Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5208–5212, May 1996
Biochemistry

Absolute mRNA levels and transcriptional initiation rates in *Saccharomyces cerevisiae*

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Communicated by Charles C. Richardson, Harvard Medical School, Boston, MA, January 25, 1996 (received for review September 28, 1995)

ABSTRACT **We quantitate the absolute levels of individual mRNAs per yeast cell by hybridizing total yeast RNA with an excess of gene-specific ^{32}P-oligonucleotides, and digesting the resulting RNA·DNA hybrids with S1 nuclease. By comparing the *his3* hybridization signal from a known amount of yeast cells to the signal generated by a known amount of *his3* RNA synthesized *in vitro*, we determine that yeast strain KY114 growing in yeast extract/peptone/glucose medium at 30°C contains seven molecules of *his3* mRNA per cell. Using a galactose shut-off procedure, we determined that the half-life of *his3* mRNA is approximately 11 min under these conditions. From these observations, we calculate that one *his3* mRNA molecule is synthesized every 140 s. Analysis of other *his3* promoter derivatives suggests that the maximal transcriptional initiation rate in yeast cells is one mRNA molecule every 6–8 s. Using *his3* as an internal standard, the number of mRNA molecules per cell have been determined for *ded1*, *trp3*, *rps4*, and *gal1* under a variety of growth conditions. From these results, the absolute mRNA level of any yeast gene can be determined in a single hybridization experiment. Moreover, the rate of transcriptional initiation can be determined for mRNAs whose decay rates are known.**

Over the past 2 decades, mRNA levels of individual eukaryotic genes have been determined by a variety of methods such as S1 nuclease protection, Northern blot analysis, RNase protection, primer extension, dot blotting, and PCR amplification. While having different advantages and limitations, these methods are essentially always utilized to determine RNA levels in relative terms. Thus, there are numerous measurements involving relative levels of different mRNAs or relative levels of a given mRNA under a variety of developmental, environmental, or genetic conditions. However, there is very limited information about the absolute number of mRNA molecules in an individual cell. Furthermore, almost nothing is known about absolute rates of transcriptional initiation *in vivo*.

In the yeast *Saccharomyces cerevisiae*, mRNA levels for individual genes vary over a range of at least three orders of magnitude. Nevertheless, three early studies suggested that mRNA levels for many yeast genes are similar. (*i*) Hybridization of poly(A)-containing RNA (in the form of ^{32}P-labeled cDNA) to a yeast genomic library resulted in similar hybridization signals for most yeast genomic DNA fragments (1). (*ii*) As determined by Northern blot analysis and cDNA hybridization, RNA levels for *his3* and five adjacent genes were extremely similar (2). (*iii*) *ura3* mRNA represents 1.3×10^{-4} of the poly(A)-containing RNA in yeast cells, a value consistent with that of an average gene (3). Based on this information, as well as estimates for the fraction of the genome that is transcribed into mRNA and for the amount of total poly(A)-containing mRNA per cell, it was estimated that yeast cells contain approximately 1–2 molecules of an average mRNA (2). In other work which relied on *in vivo* labeling of mRNA and filter hybridization, it was estimated that there was 1 fmol of *rp1* mRNA per μg of total RNA (4), which corresponds approximately to 600 molecules per cell. However, as the underlying assumptions of these estimates were difficult to verify, the absolute levels for any yeast mRNA have not been rigorously determined.

Aside from its inherent value, determination of absolute mRNA levels would be extremely useful for determining rates of transcriptional initiation *in vivo*. Direct measurements of transcriptional initiation rates *in vivo* are extremely difficult, and there are few reliable estimates. However, mRNA degradation rates for many yeast genes have been accurately determined by a variety of methods (5, 6). For any mRNA with a known decay rate, measurement of absolute mRNA levels makes it possible to calculate the rate of transcriptional initiation from the promoter.

In this paper, we describe a quantitatively rigorous approach to measure absolute mRNA levels in growing yeast cells. We directly determine the absolute level of *his3* mRNA and calculate the rate at which it is initiated from the promoter. Using *his3* as an internal standard, we determine mRNA levels for a number of other yeast genes under a variety of growth conditions. Given this information, absolute levels of any yeast mRNA can be measured in a single hybridization experiment, and transcriptional initiation rates can be determined when the mRNA half-life is known.

MATERIALS AND METHODS

Synthesis of *his3* RNA *in Vitro*. The *his3* coding sequence under the control of the bacteriophage T7 promoter in plasmid YIp55-Sc3647 (7) was amplified by PCR using TGTCCTTATTCTGGAACTTGGATTTATGGC and TACCACTTGCCACCTATCACC as primers. Half of the PCR product was digested with *Asp*718, and the resulting DNAs were extracted with phenol-chloroform and chloroform, precipitated with ethanol, purified by spinning through a Sephadex G-50 column. *In vitro* transcription reactions were carried out in a 50 μl-volume and contained 2–4 μg of the DNA, 40 mM Tris (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, 50 μg/ml BSA, 400 μM NTPs, 10 units of T7 RNA polymerase, and 10 units of RNasin (Promega). Reactions were incubated at 37°C for 45 min, and 10 units of T7 RNA polymerase was added for an additional 45 min. DNase I (2.3 units) was then added for 15 min, after which the reaction mixture was extracted with phenol-chloroform and chloroform, purified by passing through a Sephadex G-50 spin column, and precipitated with ethanol. RNA was analyzed by agarose gel electrophoresis, and its concentration determined by absorbance at 260 nm. No A_{260} was observed when the same procedure was performed on a control reaction lacking T7 RNA polymerase.

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Abbreviations: YPD, yeast extract/peptone/dextrose; YP, yeast extract/peptone.

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To independently measure the amount of *his3* RNA synthesized *in vitro*, reactions were carried out in the presence of [^{32}P]UTP (Amersham) that was purified by chromatography on DEAE-A50 and elution with NaCl. As assayed by thin layer chromatography and autoradiography, the purified material did not contain significant amounts of [^{32}P]UDP or [^{32}P]UMP, but it did contain other impurities such that [^{32}P]UTP represented only 81% of the labeled material. Taking this degree of purity into account, the specific activity of the UTP in the starting transcription reactions was measured by scintillation counting. Following the transcription reaction and subsequent purification of the RNA as described above, the concentration of RNA in three independent samples were measured by A_{260} and by scintillation counting: the difference between the two methods was ±4%.

Isolation of Total Yeast RNA from a Known Number of Cells. Strain KY114, which contains the wild-type *his3* gene (8), was grown in yeast extract/peptone/dextrose (YPD) at 30°C to an OD_{600} of 1. Dilutions in duplicate of an aliquot of the culture were made in YPD and then spread on YPD plates for determination of the number of viable cells. At the same time, 90 ml of the culture was split into six tubes containing 15 ml each, and RNA was isolated independently from each aliquot. Cells were washed once in water, resuspended in 400-μl lysis buffer (10 mM Tris, pH 7.5/10 mM EDTA/0.5% SDS), and either frozen at −20°C or used immediately for RNA isolation. Phenol (400 μl) equilibrated with H_20 (i.e., unbuffered) was mixed with the cell suspension by vortexing and then incubated at 65°C for 1 h with occasional vortexing (3 or 4 times). The tubes were placed on ice for 10 min, and centrifuged at 4°C for 10 min. The aqueous phase was re-extracted at room temperature with phenol (pH 4), chloroform, and then precipitated with 0.3 M sodium acetate and 2 vol of ethanol. RNA pellets were washed thrice with 70% ethanol, dried, and resuspended in water. RNA from strain KY320 and KY1093, which respectively contain the *his3*-Δ200 and *his3*-G17 alleles (9), was isolated using the same procedure. For the experiment in Fig. 5, the same procedure was followed except that KY114 cells were also grown in yeast extract/peptone (YP) medium containing 2% galactose, 2% raffinose, 2% lactate, or in glucose minimal medium that did or did not contain 1.2% casamino acids.

S1 Nuclease Analysis. Probes were made by end-labeling purified oligonucleotides complementary to the gene of interest. Typically, equimolar amounts (2–10 pM) of two dephosphorylated oligonucleotides were cokinased in a 50-μl reaction (50 mM Tris, pH 7.5/10 mM $MgCl_2$/5 mM DTT/50 μg/ml BSA) containing 35 pM (240 μCi; 1 Ci = 37 GBq) γ-[^{32}P]ATP (specific activity >7000 Ci/mmol) and 20 units of T4 polynucleotide kinase at 37°C for 1 h. The reaction was stopped by extracting with phenol-chloroform and chloroform, and the DNA was precipitated twice after adding ammonium acetate (to 2.5 M) and $MgCl_2$ (to 10 mM) and 2.5 vol of ethanol to remove unincorporated nucleotides. Analysis of probes on denaturing gels showed bands of equal intensity, indicating that they were labeled to the same extent in the reaction, and hence had similar specific activities.

Approximately 0.2 pM oligonucleotides were used for hybridization with approximately 40 μg of total yeast RNA. For RNAs we have measured, this represents a 100- to 1000-fold molar excess of the probe over the RNA. Hybridization was performed in a 50-μl volume containing 0.3 M NaCl, 1 mM EDTA, 38 mM Hepes (pH 7.0) and 0.5 μl of 10% Triton X-100, overnight (>12 h) at 55°C. For digestion, 150 units of S1 nuclease (Sigma) was added to each reaction in 450 μl of a solution containing 0.33 M NaCl, 66 mM sodium acetate, 2.2 mM $ZnCl_2$, 0.01% Triton X-100, and the reaction was incubated at 37°C for 30 min. Digestion was stopped by the addition of 5 μl of 0.5 M EDTA and 5 μl of 10 μg/μl tRNA, and the products precipitated with 1 ml of ice-cold ethanol. Pellets were dried, resuspended in formamide loading buffer, and analyzed on an 8% denaturing polyacrylamide gel. Dried gels were exposed with a PhosphorImager storage phosphor screen (Molecular Dynamics) or a Fujix BAS cassette (Fuji), and the bands quantitated using the appropriate instrumentation and software.

RESULTS

General Approach. The absolute level of *his3* mRNA was determined by comparing the *his3* hybridization signal from a known amount of yeast cells to the signal generated by a known amount of *his3* RNA synthesized *in vitro*. Experimentally, RNA was hybridized to completion with an excess of oligonucleotide probes labeled with ^{32}P at their 5′ ends, and the resulting RNA·DNA hybrids were digested with S1 nuclease. After electrophoretic separation, the products were quantitated by PhosphorImager analysis.

The experimental methodology was chosen for several reasons. (*i*) Solution hybridization of nucleic acids is a simple physical process that is well understood at the theoretical level. (*ii*) Large quantities of single-stranded oligonucleotides are easily obtained, thereby enabling hybridization reactions to be driven rapidly to completion by the vast excess of probe. Under these conditions, the amount of RNA·DNA hybrid is directly related to the amount of input RNA over a very large concentration range. (*iii*) Hybridization probes of equal specific activity can be generated by mixing oligonucleotides prior to 5′-end labeling. Thus, by using such a mixture of probes, the relative molar amounts of the corresponding RNA species are directly determined. (*iv*) S1 nuclease is a robust enzyme that functions efficiently under suboptimal conditions. In comparison with most enzymes, S1 nuclease is remarkably insensitive to contaminants such as phenol, chloroform, SDS, and cellular debris. (*v*) By measuring the level of a short portion of the RNA defined by the oligonucleotide, the method is essentially unaffected by RNase contamination that preferentially cleaves large RNAs over small RNAs. For these reasons, we believe that the experimental approach is superior to others for quantitative purposes. However, it is important to note that this method does not directly measure the level of full-length mRNA species.

Synthesis of *his3* RNA *in Vitro*. To obtain a known quantity of *his3* RNA for calibrating the hybridization reaction, we synthesized *his3* RNA *in vitro*. The DNA template, YIp55-Sc3647 (7), contained the entire *his3* mRNA coding region under the control of the bacteriophage T7 promoter such that T7 RNA polymerase will initiate *his3* RNA at a site that is virtually identical to that utilized *in vivo* (Fig. 1). To increase both the specificity and yield of *his3* RNA, the template was amplified by PCR to generate large amounts of DNA specifically encoding *his3*. The resulting PCR product was used

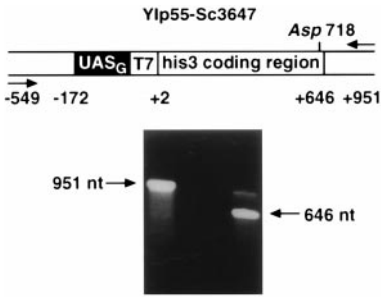

FIG. 1. Synthesis of *his3* RNA *in vitro*. Structure of Sc3647, which contains the *his3* coding region under the control of the bacteriophage T7 promoter (sequence indicated). The positions of the *Asp*718 restriction site and the PCR primers (arrows) are indicated, with nucleotide coordinates shown relative to the +1 initiation site. Shown below is the agarose gel analysis of *his3* RNA synthesized *in vitro* from DNA templates that were generated by PCR (with or without subsequent cleavage by *Asp*718).

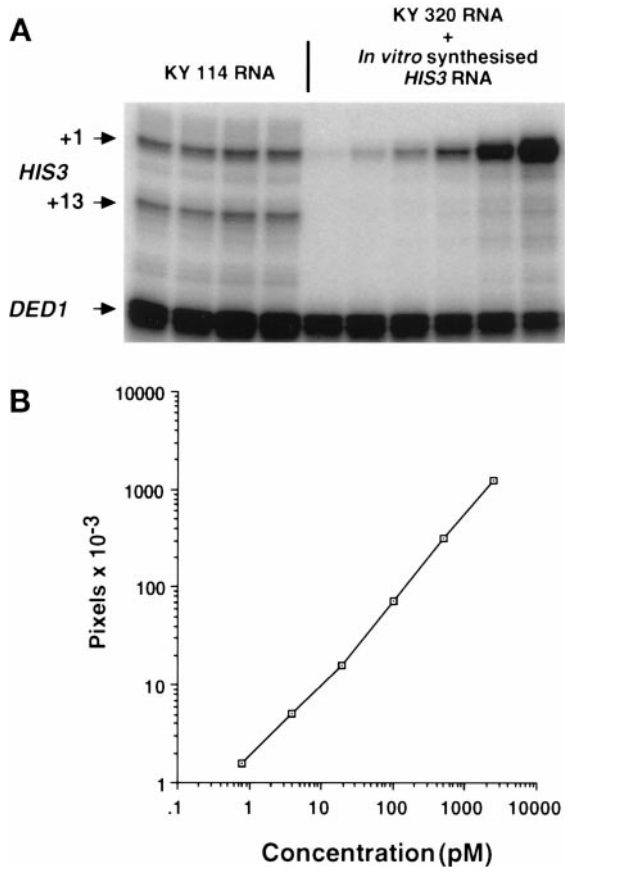

FIG. 2. Quantitative analysis of *his3* mRNA levels. (*A*) The leftmost four lanes represent independent preparations of RNA from KY114 grown at 30°C in YPD medium. The six lanes on the right represent increasing 4-fold amounts of *his3* RNA that were mixed with a constant amount of RNA from KY320. RNAs were hybridized with *his3* and *ded1* probes and treated with S1 nuclease. (*B*) Hybridization signal (in pixels) as a function of the amount of *in vitro* synthesized *his3* RNA, showing that the reaction is linear over a 3000-fold range.

directly or cleaved with *Asp*718; incubation of these products with T7 RNA polymerase generated RNAs of length 951 or 646 bases, respectively (Fig. 1). After synthesis, the reaction mix was treated with DNase I, and the RNAs were purified away from unincorporated ribonucleoside triphosphates and other small molecules by gel filtration. As a control, the identical procedure was followed except for the omission of T7 RNA polymerase.

Quantitation of *in Vitro* Synthesized *his3* RNA. We determined the amount of the *in vitro* synthesized RNA by measuring its absorbance at 260 nm (A_{260}). The measured A_{260} was entirely due to the synthesized RNA, because no detectable absorbance was observed in the control sample. The standard conversion factor for RNA is that 1 A_{260} unit equals a concentration of 40 μg/ml. However, A_{260} is also affected by RNA structure, with a high degree of secondary structure reducing the absorption that would otherwise result from the same concentration of free bases. The standard conversion factor is likely to reflect highly structured RNAs such as rRNA and tRNA which account for nearly all of the material in most bulk RNA samples. In contrast, the conversion factor for a mixture of free bases, calculated on the basis of their individual extinction coefficients, is 30 μg/ml for each unit of A_{260}.

To correct for the contribution made by the structure of *his3* RNA, we measured the increase in A_{260} (the hyperchromic effect) after it was degraded to short oligonucleotides by treatment with RNase A. The highly structured *Escherichia coli* $tRNA^{Val}$ shows a 23% increase in A_{260}, whereas the *his3* RNA shows a 10% increase. Based on this 10% increase in the A_{260} of *his3* RNA and the assumption that short oligonucleotides have the same absorbance as free bases, we estimate that each A_{260} unit of *his3* RNA represents a concentration of 33 μg/ml. This number was used for subsequent calculations. To obtain an independent measurement of the amount of *his3* RNA, transcription reactions were carried out in the presence of known amounts of purified [^{32}P]UTP (see *Materials and Methods*). Quantitation of three independently prepared RNA samples by A_{260} and radioactivity indicated that the difference between these two methods was ±4%.

Isolation of Total Yeast RNA from a Known Number of Cells. Total yeast RNA was isolated from strain KY114, which has a wild-type *his3* gene. Cells were grown to mid-logarithmic phase in rich medium (YPD), and the number of viable cells were determined by plating dilutions on the same medium. At the same time, total RNA was isolated by extraction with unbuffered phenol at 65°C. This chemical procedure is rapid and is less variable than methods involving enzymatic or mechanical breakage of yeast cells. Analysis of four independent RNA preparations (Fig. 2) indicated that *his3* and *ded1* RNA levels varied between ±10%. As this high degree of reproducibility renders it very unlikely that only a certain fraction of the RNA is being isolated, we conclude that essentially all of the RNA from yeast cells is isolated by this procedure.

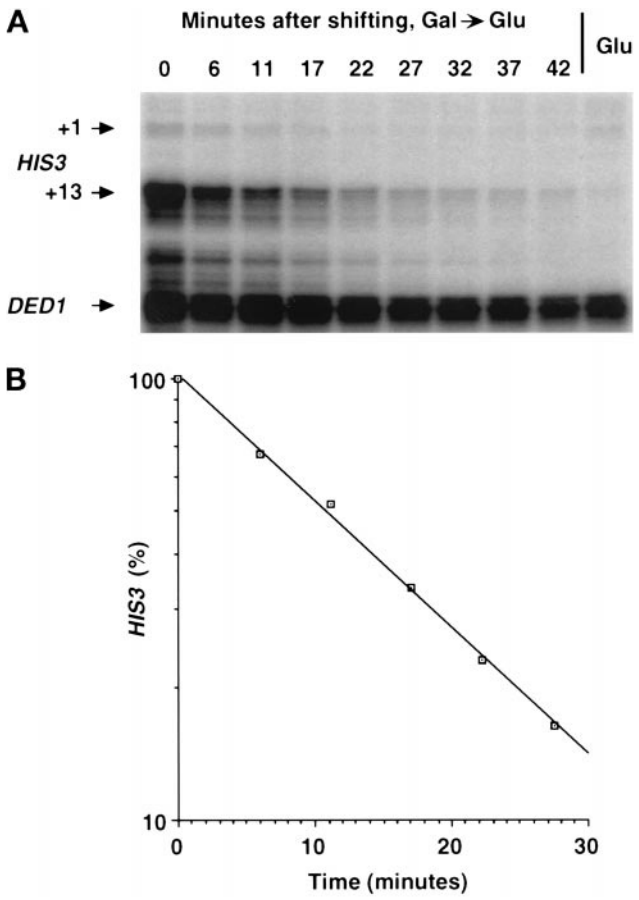

FIG. 3. Measurement of the decay rate of *his3* mRNA at 30°C. (*A*) RNAs from strain KY1093 at various times after shifting the culture from YP medium containing galactose to YPD medium were hybridized to a mixture of *his3* and *ded1* probes. The right-most lane represents an analysis of RNA from KY1093 cells grown in YPD medium. (*B*) Quantitative analysis of *his3* RNA levels (normalized to total RNA in the same sample and defined as 100% at the time of the medium shift) as a function of time after shift to YPD medium.

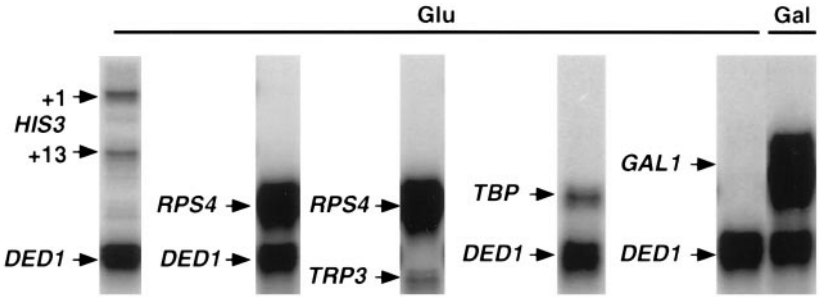

FIG. 4. Quantitative analysis of mRNA levels for various genes. RNA from KY114 cells grown in YPD or YP galactose medium were hybridized to the indicated mixtures of probes.

Quantitative Analysis of *his3* RNA. To calibrate the hybridization reactions, we mixed known amounts of *his3* RNA with total RNA from KY320, a yeast strain with a complete deletion of the *his3* locus. The resulting mixtures simulate the population of RNAs in KY114, the isogenic strain with a wild-type *his3* locus, and they permit *his3* RNA levels to be normalized to an internal *ded1* control in each hybridization reaction. As expected, such hybridization reactions are highly reproducible; equimolar amounts of the long (951 nt) and short (646 nt) *his3* RNAs synthesized *in vitro* yield hybridization signals that vary by <5%. Furthermore, the hybridization signals are linear with respect to the amount of RNA over a 3000-fold range (Fig. 2). Given this high reproducibility and linearity, the amount of *his3* RNA in KY114 cells is easily determined by comparison to the standard curve. Assuming each A_{260} unit to be equivalent to 33 μg/ml of *his3* RNA, KY114 cells grown in YPD medium contain an average of 7 ± 1 molecules of *his3* mRNA. This determination represents the sum of the +1 and +13 *his3* transcripts, which are present at similar levels when cells are grown in YPD (10).

Determining the Degradation Rate of *his3* RNA at 30°C. By measuring *his3* mRNA levels after thermal inactivation of a temperature-sensitive RNA polymerase II, it was shown previously that *his3* mRNA has a half-life of 7 min at 37°C (5). To determine the half-life of *his3* mRNA at 30°C, we used a glucose shut-off protocol (11). Specifically, strain KY1093, which contains *his3* under the control of the Gal4 activator protein, was grown in YP medium containing galactose and then shifted to YPD medium, conditions that block further initiation from this *gal-his3* promoter. Analysis of *his3* mRNA levels at various times after the shift to YPD medium revealed a rapid and exponential decay of *his3* mRNA that occurred with a half-life of 11 min (Fig. 3).

Quantitative Analysis of Other Yeast RNAs. To determine the absolute level of other mRNA species, RNA from KY114 cells grown in YPD was hybridized simultaneously to two probes (Fig. 4). To ensure that the probes had the same specific activity, equimolar amounts of the oligonucleotides (determined by A_{260}) were phosphorylated in the same reaction under conditions of excess [^{32}P]ATP; equal labeling of the oligonucleotides was verified by gel electrophoresis. Under these conditions, the relative hybridization signals are directly indicative of the relative molar amounts of the corresponding RNA species. Using the independently determined value of seven *his3* RNA molecules per cell for normalization, the results indicate that KY114 cells contain 25 molecules of *ded1* RNA, 75 molecules of *rps4* RNA, 2 molecules of *trp3* RNA, 3.5 molecules of *TBP* RNA, and < 0.1molecule of *gal1* RNA (Table 1). The absence of *gal1* RNA was expected because this gene is strongly repressed when cells are grown in glucose.

RNA Levels in Strains Grown in Various Conditions. We also measured the levels of various mRNAs in KY114 cells grown under different conditions (Fig. 5, Table 1). RNA from equivalent numbers of cells (determined by viable count) were analyzed in parallel, with the values determined relative to the seven molecules per cell of *his3* RNA in YPD grown KY114. For most of the RNAs tested, absolute levels were similar under a variety of conditions that represent fast (YPD, 90 min doubling time) and slow (YP-lactate, 5 h doubling time) growth rates. However, absolute RNA levels for these genes was decreased 3- to 5-fold when KY114 was grown in YP-galactose medium and approximately 2-fold in glucose minimal medium. This general reduction in mRNA levels under these conditions is not understood, but does not reflect the growth rate. As expected, *gal1* RNA is not detectable except in YP-galactose medium where it is expressed at a level that is 60% higher than that of *rps4*. The level of *his3* RNA is variable, with higher levels of transcription being associated with a relative increase in initiation from the +13 start site. This behavior is entirely expected, and it is due to increased Gcn4-dependent activation under conditions of suboptimal growth such as amino acid limitation (12, 13).

DISCUSSION

Generality and Accuracy of the Determinations. In this paper, we have described a general approach for accurately determining absolute mRNA levels in yeast. By comparing the hybridization signal from a known amount of cells to that signal generated by a known amount of RNA, we determine directly that there are 7 ± 1 *his3* mRNA molecules per cell. The major source of error in this value arises from the uncertainty in the conversion factor between A_{260} and amounts of RNA, which depends on the structure of the specific RNA species. We have attempted to correct for the effect of RNA structure by using RNase A to measure the hyperchromic effect. Furthermore,

Table 1. mRNA levels (molecules per cell) of various genes under different growth conditions

mRNA	YPD	YP galactose	YP raffinose	YP lactate	CAA	Min
his3	7 ± 1	1.3 ± 0.3	10 ± 2	18 ± 1	6 ± 0.5	22 ± 3
ded1	25 ± 4	5 ± 1	22 ± 4	23 ± 4	23 ± 3	12 ± 2
rps4	75 ± 10	20 ± 3	72 ± 10	77 ± 10	72 ± 10	28 ± 5
trp3	1.9 ± 0.2	0.5 ± 0.1	1.9 ± 0.3	1.5 ± 0.3	2 ± 0.2	1.2 ± 0.2
gal1	<0.1	33 ± 5	<0.1	<0.1	<0.1	<0.1

mRNA levels were determined in KY114 cells grown in YP medium containing the indicated carbon sources at 2% or in glucose minimal medium that either lacked (Min) or contained (CAA) 1.2% casamino acids.

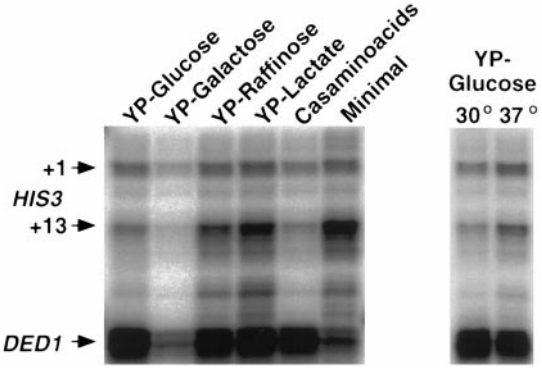

FIG. 5. Analysis of *his3* and *ded1* mRNA levels under various growth conditions. RNA from the identical amount of cells (determined by viable count) grown in the indicated media were hybridized to *his3* and *ded1* probes.

we independently determined the amount of *in vitro* synthesized RNA by performing the T7 RNA polymerase reaction with ^{32}P-labeled precursors and measuring the amount of radioactivity incorporated into the RNA product. As the difference between these two methods for quantitating RNA were within ±4%, we believe that our overall estimate of error (± 15%) is conservative.

Although our approach for measuring absolute levels of *his3* RNA is direct and can be applied to measure the level of any yeast RNA species, it is rather tedious. We therefore measured the absolute levels of other mRNAs by determining their amounts relative to that of *his3*. In addition to the errors associated with the determination of *his3* mRNA levels, this approach has an additional source of error that is due to differences in the specific activities of the hybridization probes. To minimize this problem, equimolar amounts of the oligonucleotides (determined by A_{260}) were phosphorylated in the same reaction under conditions of excess [^{32}P]ATP. In general, this procedure results in probes of equal specific activity (error ± 10%), as assessed by quantitating the amount of ^{32}P incorporated into each of the oligonucleotides. However, this must be examined in each case because, for unknown reasons, certain phosphorylation reactions lead to less equal labeling of the oligonucleotides.

Given our measurements of the number of *his3* and other mRNAs per cell in a variety of growth conditions, absolute mRNA levels for any yeast gene of interest can be easily determined in a single hybridization experiment. In this regard, *his3* is not an ideal internal standard because its levels vary according to growth conditions. Determining mRNA levels under other growth or genetic conditions is easily accomplished by quantitating parallel hybridization reactions containing RNA from the same number of cells. It is important to note that our methodology does not measure the level of full-length mRNA, but rather the amount of RNA that is complementary to the small portion of the mature species that corresponds to the oligonucleotide probe. However, as mRNA degradation intermediates are very unstable, it is likely that our measurements will reflect the level of full-length RNA. Indeed, the ratio of *his3* and *ded1* mRNAs determined in this paper is in excellent accord with the ratio determined by Northern blot analysis and cDNA hybridization (2). In addition to our preferred method, there are other procedures for measuring the relative molar amounts of mRNA species, and in conjunction with our measurements of absolute mRNA levels, these can be used to calculate the absolute level of any mRNA of interest.

Absolute Rates of Transcriptional Initiation *in Vivo*. Our measurements of the number of mRNA molecules per cell enable the calculation of transcriptional initiation rates *in vivo*, a mechanistically important parameter that is very difficult to determine directly. The steady-state level of an mRNA species depends on both its rate of initiation and degradation. The half-lives of a number of yeast mRNAs, including *his3* (7 min) and *ded1* (4 min) have been determined at 37°C by measuring RNA levels after thermal inactivation of a temperature-sensitive RNA polymerase II (5), and we determine here that the half-life of *his3* mRNA is 11 min at 30°C. Although mRNA degradation is a complex process, likely involving many nucleases and targeting factors, it appears to follow first-order kinetics (5, 6). Thus, the decay of mRNA can be expressed as $N = N_0 e^{-\lambda t}$, where N_0 is the initial mRNA concentration, N is the concentration after time t, and λ is the decay constant that is related to the half-life τ by the equation $\lambda = \ln 2/\tau$.

Given the above assumption, measurements of mRNA decay rates, and the levels of mRNA per cell measured in this paper, we calculate that the rate of *his3* transcriptional initiation in YPD medium at 30°C is one transcript per 140 s. At 37°C, *his3* transcripts are initiated every 53 s, whereas that *ded1* transcripts are initiated every 15 s. In other studies in which a wide variety of *his3* promoter derivatives were analyzed at 30°C, we have observed that *his3* RNA levels reach a maximal value (12, 13). Given the results in this paper, we now calculate that this maximal level of *his3* RNA is 120–150 molecules per cell, which corresponds to one transcript being initiated every 6–8 s. This value is in excellent accord with the initiation rate of the highly active *Drosophila hsp70* gene, which was determined by measurements of RNA polymerase density and transcriptional elongation rates *in vivo* (14, 15). Remarkably, this value is also similar to the maximal initiation rate of *E. coli* RNA polymerase *in vivo* (one transcript every 2–3 s) (16) and T7 RNA polymerase *in vitro* (one transcript every 2 s) (17). Thus, it appears that the minimal time for initiation and promoter clearance is highly conserved among prokaryotic and eukaryotic RNA polymerases.

We thank Mark Lee for suggesting the use of PCR to increase the yield and specificity of the *in vitro* synthesized *his3* RNA, Stan Tabor for advice on purifying UTP, Maria Person for calculations, and Alan Jacobson, John Lis, Will McClure, Stan Tabor, and Jonathan Warner for fruitful discussions. This work was supported by a grant to K.S. from the National Institutes of Health (GM30186).

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