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Turnover of Polyadenylate-Containing Ribonucleic Acid in *Saccharomyces cerevisiae*

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We examined the kinetics of incorporation of [^{3}H]adenine into polyadenylate-containing ribonucleic acid [poly(A)-containing RNA] in yeast. The total poly(A)-containing RNA from spheroplasts and intact cells and the polysomal poly(A)-containing RNA exhibited similar incorporation kinetics. At 30 C half-saturation of the pool of poly(A)-containing RNA with label occurred in approximately 22 min. Since precursor pools appeared to require 5 min to saturate with label, we conclude that at 30 C messenger RNA molecules in yeast decay with an average half-life of 17 min.

Knowledge of the rate of turnover of messenger ribonucleic acid (mRNA) is essential for beginning to understand the regulation of gene expression in eukaryotes. We have used *Saccharomyces cerevisiae* to study the kinetics of mRNA synthesis because it is a simple eukaryote that can be easily grown under a variety of conditions. Furthermore, there are inducible enzyme systems in this organism (see for example references 1 and 32) and there are temperature-sensitive mutants, defective in RNA or protein synthesis (9), that can be used to probe the regulation of RNA metabolism.

The functional half-life of yeast mRNA has been determined by a number of investigators. Hartwell et al. (12), using the temperature-sensitive mutant *rnal-1* in which RNA synthesis is severely restricted at the nonpermissive temperature, inferred a 23-min half-life for mRNA in yeast. This was based upon the kinetics of disappearance of polyribosomes after the cells had been placed at the restrictive temperature. Tønnesen and Friesen (30) and Cannon et al. (3), by studying the decay of protein synthetic capacity after the addition of various inhibitors of RNA synthesis, calculated a 20- to 25-min half-life for yeast mRNA. Kuo et al. (15) studied the disappearance of the capacity to synthesize various enzymes in yeast after the cells had been treated with lomofungin, an RNA polymerase inhibitor. They observed that the capacity to synthesize new enzymes disappeared 30 to 40 min after the addition of the drug. This finding is consistent with a 20- to 30-min half-life for yeast mRNA. In contrast to these observations, Lawther and Cooper (16, 17) have demonstrated that allophanate hydrolase synthesis is induced or repressed with kinetics consistent with an mRNA that turns over with a half-life of 3 min.

In our studies on the turnover of yeast mRNA, we avoided the use of inhibitors with their possible side effects on cellular metabolism and have looked directly at the chemical rather than the functional half-life of mRNA. Since the 3′ end of most yeast mRNA contains a sequence of polyadenylic acid [poly(A)] (10, 18), we isolated poly(A)-containing RNA by binding it to oligo(dT)-cellulose and examined its kinetics of synthesis. Greenberg (8) initially used this method to establish a 17-h half-life for poly(A)-containing RNA in mouse L cells. We have found that the poly(A)-containing RNA of *S. cerevisiae*, growing at 30 C, has an average half-life of approximately 17 min.

MATERIALS AND METHODS

Cell growth, spheroplast preparation, and labeling. *S. cerevisiae*, strain A364A (kindly provided by C. McLaughlin, University of California, Irvine), was grown at 23 C, unless otherwise stated, in minimal medium containing (per liter) 1 g of yeast extract, 2 g of peptone, 6.7 g of yeast nitrogen base without amino acids (Difco), 20 g of glucose, and 0.01 g of adenine. Strain S288C was grown at 30 C in minimal medium containing (per liter) 1 g of yeast nitrogen base without amino acids (Difco) and 20 g of glucose.

Preparation and culturing of spheroplasts were according to Hutchison and Hartwell (13). Spheroplasts were incubated in culture medium, containing 0.4 M $MgSO_4$, 20 min before labeling was begun with [^{3}H]adenine (final concentration, 1.5 μCi/ml). This 20-min period is sufficient for spheroplasts to resume normal rates of protein and RNA synthesis. To insure linear uptake of exogenous precursor over the duration of the experiment, carrier adenine, at a concen-

tration of 3.6 μg/ml for S288C and 5 μg/ml for A364A, was included in the medium.

Isolation of total spheroplast RNA. Ten-milliliter samples of spheroplasts were labeled for the indicated times and then harvested by pouring over a 0.5 volume of crushed ice containing 1 M sorbitol. They were collected by centrifugation (6,000 rpm, 8 min, SS34 RCII-B Sorvall rotor at 4 C), resuspended in 1 M sorbitol, and again collected by centrifugation. Spheroplast pellets were lysed in 2 ml of sodium dodecyl sulfate (SDS) buffer [0.1 M NaCl, 20 mM ethylenediaminetetraacetate, 20 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.0, and 0.5% SDS]. Recovery of the spheroplasts from culture was monitored by reading the absorbance of the lysate at 260 nm. A standard amount of [^{14}C]RNA, prepared from spheroplasts labeled for 10 min with [^{14}C]adenine (specific activity, 46.9 mCi/mmol; final concentration, 0.15 μCi/ml), was added to each sample to monitor the efficiency of RNA extraction and fractionation on oligo(dT)-cellulose.

The RNA was extracted from the lysed spheroplasts by using the hot SDS-phenol-chloroform method described by Penman (20).

Labeling and extraction of RNA from intact cells. Ten-milliliter samples of cells (strain S288C) were labeled in minimal medium containing 1.5 μCi of [^{3}H]adenine per ml and 4.8 μg of carrier adenine per ml for the indicated times. Each sample was harvested at an absorbance at 560 nm of 0.5 by pouring over 5 ml of crushed ice containing 1 M sorbitol. The cells were collected by centrifugation, washed with cold water, and resuspended in 0.4 ml of SDS buffer. A standard amount of [^{14}C]RNA was added, and each sample was quick-frozen and stored at −20 C. The pellets were thawed, brought to 0.7 ml with SDS buffer, and frozen in the chamber of an Eaton press held on dry ice. (The Eaton press was a gift from Walter Vincent, Department of Biology, University of Delaware.) The frozen cell suspension was forced through the 1.0-mm orifice of the chamber by continuous application of 10,000 lb/in^2. The extract was brought to room temperature, and cell debris was removed by centrifugation (5,000 rpm for 5 min). RNA was isolated as described above. This method of extraction yields greater than 80% of the total RNA (unpublished data).

Isolation of polyribosomal RNA. Spheroplasts were prepared from S288C cells as described above. Ten-milliliter samples were labeled with 1.5 μCi of [^{3}H]adenine per ml for the specified times. At the end of the isotope incorporation period cycloheximide, at a final concentration of 50 μg/ml, was added to each sample to minimize the loss of polyribosomes during the extraction. Each sample was harvested by pouring over a 0.5 volume of crushed ice containing 1 M sorbitol, collected by centrifugation, washed in 1 M sorbitol, and centrifuged. Each pellet was resuspended in 1 ml of buffer A of Udem and Warner (31) [0.01 M NaCl, 0.01 M 1,4-piperazine-bis-(ethanesulfonic acid), 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.01% spermidine-HCl, adjusted to pH 6.5] and held for 5 min. The detergent Nonidet P-40 was added to a final concentration of 1%, and the extract was centrifuged at 10,000 rpm for 10 min to remove nuclei, mitochondria, and any undisrupted cells. The supernatant fluid was layered over a 40-ml 10 to 50% (wt/vol) linear sucrose gradient prepared in buffer A. The gradients were centrifuged at 4 C in an SW27 Beckman rotor for 3.5 h at 26,000 rpm.

The contents of each gradient were collected from the bottom of the tube through the flow cell of a recording spectrophotometer. Fractions of the gradient that contained polyribosomes of two or more ribosomes were pooled and brought to 0.1 M NaCl and 0.2% SDS. Two volumes of 95% ethanol were added, and the samples were held overnight at −20 C. The precipitate was recovered by centrifugation (10,000 rpm for 1 h at −10 C) and suspended in SDS buffer. A standard amount of [^{14}C]RNA was added, and each sample was extracted by the hot SDS-phenol-chloroform method (20).

Preparation of oligo(dT)-cellulose and fractionation of RNA. Polymerization of thymidine 5′-monophosphate attachment to cellulose in anhydrous pyridine was accomplished as described by Gilham (7) and Edmonds (5).

The RNA was fractionated by affinity chromatography at room temperature in columns containing a bed volume of 1.5 ml of oligo(dT)-cellulose equilibrated with HS buffer (0.1 M NaCl, 5 mM ethylenediaminetetraacetate, 50 mM Tris-hydrochloride, pH 7.5, and 0.2% SDS). RNA was dissolved in 0.4 ml of HS buffer and layered on the column. The sample was permitted to flow into the column bed and held for 20 min. RNA that did not bind to oligo(dT)-cellulose was removed by elution with 14 ml of LS buffer (10 mM Tris-hydrochloride, pH 7.5, 0.2% SDS). Two-milliliter fractions were collected, and each fraction was precipitated at 4 C by the addition of 100 μg of carrier RNA and 0.5 ml of 50% trichloroacetic acid. Precipitates were collected on glass fiber filters, washed three times with 3-ml volumes of cold 5% trichloroacetic acid and once with 3 ml of cold 95% ethanol, and then dried. Radioactivity retained on the filters was determined by scintillation spectrometry in 4.4 ml of scintillation solution {0.01 g of 1,4-bis-[2]-(5-phenyloxazolyl)benzene, 6 g of 2,5-diphenyloxazole per liter of toluene}.

RESULTS

Entrance of [^{3}H]adenine into poly(A)-containing RNA of spheroplasts and intact cells. The kinetics of incorporation of labeled precursor into mRNA reflect the instability of this RNA species. Thus, the rate of incorporation deviates from the initial rate as the mRNA pool saturates with label and labeled mRNA begins to be degraded. Figure 1 presents a summary of the kinetics of incorporation of [^{3}H]adenine into spheroplast RNA from two experiments. The inset in Figure 1 demonstrates that a sufficiently high concentration of exogenous adenine was included to insure uninterrupted uptake of label over the duration of the experiment.

Poly(A)-containing RNA was isolated from samples of RNA obtained from spheroplasts labeled for the indicated times by binding to oligo(dT)-cellulose. Incorporation of [^{3}H]adenine into poly(A)-containing RNA approached a steady-state value with a half-saturation time of approximately 22 min. Incorporation of [^{3}H]adenine into RNA that did not bind to oligo(dT)-cellulose became linear after a lag of 5 min.

Each data entry in these experiments has been corrected for the variation in recovery of spheroplasts from culture and also for variations in the efficiency of RNA extraction and fractionation on oligo(dT)-cellulose. A standard amount of lysate obtained from spheroplasts labeled for 10 min with [^{14}C]adenine was added to each SDS lysate before extraction of the RNA to provide an internal standard for these corrections. Table 1 presents the raw and corrected data from one of the experiments used to generate Fig. 1. A comparison of the data in columns 3, 5, and 7 reveals that the major correction is for variations in the recovery of spheroplasts. Fractionation of the standard [^{14}C]RNA on oligo(dT)-cellulose was quite reproducible. Seventy-five to 90% of the [^{3}H]- or [^{14}C]-labeled RNA in each sample was recovered from the oligo(dT)-cellulose columns.

The half-saturation time for poly(A)-containing RNA determined from Fig. 1 includes the precursor pool saturation time. Thus a determination of the half-life of mRNA from the kinetics of incorporation requires an evaluation of the time required to saturate the intracellular nucleoside triphosphate pools used for RNA synthesis with label. This precursor pool saturation time is directly expressed as a lag in incorporation of label into stable RNA. Since RNA that does not bind to oligo(dT)-cellulose is virtually all ribosomal RNA and transfer RNA (9), the lag in incorporation of label into this fraction is a close approximation of the precursor saturation time.

Incorporation of label into RNA that did not bind to oligo(dT)-cellulose had an extrapolated lag of 5 min. Thus, if ribosomal and messenger RNA syntheses are supported by kinetically indistinguishable precursor pools, we conclude that the half-life of poly(A)-containing RNA is approximately 17 min in yeast spheroplasts cultured at 30 C. The same estimated half-life was obtained when [^{3}H]cytosine was used as labeled precursor (data not shown).

Although Hutchison and Hartwell (13) have shown that over a period of 8 h the rates per cell of protein and RNA synthesis in spheroplasts

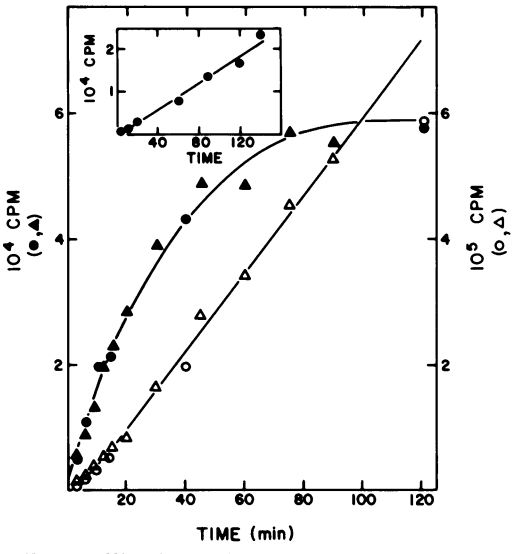

FIG. 1. *Kinetics of incorporation of [^{3}H]adenine into the RNA of S288C spheroplasts. Spheroplasts were labeled with [^{3}H]adenine (0.42 μCi/μg) for the indicated times. The RNA from 10-ml samples was extracted, fractionated on oligo(dT)-cellulose columns and monitored for isotope as described in the text. The values were then normalized to a constant input of spheroplasts as described in Table 1. Two experiments are summarized in the figure (experiment 1, ●, ○; experiment 2, ▲, △). Closed symbols designate poly(A)-containing RNA. Open symbols designate RNA that does not bind to oligo(dT)-cellulose. The insert presents incorporation of [^{3}H]adenine into spheroplast macromolecules.*

are comparable to those of intact cells, spheroplasts do not divide. Thus, the kinetics of synthesis of poly(A)-containing RNA in spheroplasts might not reflect the kinetics in intact cells. To test this possibility, the kinetics of synthesis of poly(A)-containing RNA were examined in intact cells (Fig. 2). The half-time for saturation of poly(A)-containing RNA with [^{3}H]adenine was approximately 22 min, which is the same as that obtained with the spheroplasts.

Entry of [^{3}H]adenine into polysomal RNA. In many eukaryotes there exist species of nuclear RNA that are heterogeneous in size with sedimentation coefficients between 20 and 100*S*. A high percentage of this RNA turns over rapidly within the nucleus (2, 27). Many of these molecules contain poly(A). Yeast nuclei contain species of poly(A)-containing RNA that are larger than cytoplasmic poly(A)-containing RNA (10). If these species turn over more rapidly than mRNA, they may obscure the kinetics of incorporation of [^{3}H]adenine into

TABLE 1. *[^{3}H]adenine incorporation into spheroplast RNA*[a]

Time (min)[b]	RNA[c]	^{3}H counts/min[d]	^{14}C counts/min[e]	^{3}H count/min ($\times 10^3$; yield corrected)	A_{260}[f]	Relative ^{3}H counts/min ($\times 10^3$)
3	N-B	4,577	2,075	5.5	0.299	7.6
	B	3,872	1,227	4.1		5.7
6	N-B	10,090	2,184	12.0	0.277	17.0
	B	7,817	1,373	7.5		11.0
10	N-B	21,519	2,503	22.0	0.306	29.0
	B	13,822	1,254	15.0		19.0
15	N-B	46,158	2,872	40.0	0.305	54.0
	B	16,675	1,400	16.0		21.0
40	N-B	200,011	2,664	190.0	0.393	200.0
	B	41,370	1,322	41.0		43.0
120	N-B	490,203	2.760	450.0	0.312	590.0
	B	44,791	1,351	44.0		58.0

[a] Spheroplasts of S288C were labeled as described in the legend to Fig. 1.

[b] Time that the spheroplasts were incubated with [^{3}H]adenine.

[c] N-B, RNA that does not bind to oligo(dT)-cellulose; B, RNA that binds to oligo(dT)-cellulose.

[d] Total trichloroacetic acid-precipitable ^{3}H in each fraction.

[e] Fractionation of [^{14}C]RNA standard. The average of the N-B and B fractions were taken and used to correct the ^{3}H counts per minute in column 3. For example, the average ^{14}C counts per minute in experiment 1 for B fraction is 1,321. Therefore, the corrected ^{3}H counts per minute for the 3-min B fraction = (1321/1227) × 3,872 = 4,169. The corrected values, rounded off to the second significant figure, are listed in column 5. Seventy-five to 90% of the [^{14}C]RNA added to the cell lysates was recovered from the oligo(dT)-cellulose columns.

[f] Absorbancy at 260 nm (A_{260}) of the spheroplast lysate. All ^{3}H counts per minute in column 5 were corrected to the highest A_{260} reading and are listed in column 7.

polyribosomal poly(A)-containing RNA at early times. Therefore, the uptake of radioactivity into polyribosomal poly(A)-containing RNA (functional mRNA) was examined (Fig. 3). The increased lag in incorporation of label into RNA that did not bind to oligo(dT)-cellulose was not unexpected because label found in ribosomal RNA during short labeling intervals is predominantly in precursor species, which have not had time to mature and enter polyribosomes. The half-time of saturation of polyribosomal poly(A)-containing RNA with label was 20 to 25 min. Therefore, if there were rapidly turning over nuclear species of poly(A)-containing RNA in yeast, they did not contribute significantly to our determination of the half-time of saturation of poly(A)-containing RNA with label.

Effect of different cultivation temperatures upon the half-life of poly(A)-containing RNA. Temperature-sensitive mutants have become important tools in investigations of gene expression and RNA metabolism in *S. cerevisiae*. Because metabolic rates certainly change in temperature-shift experiments, we felt it was important to examine the kinetics of incorporation of [^{3}H]adenine into poly(A)-containing RNA in wild-type (non-temperature-sensitive) cells at different temperatures. The experimental protocol was identical to that used in the experiments presented in Fig. 1. The half-saturation times for the incorporation of [^{3}H]adenine into poly(A)-containing RNA of A364A spheroplasts incubated at 17, 23, and 36 C were 74, 30, and 21, respectively. Increasing precursor pool saturation time (from 4 min at 36 C to 8 min at 17 C) only negligibly contributed to the increase in apparent half-life of poly(A)-containing RNA at lower incubation temperatures.

DISCUSSION

Previous estimates of the functional half-life of yeast mRNA have been based upon the disappearance of protein synthetic capacity in cells exposed to the drugs lomofungin (3, 15), daunomycin (30), or ethidium bromide (30). However, it is not known whether these drugs effect protein synthesis only as a consequence of their demonstrated inhibition of RNA synthesis or if they exert a direct effect upon the protein synthesis apparatus or upon mRNA decay. The effect of actinomycin D upon the assessment of the mRNA half-life in HeLa cells is an example of this type of problem (25). Since most mRNA molecules in yeast contain poly(A), we have isolated mRNA by chromatography on oligo(dT)-cellulose and have examined its kinetics of synthesis. Poly(A)-containing RNA in *S. cerevisiae* has a chemical half-life of approximately 17 min. This value has been corrected

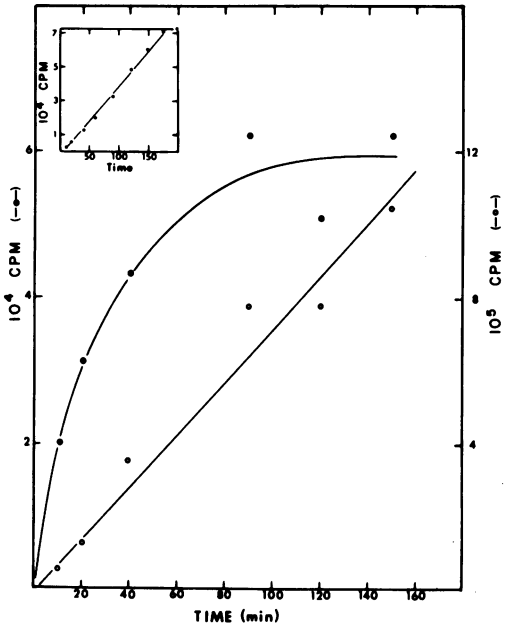

FIG. 2. *Kinetics of incorporation of [^3H]adenine into the RNA of S288C. Intact cells were labeled with [^3H]adenine for the indicated times. Ten-milliliter samples were removed and the cells were opened by passage through an Eaton press. The RNA was extracted, fractionated on oligo(dT)-cellulose columns, and monitored for isotope as described in the text. The values were normalized to a constant input of cells. Symbols: ●, Poly(A)-containing RNA; ○, RNA that does not bind oligo(dT)-cellulose. Insert: Incorporation of [^3H]adenine into intact cells in culture.*

for a 5-min lag in incorporation of [^{3}H]adenine into stable RNA species and, therefore, depends upon the assumption that kinetically indistinguishable percursor pools support the synthesis of ribosomal and messenger RNA. Although this assumption is untested in yeast, Wu and Soerio (34) have presented strong evidence for its validity in HeLa cells.

A direct assessment of the kinetics of saturation of the total cellular adenosine triphosphate and guanosine triphosphate pools in *S. cerevisiae*, using the techniques described by Winslow and Lazzarini (33), revealed complex kinetics (S. Phillips, unpublished data). Saturation of these pools with tritium from exogenous [^{3}H]adenine was approached at approximately 60 min. However, the rate of incorporation of label into ribosomal RNA was maximal long before this time. Thus, we consider it likely that cytoplasmic and nuclear compartments have kinetically distinct nucleoside triphosphate pools.

The kinetics of labeling poly(A)-containing RNA appeared to be very similar in spheroplasts and intact cells. In addition, we observed that the half-time of saturation of poly(A)-containing RNA and the growth rate of yeast were approximately proportional over a 19 C range of incubation temperature.

The shape of the uptake curve for poly(A)-containing RNA in Fig. 1 approximately corresponded to that which would be expected from a single population of mRNA that decays by a stochastic process. However, this type of analysis is quite insensitive to contributions by minor subpopulations of mRNA with faster or slower turnover rates. There is increasing evidence that the mRNA population within any eukaryotic cell may be composed of subpopulations with different half-lives (4, 6, 14, 19, 21, 22, 24, 26, 28, 35). Experiments reported in the literature suggest that the yeast mRNA population is composed of molecules with widely divergent functional half-lives. Values ranging from 3 min for allophanate hydrolase mRNA (16, 17) to 15 min for homocysteine synthase mRNA (29) and 20 min for the messengers coding for invertase,

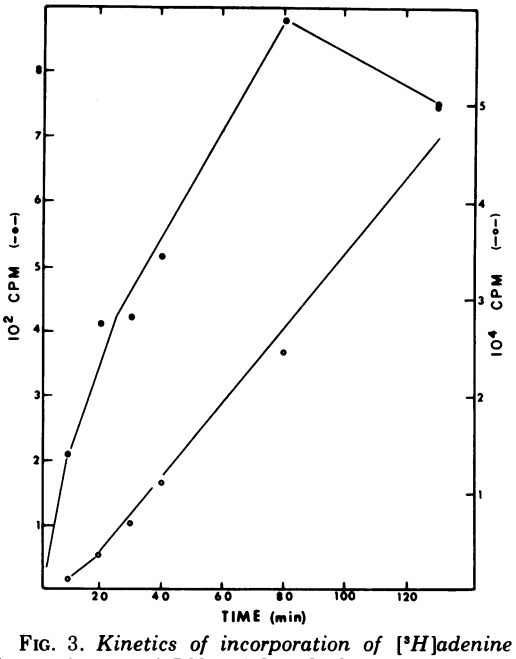

FIG. 3. *Kinetics of incorporation of [^3H]adenine into polysosomal RNA of S228C. Spheroplasts were labeled with [^3H]adenine for the indicated times. Polysomes were prepared from 10-ml samples, and the RNA was extracted, fractionated on oligo(dT)-cellulose columns, and monitored for isotope as described in the text. Symbols: ●, Poly(A)-containing RNA; ○, RNA that does not bind to oligo(dT)-cellulose.*

α-glucosidase, and acid phosphatase (15) have been reported. Thus, the 17-min half-life measured for the total poly(A)-containing RNA population of yeast likely represents an average of a spectrum of messenger half-lives.

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