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Cellular Content of Ribonucleic Acid and Protein in *Saccharomyces cerevisiae* as a Function of Exponential Growth Rate: Calculation of the Apparent Peptide Chain Elongation Rate

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The average cellular content of ribonucleic acid and protein was determined in cultures of *Saccharomyces cerevisiae* growing exponentially at different rates in a variety of media. Estimations of the proportion of total cellular ribonucleic acid that is made up of ribosomal ribonucleic acid were used to calculate the average number of ribosomes per cell at the different growth rates. The fraction of ribosomes actively engaged in translation was estimated by sucrose gradient centrifugation of ribosomes and polysomes. These data were used in a calculation of the apparent time taken for the addition of an amino acid to the growing polypeptide chain; this value was found to vary linearly with growth rate over a fivefold range of doubling times.

Simple measurements of the macromolecular content of bacterial cells in exponential growth have been ingeniously used to deduce some parameters of the biosynthesis of ribonucleic acid (RNA), deoxyribonucleic acid and protein (11). In principle a similar approach should be applicable to any organism capable of growth in single-cell culture. If in addition the organism can be cultured in different growth media supporting a variety of growth rates, one has the further opportunity of studying macromolecular metabolism as a function of balanced growth rate and during nutritional shifts. Measurements of protein and RNA content in yeast cultures growing in media supporting rapid growth have been reported (8, 16, 19), as have some of the aspects of macromolecular metabolism during nutritional shift (19), although thus far there has been no systematic study of protein and RNA content in yeast as a function of exponential growth rate. In this communication we report the cellular RNA and protein content in *Saccharomyces cerevisiae* growing exponentially over a range of rates and use these data to show that the apparent step time for amino acid addition to the growing polypeptide chain varies directly with growth rate.

MATERIALS AND METHODS

Strain and culture conditions. Cultures of *S. cerevisiae* strain A364A (obtained from Leland Hartwell) were grown at 30 C in baffled Erlenmeyer flasks in a New Brunswick gyrotary water bath (New Brunswick Scientific Co., New Brunswick, N.J.). Growth was measured by reading the absorbance (450 nm) in a Zeiss PMQII spectrophotometer. Cultures (500 ml) were grown exponentially in the following media, with doubling times indicated in parentheses: 0.5% yeast extract (YM-1) (Difco), 1% peptone (Difco), 0.67% yeast nitrogen base (YNB) (Difco), 1% succinic acid, 0.6% sodium hydroxide and 2% glucose, final pH 5.8 (reference 5) (96 min); YNB plus 1% Casamino Acids and 2% glucose (Difco) (120 min); YNB plus 2% glucose (137 min); YNB plus 2% mannose (170 min); YNB plus 2% melibiose (379 min); YNB plus 2% Casamino Acids (Difco) (389 min); YNB plus 2% sodium lactate (416 min); or YNB plus 2% sodium acetate (486 min). The growth rates were reproducible within ± 10%. All media except YM-1 also contained 50 μg of adenine, uracil, L-lysine, L-histidine, and L-tyrosine per ml.

Protein and RNA measurements. When the exponentially growing cultures reached an absorbancy at 450 nm = 0.50, they were poured over crushed ice and one-tenth volume of 50% ice-cold trichloroacetic acid was added. The cells were centrifuged for 10 min at 15,000 × g and the pellets were washed twice with 5% trichloroacetic acid. The cells were resuspended in 0.5 N NaOH and incubated at 37 C for 24 h. The RNA concentration in the NaOH extracts was determined by the orcinol reaction (2) using adenosine as a standard and correcting for the overall base composition of *S. cerevisiae* RNA (14). The protein concentration in the NaOH extracts was determined by the method of Lowry et al. (10) using bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, Mo.) as a standard. All measurements were done with

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triplicate samples taken from each of at least two independent cultures for each growth rate. Standard deviations of these measurements were approximately 5 to 7%. Cell concentrations were determined in separate samples of the same exponentially growing cultures used for RNA and protein measurements in a Petroff-Hauser counting chamber. All budding cells with a visible constriction between mother cell and bud were counted as double cells (19). This rather arbitrary definition of a cell could lead to a systematic error in estimations of cellular RNA and protein content, but will not affect calculations of the peptide chain growth rate, since in that calculation estimations of cell number do not enter (see below).

Determination of the distribution of RNA species in yeast. The distribution of RNA among ribosomal and soluble species was determined as follows. Cultures were grown exponentially for at least eight generations in the growth media described above. They were labeled during the entire growth period with [^{3}H]uracil (0.1 μCi/μg; 20 μg/ml; New England Nuclear Corp., Boston). Growth was stopped by the addition of NaN_3 to 10^{-2} M, $ZnSO_4$ to 10^{-3} M, and 200 μg of cycloheximide per ml. The cultures were poured over crushed ice and the cells were collected by centrifugation. The cells were resuspended in 2 ml of buffer containing 10^{-2} M tris(hydroxymethyl)-aminomethane, pH 7.4, 10^{-1} M NaCl, 10^{-3} M $ZnSO_4$, 10^{-2} M NaN_3, 10^{-3} M $MgCl_2$, 1% sodium dodecyl sulfate and were lysed in an Eaton press (3). After removal of lysed cell debris by centrifugation, the cell sap was sedimented through a 5 to 20% linear sucrose gradient containing 0.5% sodium dodecylsulfate in 10^{-1} M NaCl and 10^{-2} M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4. Centrifugation was for 5.5 h at 40,000 rpm at 20 C in an SB-283 rotor using an International model B-60 ultracentrifuge. After centrifugation, pin holes were punched in the bottom of the centrifuge tubes, and fractions were collected and precipitated with 5% ice-cold trichloroacetic acid. The precipitates were collected on membrane filters (Sartorius, Göttingen, W. Germany), washed with 5% ice-cold trichloroacetic acid, dried, and counted in a Beckman liquid scintillation spectrometer. The relative amount of 26*S*, 18*S*, and 4*S* RNA was obtained by estimating the area under each peak of the sedimentation profile.

Distribution of ribosomes in yeast. Twenty-five-milliliter cultures of *S. cerevisiae* strain A364A spheroplasts (formed as described by Hutchinson and Hartwell [7]) were grown for 3 h in (i) YM-1 medium, (ii) YNB medium plus Casamino Acids and glucose, or (iii) YNB medium plus glucose plus 50 μg of all growth requirements per ml and 10 × YNB medium. The culture growing in YM-1 medium was labeled for one doubling time with 5 μCi of [2-^{3}H]adenine (67 mCi/mg) per ml. The other cultures were labeled for one doubling time with [2-^{3}H]adenine (2 μCi/μg per ml). For the experiment reported in Fig. 1D, a culture of whole cells was also grown for several generations in YNB medium plus sodium acetate containing [2-^{3}H]adenine (2 μCi/μg; 10 μg/ml) and 50 μg of all other growth requirements per ml. These cells were converted to spheroplasts after growth in radioactive

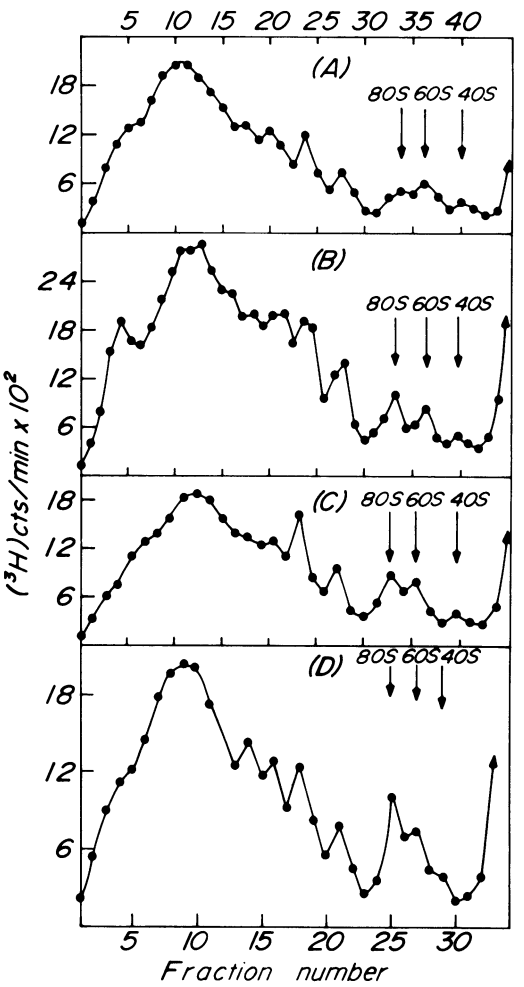

FIG. 1. *Distribution of ribosomes in yeast growing in different media. The experiment was performed as described in Materials and Methods. (A) Cells grown in YM-1 medium, (B) cells grown in YNB medium plus Casamino Acids and glucose, (C) cells grown in YNB medium plus glucose plus specific growth requirements, and (D) cells grown in YNB medium plus sodium acetate plus specific growth requirements.*

media by treatment with 2% glusulase at 30 C for 30 min. Labeling and growth was stopped by the addition of NaN_3 and cycloheximide to 10^{-2} M and 200 μg/ml final concentrations, respectively. The spheroplasts were poured over crushed ice, collected by centrifugation, and gently resuspended in 1 ml of ice-cold buffer containing 10^{-2} M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, 10^{-1} M NaCl, 2 × 10^{-2} M $MgCl_2$, 10^{-4} M $ZnSO_4$ (to inhibit ribonuclease; 13) and 100 μg of cycloheximide per ml. Brij 58 was added to 0.5%, and the suspension was mixed and allowed to stand in an ice bath for 5 min. The cell debris was removed by centrifugation for 5 min at 10,000 × *g* at 2 C. The supernatant material

(which comprised at least 90% of the initial acid-precipitable radioactivity) was layered over an 11-ml 15 to 40% linear sucrose gradient made in 10^{-2} M tris-(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, 10^{-1} M NaCl, 2×10^{-2} M $MgCl_2$. Centrifugation was for 70 min in an SB-283 rotor at 4 C using an International model B-60 ultracentrifuge run at 40,000 rpm. After centrifugation, samples were divided into equal fractions by collecting drops through a pinhole made in the bottom of the centrifuge tube. One hundred micrograms of unlabeled yeast RNA was added to each sample and the RNA was precipitated with an equal volume of 10% trichloroacetic acid. After standing for 30 min in an ice bath, the precipitates were collected on membrane filters and washed with cold 5% trichloroacetic acid by suction. The filters were then dried and the radioactivity was determined.

RESULTS AND DISCUSSION

Data collected from yeast cultures growing at a variety of rates are compiled in Table 1. The most striking point to note is the absence of a large systematic variation in RNA content per cell as a function of growth rate, although the cellular protein content is approximately 50% greater in slowly growing cultures than in rapidly growing ones. The net result is an RNA to protein ratio that is relatively invariant as a function of growth rate, in sharp contrast to findings with bacterial cultures (11). As a check on the validity of this observation, cultures growing exponentially in two growth media (YNB medium plus glucose, doubling time equals 128 min, and YNB medium plus acetate, doubling time equals 480 min) were labeled with a mixture of [^{3}H]uracil and [^{14}C]lysine for 3 generations. Samples of these cultures were precipitated with 5% trichloroacetic acid and the ratio of ^{3}H-^{14}C was determined. This ratio, which is proportional to the RNA-protein ratio in cultures growing in the two media, varied from 5.04 for the glucose culture to 3.6 for the acetate culture. The ratio of these two numbers (1.42), in turn, is proportional to the variation in RNA-protein ratios in yeast cells growing in the two media and is within 7% of the ratio measured colorimetrically (1.33; Table 1). No correction was made for that proportion of [^{3}H]uracil which entered deoxyribonucleic acid, since this represents an error of only 2 to 4% (unpublished data).

The relative proportions of 18*S*, 26*S* and 4*S*-5*S* RNA do not change significantly with growth rate (columns 5, 6, and 7). The measured values of the cellular RNA content were used to calculate the number of ribosomes per cell as follows: assuming that the molecular weight of the yeast 80*S* ribosome is 4.1×10^6 (15) and that ribosomal RNA (rRNA) constitutes 41% of the total mass of the 80*S* ribosome (15), then the amount of rRNA per ribosome is 2.79×10^{-12} μg. As shown in Table 1, columns 5, 6, and 7, 26*S* and 18*S* rRNA account for approximately 82% of the total RNA in exponentially growing cells. Therefore, from the amount of total RNA per cell (column 3), the number of ribosomes per cell can be calculated for each growth rate. These values are shown in column 8. If one assumes that the rate of protein synthesis is constant throughout the cell cycle, then the peptide chain elongation rate (dp/dt; column 9) can be calculated from the following expression: $dp/dt = P \ln 2/T R$, where P equals cellular protein content, expressed in amino acid equivalents; T equals doubling time in seconds; and R equals number of ribosomes per cell engaged in protein synthesis.

The fraction of ribosomes engaged in translation at any given time was determined from an analysis of the distribution of ribosomes in polyribosomes and ribosomal subunits in exponentially growing cells using zonal sucrose gradient centrifugation. Figure 1 shows characteristic polysome profiles obtained from cells

TABLE 1. *Cellular content of macromolecules in yeast growing in different media*

Growth media	Doubling time (min)	Total RNA/cell ($\mu g \times 10^{-7}$)	Total protein/cell ($\mu g \times 10^{-6}$) (P)	Total RNA (%)			Ribosomes per cell ($\times 10^5$) (R)	Peptide chain growth rate (amino acids per s) (dp/dt)
				26*S* rRNA	18*S* rRNA	4-5*S* RNA		
YM-1	96	4.9	2.1	55	27	17	1.5	10
Casamino Acids-glucose	120	5.8	2.9	55	26	18	1.7	9.0
YNB-glucose	137	3.8	1.9	56	26	17	1.1	8.2
YNB-mannose	170	3.9	1.9				1.1	6.3
YNB-melibiose	379	3.2	2.1	56	26	17	1.0	3.7
YNB-Casamino Acids	389	3.5	2.7				1.0	4.3
YNB-lactate	416	4.9	3.1				1.4	3.4
YNB-acetate	486	5.1	3.5	54	27	17	1.5	2.8

grown in several media. Over 90% of the radioactivity is in the 80*S* plus polysome region. We assume from these data that in these growth media approximately 90% of the ribosomes are actively engaged in protein synthesis (1). The end result of these calculations (Table 1, column 9) is that the peptide chain growth rate, dp/dt, proves to vary directly with the cell doubling time (Fig. 2) ranging from 10 amino acids per s to 2.8 amino acids per s.

The figure of 9 to 10 amino acids per s for the amino acid step time obtained for rapidly growing yeast cultures agrees well with that found by Lacroute (8) and is the same order of magnitude as has been determined in other eukaryotic organisms (9, 18) and in bacteria (14). The values of amino acid step time obtained for the slowly growing yeast cultures, however, are much lower than observed in other systems (9, 11, 18). Using our value for the amino acid step time in the rapidly growing cultures, it can be calculated that the time taken to synthesize an "average" yeast protein of 300 amino acids is 30 s, which is in good agreement with a value of 30 s determined from pulse-labeling of nascent peptide chains (6).

One can consider possible mechanisms whereby a reduction in amino acid step time as a function of growth rate could be affected, including: (i) limitation of amino acids or transfer RNA; (ii) rate-limiting translation initiation elongation or termination; and (iii) variation in the availability of messenger RNA. It is not possible at present to ascertain whether the reduction in protein chain growth is the primary rate-limiting step that sets growth rate, or is merely a reflection of other processes. It should be mentioned that a longer amino acid step time in slowly growing yeast might imply a larger mean lifetime for messenger RNA if that decay rate is coupled to translation rate (4). Direct measurements of a variety of parameters of transcription and translation in yeast cultures are needed to clarify these relationships.

It is interesting to note that the present determinations of the cellular RNA content in yeast as a function of growth rate differ from data reported by Sebastian et al. (17), where a substantial decrease in cellular RNA content of slowly growing cultures was observed. The reason for this discrepancy is not clear, but could lie in the different methods used to set the growth rate. In the experiments of Sebastian et al. (17) the growth rate was determined by nutrient limitation in a chemostat, whereas we have relied on balanced growth in different nutrients. It is conceivable that the two methods of setting growth rate invoke two different responses of the mechanism that regulates the rate of RNA synthesis in yeast.

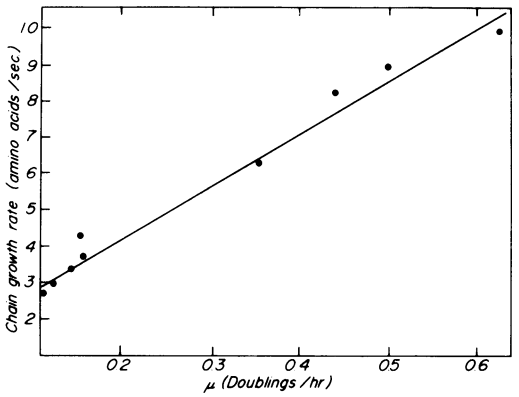

FIG. 2. *Amino acid step time as a function of growth rate. Data are taken from Table 1.*

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