Pyrimidine Utilization by Exponentially Growing and Starved Cells of *Rhodotorula gracilis*

Radioactive precursors are widely used in evaluation of the rate of synthesis and breakdown of nucleic acids and in autoradiographic techniques. The interpretation of the data thus obtained is complicated by the fact that the precursors are known to be interconverted into various nucleotides or to be degraded [1–5]. The present work studies the distribution of the radioactivity fed as ^{3}H and ^{14}C orotic acid and uridine into the nucleic acids and other macromolecular fractions in the yeast *Rhodotorula gracilis* under two different metabolic conditions: a) during exponential growth; b) under conditions of growth limited by the removal of nitrogen or carbon source.

Material and methods. The yeast *Rhodotorula gracilis*, strain Pan (ATCC 26217), was cultured into a glucose minimal mineral medium at 30 °C with eccentrical rotative agitation [6]. For label experiments were used exponentially growing cultures at a density of 3×10^{7} cells/ml or cultures under conditions of very limited growth, obtained by transferring for 1 h the exponentially growing cells into a fresh medium minus carbon source (glucose) or nitrogen source (NH_4^+). Radioactive orotic acid or uridine were fed at the concentration of 5×10^{-4} *M* (5-^{3}H uridine and 5-^{3}H orotic acid 0.4 μCi/μmole, 6-^{14}C orotic acid 0.2 μCi/μmole). After 15, 30, 60 min of incubation, the cells were harvested by filtration and rapidly stored at −20 °C. Lipids were obtained from freeze-dried material at room temperature with chloroform-methanol (1:1) according to PEDERSEN [7] and were purified by diffusion according to FOLCK et al. [8]. Acid soluble compounds, RNA, DNA, acid hydrolizable polysaccharides and alkali soluble proteins were extracted and fractionated according to the method of GEBICKI and FREED [9] modified by COCUCCI [10]. Radioactivity was determined also into a residual fraction corresponding to non-acid-hydrolizable polysaccharides and alkali insoluble proteins. The ribonucleotides from RNA were purified according to GEBICKI and FREED [9] and were analyzed according to RANDERATH [11] on polyethyleneimine cellulose-precoated sheets (Polygram cel 300 PEI, Macherey Nagel and Co., Düren, Germany). Radioactivity was assayed in a Packard liquid scintillation counter.

Results and discussion. The Figure shows the distribution of radioactivity into the main cellular fractions either of exponentially growing cells or of cells under limited growth conditions during 1 h experiments with 6-^{14}C orotic acid or 5-^{3}H orotic acid or 5-^{3}H uridine. Orotic acid is always incorporated at a rate ca. 50% higher than uridine. In exponentially growing cells (Figure A), the radioactivity of the acid-soluble fraction results immediately equilibrate with orotic acid, whereas it increases linearly in time with uridine; the incorporation of the radioactivity in the macromolecular fractions is always a linear function of time; about 90% of the radioactivity is found in RNA, regardless of the labelled precursor used. When the precursors are fed to nitrogen source deprived cultures (Figure B), the amount of label in the acid-soluble material results at least larger than in the exponentially growing cells, suggesting an expansion of nucleotide pools in spite of the dimished rate of RNA synthesis [1]; under these conditions the radioactivity of the non-RNA-acid-insoluble fractions is consistently increased, corresponding respectively to 30% with 6-^{14}C orotic acid, to 90% with 5-^{3}H orotic acid and to 60% with 5-^{3}H uridine of the total macromolecular fraction. Figure C shows the results of the incorporation of radioactive precursors in carbon source deprived cultures. The acid-soluble fraction results immediately equilibrated with either orotic acid or uridine, probably as a consequence of the dimished rate of RNA synthesis. The acid-insoluble fraction accounts for only 20% of the total

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Table I. Percentual distribution of radioactivity incorporated into macromolecular fractions different from RNA from cells incubated for 1 h with labelled pyrimidine precursors

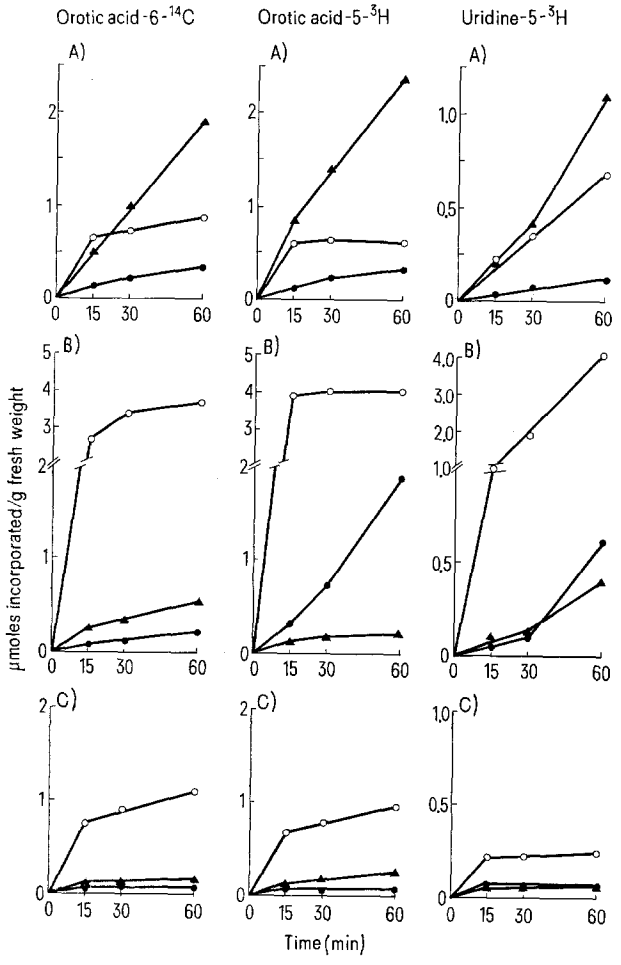
	Exponentially growing cells			Cells under limited growth conditions by depletion of nitrogen source			Cells under limited growth conditions by depletion of carbon source		
	6-^{14}C orotic acid	5-^{3}H orotic acid	5-^{3}H uridine	6-^{14}C orotic acid	5-^{3}H orotic acid	5-^{3}H uridine	6-^{14}C orotic acid	5-^{3}H orotic acid	5-^{3}H uridine
DNA	14.7 (50.8)	9.8 (33.6)	11.3 (13.8)	28.1 (64.9)	0.7 (13.8)	2.9 (18.3)	30.2 (21.6)	5.9 (9.5)	22.6 (14.5)
Lipid fraction	20.6 (70.9)	34.7 (118.8)	38.3 (46.7)	20.6 (47.6)	89.8 (1711.0)	71.5 (450.0)	16.6 (11.8)	46.7 (75.6)	31.7 (20.3)
Polysaccharides (hot acid hydrolyzable fraction)	54.6 (188.6)	44.7 (153.2)	42.3 (51.5)	43.9 (101.4)	4.2 (78.6)	9.7 (61.1)	47.2 (33.7)	33.9 (54.8)	37.6 (24.1)
Proteins (alkali soluble fraction)	0.23 (0.8)	0.06 (0.16)	0.9 (1.07)	0.3 (0.8)	0.6 (10.9)	0.58 (3.7)	1.2 (0.85)	1.2 (2.0)	2.7 (1.7)
Residual fraction	9.5 (32.8)	10.6 (36.4)	7.2 (8.86)	7.0 (16.1)	4.6 (88.6)	15.3 (96.4)	4.6 (3.25)	12.2 (19.8)	5.1 (3.3)
Total	(343.9)	(342.2)	(121.9)	(230.8)	(1902.9)	(629.5)	(71.25)	(161.7)	(63.9)

The data are the average of 3 experiments; the values in parenthesis are the nmoles incorporated/g fresh weight.

Table II. Percentual distribution of 6-^{14}C orotic acid radioactivity in the bases of RNA after 1 h poulse

	UMP	CMP	AMP	GMP
Exponentially growing cells	52.2	40.0	4.8	5.0
Cells under limited growth conditions by depletion of nitrogen source	76.2	10.0	10.3	4.0
Cells under limited growth conditions by depletion of carbon source	71.8	12.2	12.0	7.0

The data are the average of 2 experiments.



Radioactivity distribution as a function of time in the main cellular fractions of cells fed with pyrimidine precursors of RNA. A) Exponentially growing cells. B) Cells under limited growth conditions by depletion of nitrogen source. C) Cells under limited growth conditions by depletion of carbon source. ○—○, acid soluble fraction; ▲—▲, RNA; ●—●, other macromolecular fractions.

radioactivity assumed, showing that the precursors are utilized at a slower rate. In the experiments with 5-^{3}H orotic acid and 5-^{3}H uridine, 40% and 55% respectively of the total acid insoluble label is found in the fraction different from RNA; when 6-^{14}C orotic acid is used, only 28%. The distribution of the radioactivity incorporated in the macromolecular fractions different from RNA under the various experimental conditions is shown in Table I. In the experiments with 5-^{3}H uridine and 5-^{3}H orotic acid, most of the radioactivity (about 80%) of the non-RNA-insoluble fraction is equally distributed between lipids and polysaccharides, except for nitrogen source deprived cells in which more than 80% of the radioactivity of the non-RNA-insoluble fraction is found into the lipidic fraction. When 6-^{14}C orotic acid is used, the more heavily labelled fraction under all experimental conditions is that corresponding to polysaccharides (about 50% of the total radioactivity incorporated into the non-RNA-insoluble fraction).

The presence of ^{3}H label in the lipid and/or in the polysaccharides fractions is in agreement with the pattern of degradation of orotic acid and uridine through the β-alanine acetyl-CoA pathway[12]. In addition some of ^{3}H label can be lost to tritiated water in the conversion of pyrimidine precursors to TMP[13]. The distribution of the radioactivity of 6-^{14}C orotic acid can be explained by assuming that $^{14}CO_2$ deriving from its breakdown is incorporated through reductive carboxylation.

The data of Table II show the distribution of radioactivity of 6-^{14}C orotic acid in the RNA bases under all experimental conditions. The radioactivity is distributed to the same extent in uridylic acid and cytidylic acid in the RNA from exponentially growing cells, while the uridylic acid appears more heavily labelled in the RNA from cells under limited growth conditions. These findings suggest that the amount of labelled precursor incorporated in uridylic acid or cytidylic acid of the RNA depends on the nutritional condition. The data emphasize the strong limitations which are met for biochemical or autoradiographic studies in using pyrimidine precursors to RNA synthesis in an organism such as *Rhodotorula gracilis*, especially under nutritional conditions of limitation due to the deficiency of nitrogen or carbon source in the medium.

Riassunto. L'uso di precursori pirimidinici marcati in studi biochimici o autoradiografici della sintesi di RNA risulta nel lievito *Rhodotorula gracilis* fortemente limitato specie in condizioni di crescita rallentata per difetto di fonte di carbonio o di azoto.

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Transcription Products Synthesized by Purified Calf Thymus DNA-Dependent RNA Polymerase on Shope Papilloma Virus DNA

The recent purification of mammalian DNA dependent RNA polymerase has provided a tool for the study of mammalian DNA viruses[1]. The in vitro transcription of the small oncogenic DNA viruses can be the first step in the elucidation of the proteins coded by these viruses. We report here on the RNA products formed by the transcription of Shope papilloma virus (SPV) DNA using purified calf thymus RNA polymerases A and B.