UMP pyrophosphorylase activity (min medium)	Uracil uptake[b]	Feeding of a pyrimidine requiring strain	Resistance to Azauracil
min	min + arg	min + ura	min + arg + ura				
0.17	0.17	0.08	0.10	45.0	11.1	−	−
0.18	0.17	0.19	0.20	0.1	0.1	+	+
—	0.13	—	0.13	<0.05	<0.1	±	+
—	0.18	—	—	0.05	<0.1	+	+
—	0.22	—	—	<0.05	<0.1	+	+
—	—	—	—	0.1	<0.1	+	+
0.18	—	—	—	<0.05	<0.1	+	+
0.16	—	—	—	<0.05	<0.1	+	+
0.17	0.15	0.20	0.21	<0.05	<0.1	+	+
0.16	0.16	0.16	0.22	0.5	<0.1	+	+
0.21	0.19	0.25	0.26	0.05	<0.1	+	+

except for the activity of *upp* which is expressed as nmoles per hour per mg of proteins.
[c] These strains are arginine auxotrophs and could not be grown on min or min + uracil media.

C. Mapping of the upp Mutations

Phage 363, performing generalized transduction, was grown on the *upp* mutants P4XEM1, P4XCAZUR25 and P4X7531. The lysates obtained were used to transduce a Upp$^+$ guanine auxotroph, strain RC82. Gua$^+$ recombinants were selected. The *gua* defect of RC82 has not been determined but mutations giving a Gua phenotype are known to occur in either of two adjacent genes located at min 48 on the chromosome map (Taylor, 1970). For P4XEM1, P4XCAZUR25 and P4X7531, the scores of Gua$^+$ recombinants having acquired the donor *upp* marker (identified by resistance towards azauracil) were 45 among 60 (75%), 26 among 29 (90%) and 11 among 14 (70%), respectively.

The *upp* mutation of PA3305 was found to be 44% cotransducible with a *purC* marker present in that strain (116 azauracil sensitive recombinants among 265 Pur$^+$ recombinants obtained with phage grown on a wild type *E. coli K12* strain).

The *upp* mutation of PA3305 must be very closely linked to the *upp* defect of P4XEM1 since all of 238 Pur$^+$ recombinants obtained by transducing PA3305 with phage grown on P4XEM1 were azauracil sensitive.

As *purC* and the *gua* operon are known to be 24 to 25% cotransducible (Nijkamp and de Haan, 1967), a probable order of the markers is *gua—upp*—(at least for P4XEM1)—*purC*.

The situation in *Salmonella typhimurium* appears very similar although *gua* and *upp* are only 20 to 30% cotransducible in that organism (Beck and Ingraham, 1971). The orientation of the *gua-purC* segment with respect to neighbouring markers is however not known in *E. coli*.

Discussion

This work was initiated with the aim of selecting regulatory mutations affecting the synthesis of carbamoylphosphate synthetase. New mutations were not obtained but the observation was made that the *argR* mutation also affects the synthesis of GCPSase. This observation, which cannot easily be seen as an indirect metabolic consequence of these mutations, opens the route to further studies of the mechanism of cumulative repression of this enzyme. Indeed, the fact that the *argR* mutation reduces the repressibility of GCPSase by both arginine and uracil could be in favour of the assumption that repression is exerted by a complex repressor molecule built up by the interaction of product of the *argR* gene with another gene product related to the pyrimidine pathway. In this connection, it will be interesting to select mutants of *E. coli* similar to the recently isolated *pyrR* mutants of *Salmonella typhimurium* (O'Donovan and Gerhart, 1972) and determine wether these mutations influence the synthesis of GCPSase.

The mechanism by which the *upp* mutation affects the repression of the pyrimidine pathway enzymes is of a completely different nature. Uracil, although it is not an intermediate of the endogeneous pathway of pyrimidine synthesis (Fig. 1), is readily utilized by *E. coli*. UMP pyrophosphorylase, present in a wild-type strain, is able to catalyze the synthesis of UMP from uracil and PRPP. *E. coli* mutants resistant to 5-fluorouracil and 6-azauracil lack this enzyme (Brockman *et al.*, 1960; Ahmad and Pritchard, 1969; Pritchard and Ahmad, 1971).

Similar mutants assumed to lack UMP pyrophosphorylase have been obtained in *Salmonella typhimurium* by Beck and Ingraham (1970) and were assigned the symbol *upp*. The corresponding mutations map in the vicinity of the *guaAB* operon.

Quite independently from these observations, we reported some years ago (Mayné *et al.*, 1963) that in azauracil resistant strains of *E. coli*, the uptake of uracil as well as its incorporation into nucleic acids is severely reduced. These mutations which also confer resistance to 0.5 μg/ml of fluorouracil (Glansdorff, unpublished) had been mapped in the region where the *guaAB* genes have later been found. Along a chain of personal communications, our own conclusions regarding these mutations have been somewhat lost from sight so that they finally appeared in the literature under the designation *uraP* (Taylor and Trotter, 1967; Taylor, 1970; O'Donovan and Neuhard, 1970) implying the unwarranted assumption that reduced uptake of uracil was necessarily due to the loss of uracil specific "permease".

This communication establishes unambiguously that mutations at a locus which indeed should be called *upp* simultaneously bring about resistance to 6-azauracil, resistance to low concentration of 5-fluorouracil and loss of UMP pyrophosphorylase activity. In addition, these mutations invariably determine a significant excretion of pyrimidine with respect to the wild-type and reduced sensitivity of GCPSase and the other pyrimidine enzymes to repression by uracil. The mutation map very near the *guaAB* operon, just as the one assumed to affect the UMP pyrophosphorylase of *S. typhimurium*.

We also observed that the loss of a functional UMP pyrophosphorylase is accompanied by a considerable decrease of incorporation of radioactivity from uracil into the soluble pool and the non-extractible cellular material, thus repeating the observation which originally led to the confusion between *upp* and *uraP* mutations. In *Saccharomyces cerevisiae*, two classes of mutations, characterized by an apparent loss of uracil uptake have been obtained, mutations affecting directly a uracil specific permease and mutations affecting UMP pyrophosphorylase (Grenson, 1969). The loss of uracil uptake associated with the latter mutation results from feedback inhibition of the permease by its products. Such observations were not repeated in the case of *E. coli* for which no indication of a feedback control of its own uptake could be obtained. In addition, all attempts aimed at the selection of mutations affecting uracil uptake without impairing UMP pyrophosphorylase have failed so far.

Nevertheless, it has to be underlined that a significant increase of the level of ATCase is observed after growth of *upp* mutants in presence of uracil, thus implying that some uracil may enter the cell and interfere with the repression of this enzyme. This could be taken as an indication that uracil transport is not directly affected in *upp* mutants. However, such a conclusion, in order to account for all the observations, would probably require an additional assumption: for instance, that in all strains uracil is constantly excreted and reincorporated whereas in *upp*$^-$ mutants reincorporation is precluded thus resulting in an apparent increase of excretion.

A detailed study of the mechanism of uracil uptake is needed and should not neglect the possibility of a more direct involvement of UMP pyrophosphorylase

in this process. Hochstadt-Ozer and Stadtman (1971) have presented arguments in favour of the involvement of purine phosphoribosyltransferase in the uptake of adenine. On the base of osmotic shock experiments, they suggested that the latter enzyme as well as UMP pyrophosphorylase are located in the pericytoplasmic space of the cell.

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