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Regulation of Uptake of Purines, Pyrimidines and Amino Acids by *Candida utilis*

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Uptake of uracil by *Candida utilis* is increased by addition of leucine to a minimal medium in which organisms are growing. This response requires protein synthesis and has kinetics consistent with the induction of additional uracil transport by the amino acid or a derivative. Consequently, the contribution of exogenous radioactive uracil to the pyrimidine nucleotide pools increases so that RNA made after the amino acid is added is of greater specific radioactivity. Some other amino acids are as effective as leucine in increasing the incorporation of uracil into RNA. Growth with leucine present also increases to different extents the initial rates of uptake of adenine, cytosine, uridine, lysine, histidine, threonine, phenylalanine, aspartic acid and leucine itself. The action of leucine on lysine transport appears to involve induction. These effects are not restricted to leucine; growth with aspartic acid or phenylalanine in the medium gives similar results. Lysine, on the other hand, is without action on the uptake of leucine, aspartic acid, phenylalanine, threonine or uracil but decreases the initial rates of uptake of both histidine and lysine. We suggest that lysine represses its own transport. Similarly, there is a specific decrease in uracil uptake caused by growth with this pyrimidine. Thus in *C. utilis* there are complex interrelationships in the uptake of nitrogen-containing compounds.

The present work stems from an observation made by Hambleton (1968) during experiments in which the yeast *Candida utilis* was grown in a minimal medium containing radioactive uracil to label RNA. When leucine was also in the medium there was increased incorporation of radioactivity into RNA without alteration in the growth rate of the culture. In the present paper we confirm and considerably extend this finding.

Our major conclusion is that in *C. utilis* there is a pleiotypic control system for the transport of a number of different compounds in which regulation by induction and repression is involved.

Experimental

Growth of organisms

The yeasts used were *C. utilis* N.C.Y.C. 321 and a uracil-requiring mutant obtained from this after irradiation with u.v. light. Organisms were grown at 30°C in a shaking incubator in a medium, pH 6.7, containing (per litre): glucose, 10g; KH_2PO_4, 2g; K_2HPO_4, 1.5g; NH_4Cl, 1g; $MgCl_2,6H_2O$, 500mg; K_2SO_4, 50mg; thiamin hydrochloride, 1mg; calcium pantothenate, 1mg; nicotinic acid, 1mg; pyridoxine, 1mg; folic acid, 50μg; biotin, 5μg; 20mg of uracil/l was added to the medium when required. L-Amino acids were also added, either singly at a final concn. of 50mg/l, or as an amino acid mixture (final concn. of each amino acid 50mg/l) made from leucine, valine, isoleucine, serine, threonine, phenylalanine, tryptophan, proline, aspartic acid, glutamic acid, lysine, histidine, arginine and glycine. When [^{32}P]phosphate was used to label nucleic acids or nucleotides, potassium phosphate salts in the medium were replaced by 140mg of KH_2PO_4/l and 3.9g of 2-(*N*-morpholino)ethanesulphonic acid (MES)/l; the pH was adjusted to 6.7 with 0.1M-KOH.

Cultures in the exponential growth phase were used as inocula for experiments. Extinctions of cultures were measured at 450nm with a Unicam SP.500 spectrophotometer and microcuvettes (capacity 0.4ml) of 1cm light-path. Where necessary extinctions were corrected for deviations from Beer's Law. Resuspension in fresh medium was by filtering up to 100ml of an exponentially growing culture through an Oxoid membrane filter (6cm diam.). Filtered cells were washed with 250ml of medium at 30°C and resuspended in the original volume of fresh medium also at 30°C. Exponential growth then resumed without a lag.

Chemical determination of RNA and protein

Samples (20ml) of culture were cooled rapidly to about 3°C and then added to 2ml of 50% (w/v) trichloroacetic acid. After 30min a mixture was centrifuged at 3°C; the pellet was resuspended at 0°C in

2ml of 5% (w/v) trichloroacetic acid and re-centrifuged. Precipitated material was extracted at 60°C for 30min with 2ml of 5% (w/v) trichloroacetic acid and the supernatant collected after centrifuging. The extraction at 60°C was repeated twice and the combined supernatants were used for the determination of RNA both by an orcinol method (Schneider, 1957) and by measurement of the E_{260} of the supernatants (De Deken-Grenson & De Deken, 1959). Material remaining after the extractions was suspended in 2ml of 0.1M-NaOH and heated at 100°C for 10min. After being centrifuged, protein in the supernatant was determined by the Folin method (Lowry *et al.*, 1951) with bovine serum albumin as standard.

Radiochemical methods

The total incorporation of purines, pyrimidines and L-amino acids by whole cells was measured essentially as described by Grenson *et al.* (1966). A portion (10ml) of an exponentially growing culture was added to radioactive solution (0.2ml) in a 50ml flask in a shaking water bath at 30°C. Samples (2.0ml) removed with a spring-loaded syringe were immediately filtered under vacuum on Whatman GF/C glass-fibre circles (2.1cm diam.) in a chilled stainless-steel filter tower and washed ten times with 10ml of water at 0°C. Less than 5s was required to remove and filter a sample; washing of the filtered material took under 30s.

The incorporation of radioactivity into material insoluble at 0°C in 5% (w/v) trichloroacetic acid was used as a measure of synthesis of RNA. To cultures were added 20μg of uracil/ml and 1.0μCi of [5-^{3}H]-uracil/ml. Protein synthesis was often measured in the same cultures by the addition of about 0.1μCi of [^{35}S]sulphate/ml. For the determination of radioactivities, samples (1.0ml) were added to 1ml of 10% (w/v) trichloroacetic acid at 0°C. After standing for at least 15min, precipitates were collected by filtration on Whatman GF/B glass-fibre circles, washed four times at 0°C with 10ml of 5% (w/v) trichloroacetic acid and then with 10ml of diethyl ether–ethanol (1:1, v/v).

Filters in glass sample tubes were dried at 80°C for 1h in a vacuum oven. Scintillator solution (4ml; 5g of butyl-PBD[5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole]/litre of toluene) was added and radioactivity measured in a Beckman liquid-scintillation counter.

Extraction of RNA

Organisms were grown to E_{450} 0.4 in medium buffered with MES and containing 100nCi of [^{32}P]-phosphate/ml, 20μg of uracil/ml and 500nCi of [5-^{3}H]uracil/ml. Samples (100ml) were rapidly cooled to 0°C; cells were collected by centrifuging in the cold, resuspended at 0°C in 4ml of buffer (10mM-Tris–HCl–0.1mM-magnesium acetate, pH7.4) and broken by using an Eaton (1962) press operated at about −20°C. The suspension was centrifuged at 0°C for 10min at 24000$g_{av.}$. Sodium dodecyl sulphate (2mg/ml) was added to the supernatant which was shaken for 10min at room temperature with 1vol. of phenol saturated with buffer (15mM-potassium phosphate–0.1mM-magnesium acetate, pH7.4). The mixture was centrifuged at low speed, the aqueous phase recovered and the extraction with phenol repeated twice. RNA was precipitated from the aqueous layer by the addition of 2vol. of ethanol in the cold, collected by centrifuging and dissolved in 10mM-acetate buffer, pH4.6, containing 100mM-NaCl. The ethanol precipitation was repeated twice and the RNA then dissolved in 2ml of acetate buffer.

Nucleotide pools

Organisms were grown for several generations to E_{450} 0.4 in medium buffered with MES and containing about 2.5μCi of [^{32}P]phosphate/ml, 20μg of uracil/ml and 10μCi of [5-^{3}H]uracil/ml. Samples (10ml) were rapidly filtered through Whatman GF/C glass-fibre circles (2.1cm diam.). A filter was washed with 20ml of water at 0°C and then immersed at 0°C in 4ml of 5% (w/v) trichloroacetic acid containing 25μg of each of twelve nucleotides (AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, GTP, UMP, UDP, UTP). Samples were frozen and thawed once to aid liberation of nucleotides, then centrifuged at 24000*g* for 10min at 3°C and the pellets discarded. Trichloroacetic acid was removed by five extractions each with 5ml of diethyl ether. The remaining ether was blown off with air. A portion (2.5ml) of each sample was freeze-dried and solids were redissolved in 100μl of water.

Components in 10μl portions of this nucleotide pool were separated by t.l.c. in two dimensions by using polyethyleneimine-impregnated cellulose layers (PEI-cellulose F; E. Merck A.-G., Darmstadt, Germany), as described by Randerath & Randerath (1964); the markers (made visible under a short-wavelength u.v. lamp) were identified by their relative positions. Areas of a layer were scraped into glass sample tubes, scintillant (4ml) was added and radioactivity measured.

High-voltage electrophoresis

Electrophoresis of soluble cell contents was carried out as follows. Three cultures were grown for about three generations to E_{450} 0.40 in media containing (i) no additions, (ii) 50μg of lysine/ml and (iii) 50μg of leucine/ml. Cultures were filtered, washed and resuspended in the same volume of medium with-

out additions. Portions (3.0ml) were added to [^{14}C]-lysine (final concn. 0.1mM; 0.28μCi/ml). After 2.5min a sample (2.0ml) was filtered rapidly through a Whatman GF/C glass-fibre circle and washed ten times with 10ml of water at 0°C. The filter was immersed in 1.5ml of ice-cold 5% (w/v) trichloroacetic acid, containing 5nmol of lysine/ml, and washed twice with 1ml of 5% trichloroacetic acid. The trichloroacetic acid was removed by ether extraction, the combined extracts were freeze-dried and solids were dissolved in 200μl of water. Samples (20μl) were subjected to high-voltage electrophoresis (3kV; 1h) on Whatman no. 1 paper with the buffer system of Ambler (1963). An amino acid mixture was run with

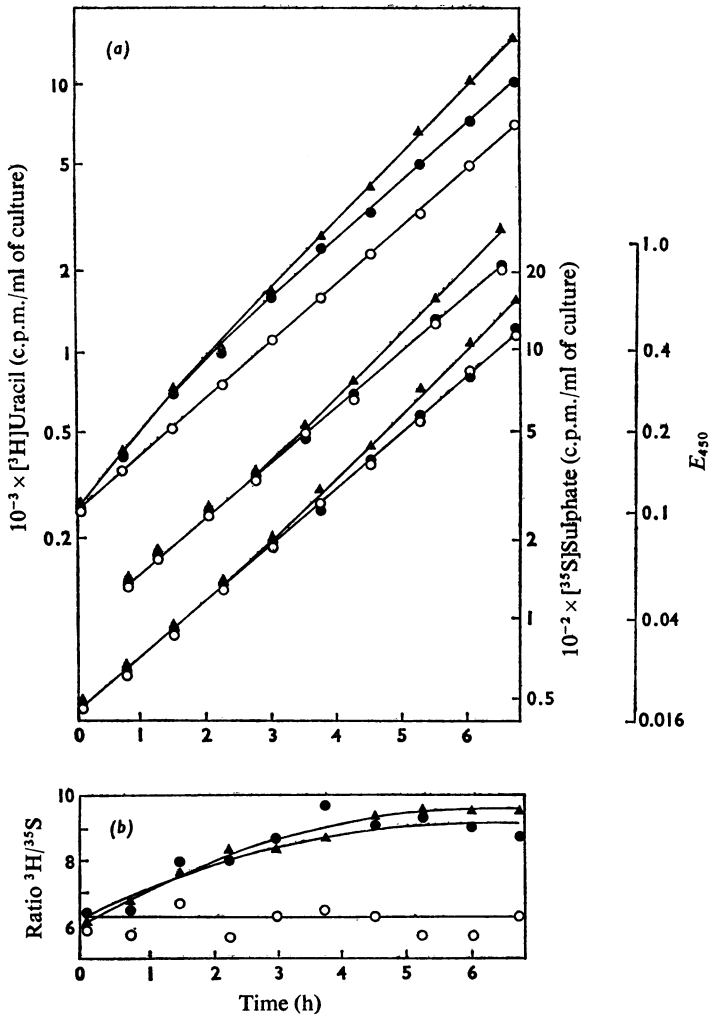

Fig. 1. *Growth of C. utilis with leucine or an amino acid mixture*

The yeast was grown in medium (200ml) containing [^{3}H]uracil (20μg/ml; 1.0μCi/ml) and [^{35}S]sulphate (0.1μCi/ml) from E_{450} about 0.005. At E_{450} about 0.04, portions (20ml) were transferred to flasks containing (i) a mixture of 14 amino acids (see the Experimental section) (▲), (ii) leucine (final concn. 50μg/ml) (●), or (iii) water (○). (*a*) shows the incorporation into material insoluble at 0°C in 5% trichloroacetic acid of ^{3}H radioactivity (upper set of curves) and ^{35}S radioactivity (lower set of curves) during further growth, which is plotted in the middle set of curves. In (*b*) is shown the ^{3}H/^{35}S radioactivity ratio of the samples from the three cultures.

each sample and amino acids were detected with the ninhydrin–cadmium reagent of Heilmann *et al.* (1957). Areas of strips containing radioactive lysine were cut out and radioactivity was assayed.

Chemicals

Radioactive chemicals from The Radiochemical Centre, Amersham, Bucks., U.K. were [2-^{14}C]uracil, [5-^{3}H]uracil, [2,8-^{3}H]adenine, [2-^{14}C]cytosine sulphate, [8-^{14}C]guanine, [5-^{3}H]uridine, L-[1-^{14}C]-leucine, L-[U-^{14}C]lysine, L-[4,5-^{3}H]lysine, L-[2,5-^{3}H]-histidine, L-[U-^{14}C]threonine and L-[G-^{3}H]aspartic acid. Butyl-PBD was from CIBA (A.R.L.) Ltd., Duxford, Cambs., U.K.

Results

Effects of amino acids on the incorporation of uracil into RNA

Medium containing [^{35}S]sulphate and [^{3}H]uracil was inoculated with *C. utilis* to give E_{450} about 0.005. After three generations of growth of the culture, portions were transferred to flasks containing either leucine or a mixture of 14 amino acids or (the control) an equivalent volume of water. The control culture grew exponentially with a mean generation time of 80min; the semilogarithmic plots of radioactivity incorporated into both RNA and protein were also linear, with the same slope as that of the growth curve (Fig. 1*a*); the ratio of radioactivity incorporated into RNA/radioactivity incorporated into protein was therefore constant (Fig. 1*b*). Within 45min of the addition of the amino acid mixture an increased rate of uracil incorporation into RNA was detectable; after about 3h there were increases in the rates of growth and [^{35}S]sulphate incorporation into protein. The addition of leucine also increased the radioactivity incorporated into RNA, but there was no subsequent increase in protein synthesis or growth rate; instead, after about three generations the culture entered a new steady state in which the ratio of [^{3}H]uracil/[^{35}S]sulphate incorporated into acid-precipitable material was about 45% greater than the control (Fig. 1*b*). Thus leucine altered uracil incorporation into RNA in a way similar to the amino acid mixture, but without the concomitant 'shift-up' observed with the latter.

In experiments similar to that of Fig. 1 other amino acids added singly were shown to vary in their effects on uracil incorporation, although in no case did addition alter the growth rate or incorporation of [^{35}S]sulphate. (Protein synthesis was not measured when methionine was used.) Four amino acids (leucine, methionine, asparagine and aspartic acid) gave 30–50% increases in uracil incorporation after growth for three generations; six (phenylalanine, tyrosine, serine, alanine, valine and isoleucine) gave increases of 13–23%, whereas eight (tryptophan, histidine, lysine, arginine, glycine, proline, threonine and glutamic acid) had no significant effect.

The increased incorporation of uracil caused by growth of *C. utilis* with leucine could represent the synthesis of additional stable RNA. However, a number of experiments suggested that this is not so. A culture of *C. utilis* was grown for three generations to E_{450} 0.05 in the presence of both [2-^{14}C]uracil and [2,8-^{3}H]adenine; the culture was then divided and leucine added to one portion. During further growth with leucine, the incorporation of uracil into trichloroacetic acid-precipitable material rose to a value 33% higher than the control, but there was only a 15% increase in the incorporation of labelled adenine. Moreover, another experiment showed that guanine incorporation was unaffected by growth with this amino acid. In addition, chemical analysis of organisms showed that after growth with leucine cells have a RNA/protein ratio that is not significantly different from that of a control culture.

Contribution of exogenous uracil to the pyrimidine bases of RNA

Another explanation for the effect of leucine (and other amino acids) on the incorporation of uracil into RNA is that growth with leucine might increase the ability of *C. utilis* to incorporate exogenous uracil into RNA. In a uracil-requiring mutant of *C. utilis* pyrimidine bases in RNA must be made from exogenous uracil; a measure of this is obtained by growing the mutant in medium containing [^{3}H]uracil and [^{32}P]phosphate, then isolating RNA and determining the ratio of the two radioactivities. In the wild-type grown in the same batch of medium the ratio of ^{3}H radioactivity/^{32}P radioactivity in RNA will be less because external [^{3}H]uracil competes with endogenous nucleotides for incorporation into RNA. When the wild-type is grown with leucine, a greater incorporation of exogenous uracil would give an increased ^{3}H/^{32}P radioactivity ratio in RNA; in the mutant, there should be no such increase.

Table 1 shows that these expectations are realised. Growth of the mutant with leucine has no effect on the ratio of radioactivities. The ratio for the wild-type grown without leucine is 38% of that for the mutant so that this percentage of the pyrimidines in RNA comes from uracil in the medium. Growth with leucine increases the contribution of exogenous uracil to 57%; this agrees well with the increased incorporation of [^{3}H]uracil into trichloroacetic acid-precipitable material.

A further feature is the effect of leucine on the growth rate of the uracil-requiring mutant. Like pyrimidine-requiring mutants of *Neurospora crassa* (O'Donovan & Neuhard, 1970), this mutant grew

Table 1. *Contribution of uracil in the medium to the pyrimidine bases of RNA*

Medium buffered with MES (1 litre) and containing uracil (20μg/ml), [^{3}H]uracil (0.5μCi/ml) and [^{32}P]phosphate (0.1μCi/ml) was divided. One portion was inoculated with *C. utilis* and the other with its uracil-requiring mutant. After about three generations of growth, the cultures (E_{450} 0.05) were divided and 50μg of leucine/ml was added to one portion of each. Three generations later (E_{450} 0.4) samples (1ml) were taken for the determination of ^{3}H radioactivity insoluble at 0°C in 5% trichloroacetic acid; duplicate samples (100ml) were also taken for the extraction of RNA and measurement of its radioactivity. The average ratio (13.1) of ^{3}H/^{32}P radioactivity in the RNA from the uracil-requiring mutant is assumed to represent complete synthesis of the pyrimidine bases in RNA from exogenous uracil.

Organism	Addition to medium	Mean generation time (min)	^{3}H radioactivity in whole cells (c.p.m./ml)	RNA				Exogenous uracil in pyrimidine bases of RNA (%)
				Sample no.	Radioactivity (c.p.m./ml) ^{3}H	^{32}P	^{3}H/^{32}P ratio	
Wild-type	None	80	5050	1	14303	2894	5.0	38
				2	19828	4060	4.9	
	Leucine	80	7720	1	29820	4175	7.2	57
				2	21267	2746	7.7	
Uracil-requiring mutant	None	300	6370	1	32048	2475	13.0	100
				2	28781	2170	13.2	
	Leucine	210*	8570	1	30716	2350	13.0	102
				2	39705	2874	13.8	

* Measurement after more than one generation of growth with leucine present.

slowly with a generation time of 5h. About one generation after the addition of leucine the rate of growth increased by about 30%. This increase probably causes the increased incorporation of uracil into trichloroacetic acid-precipitable material. Although it is not known whether this mutant is typical of uracil-requiring mutants of *C. utilis*, this behaviour would be expected if, as in the wild-type, the ability to take up uracil from the medium were restricted and was increased after growth with leucine.

In a further experiment, *C. utilis* was grown for three generations to E_{450} 0.05 in medium containing [^{3}H]uracil and [^{32}P]phosphate. The culture was then divided and 50μg of leucine/ml added to one portion. At E_{450} 0.4, samples were taken for the extraction of nucleotide pools, which were chromatographed and the ^{3}H/^{32}P radioactivity ratios in UTP and CTP measured. Growth with leucine caused increases of 45 and 30% in the ratios in UTP and CTP respectively. Similarly, growth with methionine, which also stimulates the incorporation of uracil into RNA, caused 50% increases in the ratio of radioactivities in both UTP and CTP; conversely, histidine, an amino acid without effect on uracil incorporation into RNA, produced no significant change in ratio in either nucleotide.

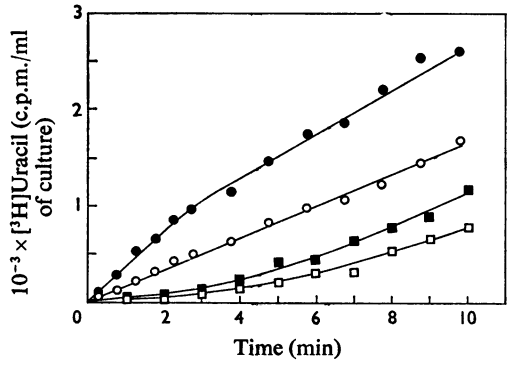

Fig. 2. *Total uptake of uracil and its incorporation into RNA by cultures grown with and without leucine*

Two portions of medium (50ml), one containing 50μg of leucine/ml, were inoculated with *C. utilis*. After about three generations of growth of culture and at E_{450} 0.40, [^{3}H]uracil (20μg/ml; 1.0μCi/ml) was added to each culture. Samples (2.0ml) were either (a) filtered to measure total radioactivity taken up or (b) added to 2ml of 10% (w/v) trichloroacetic acid at 0°C to measure radioactivity in RNA. ●, Total uptake after growth with leucine; ■, incorporation into RNA after growth with leucine; ○, total uptake by control; □, incorporation into RNA by control.

Effect of leucine on the uptake of uracil and other compounds

To show directly an effect of leucine on uracil uptake, two cultures of *C. utilis* were grown without uracil and in the presence and in the absence of the amino acid for three generations. [^{3}H]Uracil was then added. Both the total radioactivity taken up and radioactivity incorporated into trichloroacetic acid-precipitable material were measured at intervals for 10min. Fig. 2 shows that the rate of incorporation into acid-precipitable material increases gradually and is greater in the culture grown with leucine. Total uracil uptake shows very different kinetics in the two cultures. In the control without leucine uptake is linear. With leucine present uracil is at first taken up at about twice the rate of the control; however, this rate is not maintained and falls fairly abruptly, after about 2.5min, to a value about 35% greater than in the absence of leucine. This experiment directly confirms that cells grown with leucine are better able to take up uracil from the medium. The rate of uptake of uracil was measured over 2.5min

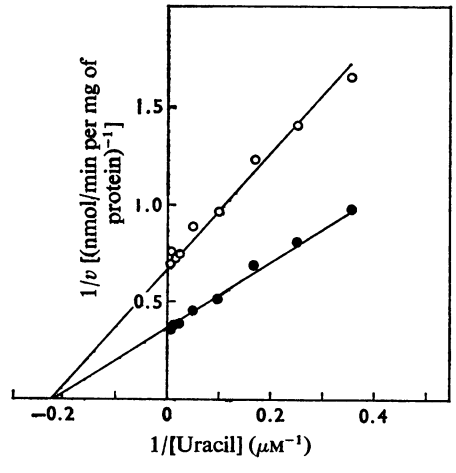

Fig. 3. *Uracil uptake at different uracil concentrations by cultures grown with or without leucine*

Portions (50ml) of medium without or with 50μg of leucine/ml were inoculated with *C. utilis*. After about three generations of growth of the culture and at E_{450} 0.40, 25ml of a culture was added to 1.0ml of [^{3}H]uracil (sp. radioactivity 6μCi/μmol) at a range of uracil concentrations. The total uptake of radioactivity by a sample was measured at intervals for 2.5min (cf. Fig. 2). ○, Samples grown without leucine, K_m = 4.5μM, maximum velocity = 1.45nmol/min per mg of protein; ●, samples grown with leucine, K_m = 4.5μM, maximum velocity = 2.2nmol/min per mg of protein.

at a number of different uracil concentrations by using cultures that had been grown with and without leucine. In both cases uptake shows simple Michaelis kinetics (Fig. 3); the Michaelis constants are the same and about 4.5μM. However, growth of organisms in the presence of leucine increases the maximum velocity of uracil uptake from 1.45 to 2.2nmol/min per mg of protein. Under these conditions therefore leucine appears to increase the number of sites available for transport without changing their affinity for uracil.

The effect of leucine on the uptake of adenine, cytosine or uridine is similar to that on uracil. In experiments similar to that described in Fig. 2, the rate of uptake by organisms grown without leucine was constant; growth with leucine approximately doubled the initial rates of uptake, but after about 2.5min rates declined to values that were still about 30% (uridine) or 45% (adenine and cytosine) greater than the controls.

Mechanisms of increased uracil-transport activity caused by growth with leucine

An exponentially growing culture was divided into two portions, to one of which leucine was added. At intervals, the ability of portions of the cultures to take up uracil was measured over 2.5min. Initial rates of uptake are plotted in Fig. 4(*a*) against the extinctions of cultures. The ability of the control to take up uracil increases linearly throughout; about 5min after the addition of leucine (an increase of E_{450} of about 0.02) a new constant differential rate is established which is about twice that of the control.

In another experiment two cultures, one containing leucine, were grown in parallel to E_{450} 0.20, when they were simultaneously filtered and washed; the cells were then resuspended in fresh medium without leucine. The differential plot (Fig. 4*b*) shows that at the time of filtration organisms grown with leucine are, as expected, better able to take up uracil, but after the removal of leucine the rate of increase of uracil-transport activity is the same in the two cultures.

These kinetics suggest that leucine induces a component of the uracil-transport system. This is also consistent with the measurements (Fig. 3) in which growth with leucine increased the maximum velocity of uptake but was without effect on the Michaelis constant. If induction is involved, then the stimulation of uracil uptake by leucine should depend on protein synthesis.

Three cultures of *C. utilis*, one (C) with leucine added, were grown in parallel to E_{450} 0.40. Cycloheximide (10μg/ml) was then added to each culture and leucine to one of those (B) grown without the amino acid. Rates of uptake of uracil were measured

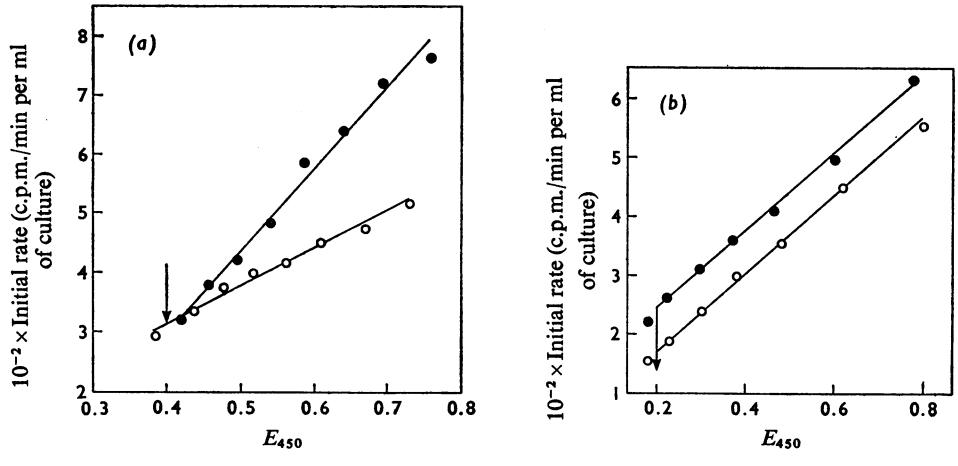

Fig. 4. *Initial rates of uracil uptake after leucine is added to, or removed from, a culture*

For (*a*), minimal medium (220ml) was inoculated with *C. utilis*. After three generations of growth to E_{450} 0.40, the culture was divided and 50μg of leucine/ml added to one portion (shown by the arrow). At intervals, samples (10ml) were removed from the cultures, added to flasks containing [^{3}H]uracil (20μg/ml; 1μCi/ml) and the initial rates of uptake of uracil obtained from measurements on total incorporation made over 2.5min as in Fig. 2. ●, Growth with leucine; ○, no leucine added. For (*b*), two portions (100ml) of medium, one containing 50μg of leucine/ml, were inoculated with *C. utilis*. After three generations of growth to E_{450} 0.20, the organisms were filtered (shown by the arrow), washed and resuspended in the original volume of medium without leucine. Initial rates of uptake of uracil by samples from these cultures were measured as in (*a*). ●, Growth (to E_{450} 0.2) with leucine; ○, growth without leucine.

10min before and 45min after addition of antibiotic. After exposure to cycloheximide each culture took up uracil at the same rate as before the antibiotic was added. Cycloheximide therefore prevents increased uracil uptake during exposure of culture (B) to leucine. This is not due to inhibition of uracil uptake, because the ability to take up uracil is unchanged after the addition of cycloheximide to cultures grown both with (C) and without (A) leucine. Although the concentration of cycloheximide used inhibits protein synthesis in this organism by about 95%, a separate experiment showed that after inhibition for 45min the incorporation of leucine into acid-soluble material (measured as the difference between the total radioactivity taken up by the cells and the incorporation into acid-precipitable radioactivity) was about 60% of that of a culture without added antibiotic. It is therefore unlikely that the failure of leucine to affect uracil uptake in cycloheximide-treated cultures is caused by inability of the amino acid to enter cells.

Effect of growth with uracil on uracil uptake

Uracil was added to an exponentially growing culture. At intervals, portions of culture were filtered, washed, resuspended in uracil-free medium and the initial rate of uptake of uracil was measured 5min after resuspension. The addition of uracil prevents increase in activity of the uracil-uptake system for about one-half of a generation (Fig. 5*a*); after this, the activity rises, but at about one-half the rate before uracil was added. This could represent an initially severe repression of synthesis, which is then released during further growth. It is noteworthy that uracil causes no decrease in the rate of uptake such as is found when there is transinhibition by the compound transported (Crabeel & Grenson, 1970).

In a second experiment, two cultures, one with added uracil, were grown for three generations. Both were filtered, washed and resuspended in medium without uracil; the ability of organisms to take up uracil was then measured at intervals. The differential plot (Fig. 5*b*) shows that shortly after resuspension the culture grown with uracil has about 55% of the uptake activity of the control. This confirms that growth with uracil decreases the ability to use uracil. As growth continues in the absence of uracil, the ability of both cultures to take uracil up increases linearly. Surprisingly, the rate of synthesis of uracil-transport activity in the culture originally grown with uracil is now somewhat greater than the control. Thus although this linear increase is consistent with de-repression of the transport activity by removal of uracil, other factors may complicate the situation.

Another experiment differed from the last only in that leucine was present in the two cultures both before and after resuspension. Again, growth with uracil decreased by 45% the ability of the cells to take up uracil, and the rate of synthesis of the transport system after uracil was removed was constant but about 25% greater than in the control.

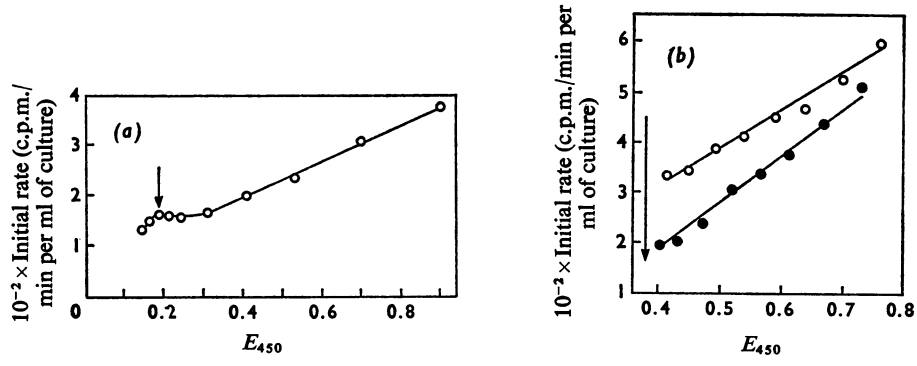

Fig. 5. *Effect of growth with uracil on initial rates of uracil uptake*

For (*a*), a culture (200ml) of *C. utilis* in minimal medium was grown for three generations to E_{450} 0.2 and then 20μg of uracil/ml was added (as shown by the arrow). At times before and after the addition of uracil, portions (20ml) of the culture were filtered, the organisms washed and resuspended in fresh medium without uracil. At 5min after filtration the uptake of [^{3}H]uracil (20μg/ml; 1μCi/ml) by a portion (10ml) of the resuspended culture was measured over 2.5min as in Fig. 2. For (*b*), two portions (100ml) of minimal medium, one containing 20μg of uracil/ml, were inoculated with *C. utilis*. After three generations of growth to E_{450} 0.38, both cultures were filtered (as shown by the arrow), washed and resuspended in the same volume of medium without uracil. Portions (10ml) of these cultures were taken at intervals to measure the initial rate of uptake of uracil as in (*a*). ●, Previous growth with uracil; ○, previous growth without uracil.

Uracil and leucine are thus not mutually exclusive in their effects and so might regulate the same step in uracil uptake. This conclusion was strengthened by measurement of initial rates of uracil uptake at different concentrations of uracil by using cultures that had been grown for three generations with and without the pyrimidine. The maximum velocity of uptake by the culture grown with uracil was one-half that of the culture grown without it, whereas the Michaelis constants were the same.

Leucine increases the uptake not only of uracil, but also of cytosine and adenine; growth with uracil decreases the ability to take up uracil. However, cytosine uptake was unaffected by previous growth with uracil, adenine or cytosine itself. Thus cytosine uptake, although increased by amino acids, is not decreased by growth with either its substrate or uracil. This suggests that in *C. utilis*, as in *Saccharomyces cerevisiae* (Grenson, 1969), these two pyrimidines are taken up separately.

Effects of leucine and lysine on lysine uptake

Because growth with an amino acid regulates unrelated transport systems, amino acids might also affect their own transport or that of other amino acids.

Three cultures were grown in either minimal medium or the same supplemented with either leucine or lysine. At E_{450} 0.35 the cells were filtered, washed and resuspended in medium without amino acids. The uptake of radioactive lysine was measured 10min after resuspension. Growth with leucine doubles the initial rate of lysine uptake (Fig. 6); conversely, growth with lysine halves the rate.

An examination of the radioactive material taken up by cells confirmed that lysine transport is being measured in these experiments. Three cultures were grown in parallel to E_{450} 0.40 without additions or with leucine or lysine present. After filtration and washing of the cultures a portion of each was incubated for 2.5min with [^{14}C]lysine. The cells were collected and their acid-soluble contents subjected to high-voltage electrophoresis. In the control culture 89% of the radioactivity applied was recovered as material with the same mobility as lysine markers; values for the leucine-grown and lysine-grown cultures were 85 and 90% respectively.

Measurements were made of initial rates of uptake of different concentrations of lysine by cultures that had been grown without additions or with either leucine or lysine present. Uptake showed simple Michaelis kinetics. In two experiments cultures grown without additions gave Michaelis constants of 7.8 and 8.3 μM; maximum velocities of uptake were 11 and 12.5nmol/min per mg of protein. Growth with leucine did not alter the Michaelis constant for lysine uptake, but increased the maximum velocity to 27nmol/min per mg of protein; growth with lysine decreased the maximum velocity to 4.9nmol/min per mg of protein without affecting the Michaelis constant. This result suggests that leucine may induce, and lysine repress, a lysine-transport system.

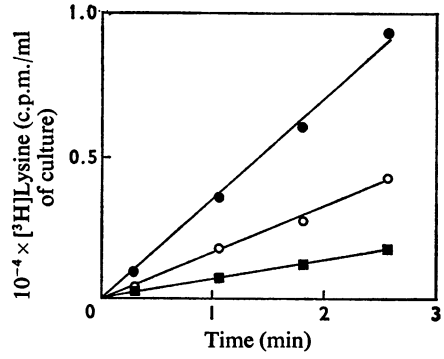

Fig. 6. *Effect of growth with leucine or lysine on lysine uptake*

Three portions (50ml) of medium, one containing 50μg of leucine/ml and another 50μg of lysine/ml, were inoculated with *C. utilis*. The cultures were grown for three generations to E_{450} 0.35, then filtered, washed and resuspended in the same volume of medium without additions. After 10min the uptake of [^{3}H]lysine (14.6μg/ml; 0.5μCi/ml) by 10ml of a resuspended culture was measured. ●, Cells previously grown with leucine; ■, cells previously grown with lysine; ○, cells grown without additions.

Further experiments supported this suggestion. An exponentially growing culture was divided and leucine added to one portion. Lysine uptake was measured in samples of both cultures during continued growth. Fig. 7 shows that leucine more than doubles the differential rate of synthesis of the lysine-transport system; this effect occurs about 5min (an increase in E_{450} of 0.02) after leucine is added.

Removal of lysine from a culture grown with it gave kinetics consistent with the release of repression. Two cultures grown in parallel, one with lysine, were filtered, washed and resuspended in lysine-free medium. Immediately after the removal of lysine the culture grown with the amino acid had about one-half the ability of the control to take up this amino acid. During subsequent growth the differential rate of synthesis of the lysine-transport system was constant and the same in both cultures.

Further studies on the uptake of amino acids and uracil

Table 2 surveys the effects of growth of *C. utilis* with amino acids or uracil on their own and on each other's uptake. The amino acids were chosen partly on the basis of the experiments above and partly because

in *S. cerevisiae* strain Σ1278b, lysine (Grenson, 1966), dicarboxylic acids including aspartic acid (Joiris & Grenson, 1969), leucine and threonine (Gits & Grenson, 1969) as well as histidine (Crabeel & Grenson, 1970) are taken up by separate permeases. Cultures of *C. utilis* were grown for three generations with an amino acid or uracil in the medium, filtered, the cells washed and resuspended in fresh medium without additions. Initial rates of uptake of radioactive amino acids or uracil were measured 10min after resuspension.

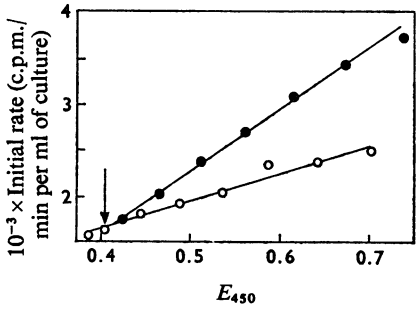

Fig. 7. *Lysine uptake after addition of leucine*

A culture (200ml) of *C. utilis* in minimal medium was grown to E_{450} 0.41 and then divided into two portions, to one of which was added 50μg of leucine/ml (shown by the arrow). At intervals portions (10ml) of the cultures were removed and initial rates of uptake of [^{3}H]lysine (14.6μg/ml; 1μCi/ml) measured over 2.5min as in Fig. 6. ●, Initial rate of uptake in the culture with added leucine; ○, control with no leucine added.

With some exceptions, relationships expected from the more-detailed studies of growth with leucine, lysine and uracil can be extended. Thus growth with leucine, aspartic acid or phenylalanine, in decreasing order of effectiveness, increases uracil uptake. Moreover, these amino acids also increase all uptake activities measured, usually in the same order of effectiveness so that, for example, leucine increases aspartic acid uptake more than aspartic acid itself.

Uracil decreases its own uptake but not that of the three amino acids tested. Similarly, growth with lysine decreases the ability of organisms to take up lysine and histidine but is without effect on the other four amino acids or uracil. Histidine decreases its own uptake and that of lysine and is without effect on uracil, aspartic acid and phenylalanine transport; however, growth with histidine does cause small and probably significant increases in the ability of *C. utilis* to take up leucine and threonine.

Discussion

In *C. utilis* the uptake of radioactive uracil is rate-limiting in its incorporation into RNA. In a 'step-up' an increase in the synthesis of stable RNA will be obscured. In other cases increased RNA synthesis may not be accompanied by increased incorporation of radioactivity. Radioactive pyrimidines and purines therefore cannot be reliably used to monitor changes in rates of RNA synthesis by this yeast. Moreover, growth with uracil decreases its own uptake. This transport system is therefore singularly ill-suited for replacement of synthesis of nucleotides *de novo* by using exogenous uracil.

The addition of a single amino acid can affect a wide range of transport activities in *C. utilis*. Those

Table 2. *Effect of growth with amino acids or uracil on the transport of these compounds*

Cultures (50ml) of *C. utilis* were grown for at least three generations in the presence of 50μg of an amino acid/ml or 20μg of uracil/ml. At E_{450} 0.35, a portion (25ml) of a culture was filtered, washed and resuspended in the same volume of fresh medium without additions. At 10min after resuspension uptake of a radioactive amino acid (0.1mM) or uracil (20μg/ml) was measured over 2.5min.

Addition to medium	Initial rate of uptake (nmol/min per mg of protein)						
	Leu	Asp	Phe	Thr	Lys	His	Uracil
None	0.36	0.10	0.11	0.83	9.80	8.75	1.75
Leu	3.04	0.88	0.46	11.0	20.0	12.4	3.25
Asp	3.00	0.43	0.36	14.2	14.2	11.0	2.6
Phe	2.48	0.33	0.31	11.5	12.5	10.9	2.1
Thr	0.82	—	0.14	2.76	—	8.20	1.78
Lys	0.34	0.10	0.10	0.83	4.10	6.10	1.78
His	0.61	0.10	0.10	1.14	6.40	2.50	1.8
Uracil	0.36	0.10	—	—	10.8	—	1.05

examples studied in detail suggest that induction and repression are involved in the maintenance of altered rates of uptake.

Although both uracil and lysine transport are increased by growth with leucine, it seems unlikely that the entry of such dissimilar compounds occurs by a common transport system. The specific decrease of uracil transport by uracil and lysine transport by lysine also argues strongly against this possibility. Moreover, it is probable that most of the amino acids in Table 2 are taken up separately by *C. utilis*. The very disparate increases in activity caused by growth with a particular amino acid argues against there being a single general transport system. In addition, we (R. W. Jones & D. G. Wild, unpublished work), have been unable to detect significant inhibition of the uptake of one of the amino acids in Table 2 by another of those listed. Thus the most economical explanation for the effects of amino acids that increase incorporation is that they do so by inducing separate transport systems. (The uptake of histidine and lysine might be exceptional in that growth with one of these amino acids decreases the ability to take up both. This situation merits further enquiry.)

Studies on the transport of amino acids, purines and pyrimidines by yeasts have mainly used strains of *S. cerevisiae*. Grenson and co-workers (Grenson, 1966; Joiris & Grenson, 1969; Gits & Grenson, 1969; Crabeel & Grenson, 1970) have shown that the uptake of amino acids by *S. cerevisiae* strain Σ1278b is mediated by specific permeases whose specificities sometimes overlap. In this strain, control of amino acid uptake can involve transinhibition. Thus histidine inhibits its own uptake (Crabeel & Grenson, 1970) and growth with glutamic acid inhibits uptake of aspartic acid and glutamic acid by the dicarboxylic acid permease (Joiris & Grenson, 1969). In a mutant strain, the ability to transport amino acids by both the specific and general permease is impaired (Grenson & Hennaut, 1971). The uptakes of uracil, cytosine and uridine were unaffected in this mutant; however, the implication is that the transport of amino acids, although mediated by a number of separate permeases, shares a common element or elements and so, under certain circumstances, might be regulated in parallel. Moreover, Gits & Grenson (1969) reported briefly that 'methylated' amino acids (methionine, leucine, valine, isoleucine, alanine and threonine) induce their own and each other's uptake. Because the transport of these amino acids involves specific permeases, these authors suggest that induction involves a component necessary for the functioning of them all.

The effects of single amino acids on transport processes in *C. utilis* therefore appear somewhat similar to events in at least one strain of *S. cerevisiae*. There are, however, differences. For example, Gits & Grenson (1969) note that, although growth with threonine induces the uptake of other methylated amino acids, it fails to induce its own uptake and so functions as a gratuitous inducer. More importantly, there are no previous reports of which we are aware of an effect of growth with an amino acid on the transport of other nitrogen-containing compounds into yeast.

Our working hypothesis is therefore that in *C. utilis* there is a pleiotypic control mechanism by which an amino acid can induce a component or components common to transport processes involving different classes of compound. This control mechanism may be allied to one in which there is sometimes more specific repression of uptake. The generality of this scheme needs to be established in much more detail. Because different amino acids appear to function similarly, it is unlikely that the amino acids themselves act directly as inducers; some molecules related to pathways of amino acid metabolism may be more intimately involved. Similarly, the hypothetical element common to a wide variety of transport processes remains to be identified.

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