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Regulation of Translation Rate during Morphogenesis in the Fungus *Mucor*†

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ABSTRACT: The present study was undertaken in order to elucidate the molecular mechanisms responsible for regulating changes in the specific rate of protein synthesis during the yeast-to-hyphae morphogenesis in the fungus, *Mucor racemosus.* The distribution of ribosomes between active polysomes and monosomes and inactive subunits was determined by means of pulse-labeling and density gradient fractionation techniques. The percentage of ribosomes active in protein synthesis was observed to decrease throughout the morphological transition. The rate of amino acid addition to nascent polypeptide chains was calculated and the transit time of messenger RNA translation was measured. The results showed a significant increase in the velocity of ribosome movement along the message which was continuously adjusted throughout hyphal development.

Mucor racemosus is a dimorphic phycomycete which can be stimulated to undergo a rapid and synchronous morphological change by altering the composition of the atmosphere under which the cells are grown. The cells grow as budding yeasts under a CO_2 atmosphere in the presence of a hexose. Changing the atmosphere from CO_2 to air results in the emergence of hyphae from the yeast cells (Bartnicki-Garcia and Nickerson, 1962; Larsen and Sypherd, 1974). Measurements of the kinetics of radioactive amino acid incorporation into protein indicate that there is an acceleration in the specific rate of protein synthesis during initial germ tube emergence which later declines during further hyphal elongation (Orlowski and Sypherd, 1977). Protein is synthesized throughout

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the entire developing cell structure (Orlowski and Sypherd, 1978) and may include some portion that represents new gene products (Orlowski and Sypherd, 1977). The present experiments were performed in order to investigate the quantitative regulation of protein synthesis during the yeast-to-hyphae morphogenesis of *Mucor*. The distribution of ribosomes between active polysomes and inactive subunits was determined. The rate of amino acid addition to growing polypeptide chains was calculated and the transit time of mRNA[1] translation was measured. The data presented here show that the percentage of ribosomes active in protein synthesis and the velocity of ribosome movement along the mRNA are regulated during morphogenesis.

Materials and Methods

Organism, Cultivation, and Morphological Conversion. *Mucor racemosus* (*M. lusitanicus*) ATCC strain 1216B was used in all experiments. The culture medium used and all procedures involved in the propagation and morphogenetic conversion of the organism were the same as previously described (Larsen and Sypherd, 1974; Orlowski and Sypherd, 1977), except that stationary phase yeast cells (CO_2 atmosphere) were used as an inoculum rather than sporangiospores. The characteristics, time course, and percent of morphological conversion from yeast to hyphae were identical with our previous results (Orlowski and Sypherd, 1977).

Polysome Profiles. Cells at the appropriate stage of development were incubated in the presence of cycloheximide (200 μg/mL) for 5 min, rapidly collected on a Teflon filter (Millipore Corp., Type LC, pore size 10.0 μm), washed briefly with cold TMK buffer (see below) containing cycloheximide, quickly placed into a mortar full of liquid N_2 and vigorously ground with a pestle for 2 min. Cell breakage with this method was always greater than 50% (as determined by the percent release of incorporated radioactive amino acids into a 30 000*g* supernatant fraction) and microscopically appeared representative of all cell types present (i.e., yeast mother cells, buds, and germ tubes broke with equal efficiency). The broken cells were resuspended in cold, sterile buffer (TMK) composed of 50 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 7.25), 10 mM magnesium acetate, and 500 mM KCl which contained 200 μg/mL cycloheximide. Cold, sterile glassware and solutions were used throughout these procedures. The suspension was centrifuged at 15 000*g* for 10 min at 4 °C. The supernatant fraction (S-15) was gently decanted away from the pellet. Portions of the S-15 fraction were incubated on ice for 20 min in the presence of RNase (10 μg/mL), RNase-free DNase (10 μg/mL), or RNase plus DNase or were left untreated. A volume of the S-15 fraction sufficient to contain 4.0 $A_{260\ nm}$ units was layered on top of a 10–40% (w/w) linear sucrose gradient (in TMK buffer, 11.0-mL volume) which rested on a 2 M sucrose cushion (0.8-mL volume). The gradients were centrifuged in a Beckman SW41 rotor at 150 000*g* for 60 min at 4 °C. The gradients were scanned at 254 nm using an ISCO density gradient fractionator, Model 180, attached to a chart recorder.

Protein and RNA Measurements. Total cellular protein was determined by the method of Lowry et al. (1951). Total cellular RNA was measured by the orcinol procedure (Dische, 1953) or the method of Cheung et al. (1974). The resolution of total cellular RNA into the soluble and ribosomal forms was done using the following procedure. Cells were rapidly filtered, washed with, and resuspended in cold TMK buffer. The suspension was passed through a chilled French press. Sodium dodecyl sulfate was immediately added to the broken cell suspension to a final concentration of 1% (w/v). The preparation was extracted three times with cold, water-saturated redistilled phenol containing 0.1% 8-hydroxyquinoline. RNA was precipitated from the aqueous phase by the addition of 2 volumes of absolute ethanol and 0.3 M ammonium formate (final concentration) at −20 °C. The RNA was collected by centrifugation, lyophilized, resuspended in ice-cold TMK buffer, and treated with DNase (10 μg/mL) for 20 min. This preparation was again extracted with water-saturated phenol and precipitated with ethanol and ammonium formate. The precipitate was lyophilized and then dissolved in a buffer (ANE) composed of 10 mM sodium acetate, 100 mM NaCl, and 1 mM ethylenediaminetetraacetic acid (pH 5.3). A volume of solution containing 1.5 A_{260nm} units was layered on top of a 5–20% (w/w) linear sucrose gradient (in ANE buffer, 11.0-mL volume) which rested on a 2 M sucrose cushion (0.8 mL). The gradients were centrifuged in a Beckman SW41 rotor at 77 000*g* for 18 h at 4 °C. The gradients were scanned at 254 nm on the ISCO fractionator and the areas under the peaks integrated gravimetrically to determine the percentage of each RNA species.

Transit Time Determinations. Two hundred milliliters of cells at the appropriate stage of development were collected on a Teflon filter and resuspended in 50 mL of fresh medium contained in a large syringe barrel fixed vertically and fitted with a three-way stopcock. The appropriate gas (CO_2 or air) was bubbled vigorously through the medium to keep the cells homogeneously suspended. L-[^{3}H]Leucine, 200 μCi (45 Ci/mmol), was rapidly injected into the culture and 5-mL portions were drawn at 20-s intervals into 5 mL of ice-cold aqueous cycloheximide (500 μg/mL). The labelled cells were collected on a membrane filter (pore size 0.45 μm), washed with cold TMK buffer containing cycloheximide, and broken under liquid N_2. The broken cells were resuspended in TMK buffer containing cycloheximide and centrifuged at 15 000*g* for 10 min at 4 °C. The supernatant fluid (S-15) was recovered and a portion of it subjected to centrifugation at 150 000*g* for 3 h at 4 °C which yielded a second supernatant fraction (S-150). This centrifugation step separated labeled nascent polypeptides (ribosome bound, in the pellet) from labeled completed protein molecules (released into the S-150). Portions of both the S-15 and S-150 fractions were heated for 20 min at 90 °C in 10% (w/w) trichloroacetic acid. The precipitates were collected on membrane filters, washed with 10% trichloroacetic acid and diethyl ether, dried, and assayed for radioactivity by scintillation spectroscopy. The experimental data were plotted and interpreted according to Scornik (1974).

Results

Polysome Profiles. Figure 1 represents the characteristic patterns obtained on 10–40% linear sucrose gradients from a typical S-15 fraction which had been treated in several specific ways. The profile in Figure 1A represents the "standard preparation," that is, the treatment used in most of the experiments described and which included incubation of the cells in the presence of cycloheximide prior to breaking them (see Materials and Methods). The top of the gradient is at the far right in each gradient-scan shown. Low molecular weight, UV-absorbing material which never entered the gradient is the first peak on the right, observed off-scale. Proceeding left are the ribosome peaks identified as "40S subunits," "60S sub-

[1] Abbreviations used: mRNA, messenger RNA; rRNA, ribosomal RNA; RNase, ribonuclease; DNase, deoxyribonuclease; cAMP, cyclic adenosine 3′,5′-monophosphate; ATP, adenosine 5′-triphosphate; mol wt, molecular weight; UV, ultraviolet; S-15, S-100, and S-150, supernatant fractions resulting from centrifugation at 15 000*g*, 100 000*g*, and 150 000*g*, respectively.

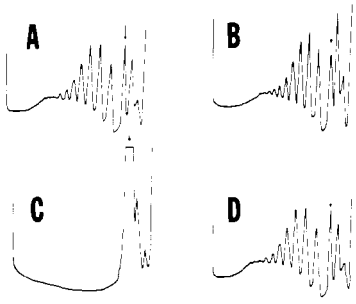

FIGURE 1: A_{254nm} trace of S-15 fraction from CO_2-grown yeast, subjected to specified treatments, fractionated by centrifugation (150 000*g* for 60 min) on 10–40% linear sucrose density gradients. (A) "Standard preparation," cells exposed to cycloheximide (200 μg/mL) before breakage (see Materials and Methods). (B) No cycloheximide treatment of cells before breakage. (C) S-15 fraction incubated on ice with RNase (10 μg/mL) for 20 min. (D) S-15 fraction incubated on ice with DNase (10 μg/mL) for 20 min. The arrow indicates the 80S peak in each gradient scan.

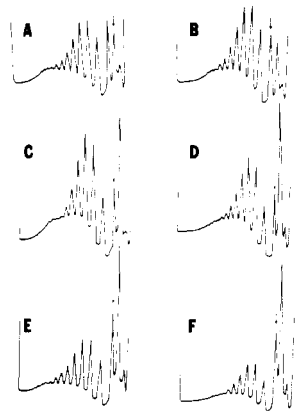

FIGURE 2: Ribosome distribution in cells sampled at specified times throughout the yeast-to-hyphae conversion of *Mucor*. A_{254nm} trace of standard S-15 fractions subjected to centrifugation (150 000*g* for 60 min) on 10–40% linear sucrose density gradients. (A) CO_2-grown yeasts. (B) Yeasts, 5 min after exposure to air. (C) Emerging germ tubes, 2 h after exposure to air. (D) Emerging germ tubes and short hyphae, 4 h after exposure to air. (E) Elongating hyphae, 6 h after exposure to air. (F) Elongating hyphae, 8 h after exposure to air. The arrow indicates the 80S peak.

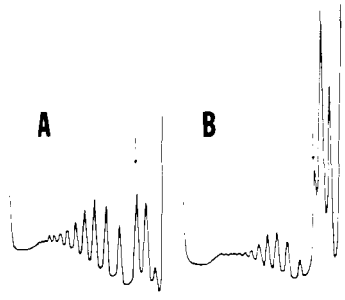

FIGURE 3: Ribosome distribution in cells grown under N_2. Standard preparation of S-15 fractions from exponentially growing cells and sucrose density gradient fractionation used (see Materials and Methods). (A) Yeast cells. (B) Hyphae. The arrow indicates the 80S peak in each gradient scan.

units," "80S monosomes," and polysomes of increasing size up to about 12–14 monosome equivalents in the best resolved gradients. The profile in Figure 1B derives from the same culture as in Figure 1A, but without the addition of cycloheximide. Since only a few seconds elapsed between filtration of the cells and their immersion in liquid N_2 most of the polysomes were preserved, but sufficient run-off did occur to dictate routine preincubation with cycloheximide. The profile in Figure 1C represents the S-15 fraction from Figure 1A treated with RNase. Most of the absorbance from the polysome region of the gradient has shifted into the 80S region. This behavior is typical of polysomal material. Single-stranded RNA (mRNA) has been cleaved, with the resulting degradation of polysomes into 80S ribosomes. Treatment of the S-15 fraction with DNase did not change the profile (Figure 1D), indicating no appreciable DNA component in the profiles. Treatment with RNase plus DNase gave results identical with treatment with RNase alone (not shown). Preincubation of the cells with puromycin caused the complete absence of all absorbance on the gradients heavier than 60 S (not shown). This behavior is also indicative that the material in question is polysomal (Pestka, 1971).

The ribosome distribution between polysomes, monosomes, and subunits in cells before and throughout the yeast-to-hyphae morphogenesis is presented in Figure 2. Budding yeasts, growing under CO_2 and immediately after a shift to an air atmosphere, were observed to have the highest ratio of polysomes to subunits of all cells examined (Figure 2A,B). As the cells remained in air for progressively longer periods of time, and cellular morphology became more hyphal in character, the polysome-to-subunit ratio became consistently lower (Figure 2C–F). The same relationship between cellular morphology and ribosome distribution was observed in cells that had been grown as yeast or hyphae under nitrogen gas according to the conditions of Mooney and Sypherd (1976) (Figure 3). Mixing experiments were performed in which an S-15 fraction from CO_2-grown yeast cells (high polysome/subunit ratio) was mixed with a ribosome-free S-100 fraction from hyphal cells that had been exposed to air for 6 h (low polysome/subunit ratio). No change in the distribution of ribosomes from the pattern in the original S-15 was observed and there was no degradation of polysomes to 80S particles or subunits (not shown). The ribosome profiles from differentiating hyphal cells were not generated by the action of a RNase or other soluble agent which cleaved mRNA or caused ribosomal run-off or release from the message. Polysomes in S-15 fractions prepared from this organism at any stage of morphogenesis were observed to be completely stable for at least 24 h at 4 °C. Although fewer polysomes were present at progressive times, the weight distribution of the polysomes did not change appreciably throughout the period of morphogenesis. The most frequent class of polysomes during morphogenesis was always the tetramers (Figure 2). Discrete peaks of up to 12–14 monosome equiv in size were usually observable. An alternative, more gentle method of breaking the cells was employed to determine if larger polysomes than those described could be obtained. Spheroplasts were prepared by exposing cells (treated with cycloheximide and suspended in buffer plus 0.35 M sorbitol, or growing in culture medium plus 0.35 M sorbitol) to a mixture of chitinase (Calbiochem) and chitosanase (purified from *Myxobacter* AL-1, procedure developed by P. T. Borgia, personal communication). The spheroplasts were extensively washed and gently lysed in low ionic strength buffer excluding sorbitol. After addition of TMK buffer, an S-15 fraction was prepared and polysome profiles were obtained as above. The polysomes obtained with this procedure failed to show a heavier distribution than those prepared in the usual way.

Determination of Percent Active Ribosomes. It was assumed that all ribosomes in the form of polysomes were actively engaged in protein synthesis. The high salt concentration used in all buffers and gradients should have caused any run-off 80S ribosomes (i.e., not attached to mRNA) to dissociate into 60S

TABLE I: Polypeptide Chain Elongation Rate and Other Measured Parameters during the Yeast-to-Hyphae Conversion of *Mucor racemosus*.[a]

Time rel to shift (h)	μ (doublings/h)	Ratio, RNA/protein	% rRNA	Ribosomes/ mg of protein	% active ribosomes	Polypeptide chain elongation rate (AA per s per ribosome)[b]
−4	0.179	0.362	83.2	8.9×10^{13}	82.4	2.35
−2	0.179	0.385	83.2	9.4×10^{13}	82.4	2.22
0	0.476	0.416	83.2	10.2×10^{13}	87.1	5.16
1	0.476	0.420	87.5	10.8×10^{13}	80.5	5.16
2	0.476	0.420	87.9	10.9×10^{13}	80.0	5.27
4	0.476	0.420	86.7	10.7×10^{13}	62.0	6.85
6	0.476	0.420	88.6	11.0×10^{13}	51.0	8.19
8	0.476	0.480	85.5	12.1×10^{13}	45.0	8.32

[a] Cells were sampled at the times indicated in column 1. At time zero the atmosphere to which the cells were exposed was changed from CO_2 to air. The growth rate was measured on the basis of total protein accumulation in the culture with time. The number of ribosomes/mg of protein was calculated using the combined molecular weights of *M. racemosus* rRNA reported by Lovett and Haselby (1971) (i.e., 2.04×10^6). Dividing this value by Avogadro's number yields the weight of rRNA per 80S ribosome (i.e., 3.38×10^{-18} g/ribosome). The data in columns 3 and 4 permit the final calculation of this value (shown in column 5). All other values were determined as described in the text. [b] Abbreviation used: (AA per s per ribosome), amino acids added per second per ribosome.

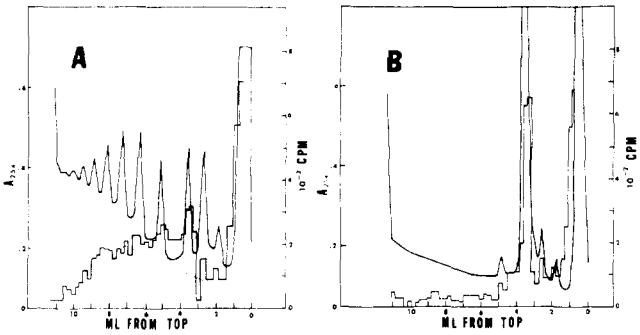

FIGURE 4: Ribosome profile and distribution of radioactivity incorporated into trichloroacetic acid insoluble material from cells following a short (<60 s) pulse with L-[^{3}H]leucine. Standard S-15 fractions and sucrose density gradient fractionation were used. The centrifuge run-time at 150 000*g* was 90 min rather than the usual 60 min. Data presented are from CO_2-grown yeast cells. The arrow indicates the 80S peak. (A) Standard preparation. (B) RNase-treated S-15 fraction. Notice that the radioactivity entering the gradients mainly accompanies the ribosomes, whether in polysomal form or RNase-generated 80S particles.

and 40S subunits (Martin, 1973; Martin and Hartwell, 1970). Consequently, all 80S particles occurring in the sucrose gradients should theoretically represent either breakdown products of polysomes or single actively translating ribosomes, perhaps beginning or ending translation of an mRNA molecule. However, an experimental measurement was made to get an actual determination of the percentage of 80S ribosomes active in protein synthesis. The procedure used was basically that of Forchhammer and Lindahl (1971). Cells were pulsed with L-[^{3}H]leucine (40 μCi/mL; 45 Ci/mmol) for <60 s, exposed to cycloheximide (200 μg/mL) for 5 min, filtered, washed, broken under liquid N_2, and fractionated on sucrose density gradients as described in the Materials and Methods. Fractions of the sucrose gradients were collected as the gradients were being scanned for A_{254nm}. The fractions were treated with trichloroacetic acid, filtered, washed, and assayed for radioactivity as described in the Materials and Methods. The results, some of which are depicted in Figure 4, showed that in all stages of morphogenesis the 80S peaks were labeled to the same extent as the polysomal regions of the gradients. This indicated that all of the ribosomes in the 80S peak can be considered active. The percentage of all cellular ribosomes which are active in protein synthesis is therefore taken to be the sum of all polysomes plus all 80S ribosomes. Some of the 80S peaks shown in Figures 1–3, in which the centrifugation run-time was 60 min, are not well separated from the 60S subunit peaks. This was the case mainly in extracts with a large proportion of ribosomal subunits relative to polysomes, i.e., in extracts from developing hyphae. In order to more accurately quantify the amount of 80S material present in these cell extracts, centrifugation run-time was increased to 120 min. This increased run-time better resolved the 80S from the 60S peaks but compressed the polysomal region against the 2 M sucrose cushion (not shown). The compression of the polysome region obscured the polysome weight distribution but did not interfere with quantitation of the total amount of this material. An accurate determination of the percentage of total cellular ribosomes actively engaged in protein synthesis could be made at every stage of cellular differentiation and is presented in column 6 of Table I.

Rate of Nascent Polypeptide Chain Elongation. Calculation of the rate of polypeptide chain elongation was determined using the basic procedures and theory of Forchhammer and Lindahl (1971). The parameters measured were those necessary to complete the following relationship:

$$\frac{\text{protein}/120 \times \mu \times \ln 2}{\text{rRNA}/\text{mol wt of rRNA} \times \%\text{ active ribosomes} \times 3600} = \text{amino acids per s per ribosome}$$

where "μ" is the growth rate in doublings/h, "ln 2" is from the growth rate equation, "3600" is the number of s per h, and "120" represents the average molecular weight of the 20 naturally occurring L-amino acids. Total cellular protein, rRNA, and the percent of ribosomes active in protein synthesis were measured as described above. The molecular weights of the rRNA species from *M. racemosus* were previously reported (Lovett and Haselby, 1971). Intracellular protein turnover must be negligible in order for the calculated elongation rates to be accurate. We have previously reported the absence of detectable protein turnover at all stages of *Mucor* morphogenesis (Orlowski and Sypherd, 1977). The calculated rates of polypeptide elongation in cells throughout the morphogenetic processes are presented in column 7 of Table I. The values are constant, at about 2.3 amino acids added per s per ribosome, during yeast-like growth under CO_2. Upon changing the atmosphere to air, the rate immediately increased to about 5.2 amino acids per s per ribosome during germ tube emergence and later gradually increased to about 8.2 amino acids

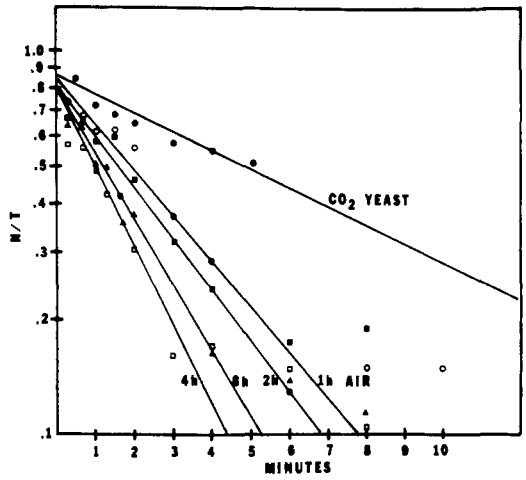

FIGURE 5: Kinetics of radioactive amino acid flow from nascent polypeptides to completed and released protein molecules. Represented as the rate of decay of the percent of total incorporated label occurring in the ribosome-bound (nascent protein) fraction (N/T). Cells were examined at the times indicated: (●) CO_2-grown yeast; (○) yeast cells, exposed to air for 1 h; (■) emerging germ tubes, in air for 2 h; (□) emerging germ tubes and short hyphae, in air for 4 h; (▲) elongating hyphae, in air for 8 h.

per s per ribosome during further hyphal elongation. Several other measured parameters and calculated values are also presented in Table I. The growth rates shown (column 2) are similar to published values (Larsen and Sypherd, 1974; Paznokas and Sypherd, 1975; Orlowski and Sypherd, 1976). The total cellular RNA/protein ratio (column 3), percent rRNA (column 4), and number of ribosomes/mg of cellular protein (column 5) did not change significantly during the morphogenesis. The percent of ribosomes active in protein synthesis (column 6) decreased throughout hyphal development.

Transit Time of mRNA Translation. The transit time is the period of time required for a ribosome to bind to an mRNA, complete translation, and release a finished polypeptide. The kinetics of radioactive amino acid transfer from nascent polypeptide chains to completed proteins are directly related to the transit time (Haschemeyer, 1969; Fan and Penman, 1970; Scornik, 1974). Centrifugation at 150 000*g* was used to separate broken cells into a ribosome fraction, where labeled nascent polypeptides were located, and a supernatant fraction, where labeled finished proteins were found (see Materials and Methods). The distribution of label between these two fractions following a pulse of L-[^{3}H]leucine was quantitated over very short time intervals and plotted as the percent of radioactivity in nascent protein (*N/T*). We have previously shown that the kinetics of label incorporation into total cellular protein attain linearity within 1–2 min at all stages of *Mucor* morphogenesis (Orlowski and Sypherd, 1977). Budding yeasts and cells throughout hyphal development were examined (Figure 5). Transit times were calculated from the kinetics presented in Figure 5 according to the analysis of Scornik (1974). The transit time was determined as the period of time in which *N/T* decreased by one-half (from 0.5 to 0.25). The calculated transit time values are presented in Table II. Also presented in Table II for comparative purposes are the polypeptide chain elongation rates from Table I and the average molecular weights of cellular proteins synthesized at the times indicated which were calculated from the elongation rates and transit time data (transmit time × elongation rate × 60 s × 120 = average protein molecular weight). Assuming there is no major change in the actual molecular weight distribution of proteins made

TABLE II: Calculated Transit Times of mRNA Translation during the Yeast-to-Hyphae Conversion of *Mucor racemosus*.[a]

Time rel to shift (h)	Transit time[b] (min)	Polypeptide chain elongation rate (AA per s per ribosome)	Av protein mol wt[c,d]
Preshift	6.1	2.3	101 000
1	2.6	5.25	98 000
2	2.2	5.3	84 000
4	1.5	6.9	75 000
8	1.7	8.3	101 000

[a] Cells were sampled at the time indicated in column 1. At time zero the atmosphere to which the cells were exposed was changed from CO_2 to air. [b] Calculated from the kinetic data presented in Figure 5. [c] Abbreviations used: (AA per s per ribosome), amino acids added per second per ribosome; av protein mol wt, average protein molecular weight. [d] Calculated from the following product: transit time × polypeptide chain elongation rate × 60 s/min × 120.

at the different times, the elongation rate and transit time values should change only in strict coordination with one another. Since the calculated average protein molecular weights are a product of these two measured parameters, they should not vary significantly from one another if the methods used are reliable and the measurements accurate. The values presented in Table II indeed did not vary more than 18% from the 92 000 molecular weight average calculated. The data collected using the two different methods therefore correlate well and indicate that the rate of polypeptide chain elongation increases substantially during the yeast-to-hyphae morphogenesis of *Mucor*.

Discussion

We have previously shown that a rapid increase in the differential rate of protein synthesis accompanies the yeast-to-hyphae morphogenesis in *M. racemosus* (Orlowski and Sypherd, 1977). This acceleration in the rate of protein synthesis was not due simply to a change from anaerobic to aerobic metabolism, since it occurred when morphogenesis proceeded in CO_2 to N_2 shifts. The strong correlation of this acceleration of protein synthesis with morphogenesis was the impetus for the present studies. Although many possibilities exist for the mechanism of this acceleration, the results show that there is a substantial increase in the rate of amino acid polymerization per ribosome. These results were obtained by two independent methods: (i) determination of the rate of amino acid addition to the growing polypeptide chain; and (ii) measurement of the transit time of ribosome movement across the mRNA. The results from both sets of measurements indicated the same findings; that is, the velocity of translation increased about 2.3-fold immediately after the change of atmosphere from CO_2 to air and approximately another fold in gradual increments during the ensuing morphological change. The two sets of values, which are in essence reciprocal expressions of the same parameter, should fluctuate in an entirely dependent relationship. This prediction was completely fulfilled by the data. Our conclusion must therefore be that the velocity of ribosome movement along mRNA is regulated during hyphal development in the fungus, *Mucor*.

The potential role of the polypeptide chain elongation rate in regulating the overall rate of protein synthesis has been extensively investigated in both prokaryotes (Coffman et al.,

1971; Forchhammer and Lindahl, 1971; Engbaek et al., 1973; Dalbow and Young, 1975; Martin and Iandolo, 1975; Young and Bremer, 1976) and eukaryotes (Haschemeyer, 1969; Fan and Penman, 1970; Lacroute, 1973; Petersen and McLaughlin, 1973; Scornik, 1974; Alberghina et al., 1975; Boehlke and Friesen, 1975). In prokaryotes, the rate of amino acid addition to growing polypeptide chains remains relatively constant over a wide range of growth rates and nutritional conditions (Coffman et al., 1971; Forchhammer and Lindahl, 1971; Engbaek et al., 1973; Dalbow and Young, 1975; Young and Bremer, 1976). It is only at very slow growth rates or under starvation conditions that any alteration of this parameter is observed (Forchhammer and Lindahl, 1971; Engbaek et al., 1973; Martin and Iandolo, 1975; Young and Bremer, 1976) and even under these conditions the differences are small. For example, a threefold reduction in growth rate of *Escherichia coli* yields a 30% reduction in polypeptide chain elongation rate (Young and Bremer, 1976); a fivefold reduction yields a 43% drop in the same organism (Forchhammer and Lindahl, 1971); and a threefold lowering of growth rate in *Staphylococcus aureus* causes only a 36% change in step time (Martin and Iandolo, 1975). Only during polyamine limited growth in *E. coli*, showing a 40% reduction in growth rate from the control, is a commensurate decrease in the polypeptide elongation rate reported (Jorstad and Morris, 1974). If the general observation is true that the step time of amino acid addition to growing polypeptides is not adjusted outside of rather narrow limits in prokaryotes, then these organisms must mainly control the rate of initiation of new nascent chains.

In eukaryotes, the observations are highly variable. Regenerating rat liver exhibits a measured transit time (1.06 min) identical with that in control organs (Scornik, 1974). Glucagon causes a 38% increase in the transit time (slows polypeptide elongation) in rat liver (Ayuso-Parrilla et al., 1976). Transit time remains constant but the binding of ribosomes to mRNA (initiation) becomes inhibited during mitosis in chinese hamster ovary cells (Fan and Penman, 1970). Toadfish exposed to a large temperature drop (21 to 10 °C) doubled the rate of polypeptide elongation (transit times changed from 6.3 min to 3.4 min) (Haschemeyer, 1969). In fungi, the polypeptide rate is sometimes found to be very responsive to cellular growth rate, as in *Saccharomyces cerevisiae* in which a fivefold change in cellular growth rate (486–96 min doubling times) elicits an approximate fourfold change in the rate of amino acid addition to growing protein chains (2.8–10 amino acids per s per ribosome) (Boehlke and Friesen, 1975). In other cases the response is much less, as in *Neurospora crassa* in which a sevenfold range in cellular growth rate (0.13–0.91 doublings/h) shows only a 50% difference in the rate of polypeptide elongation (12–18 amino acids per s per ribosome) (Alberghina et al., 1975).

Allowing for the simple thermodynamic effect of temperature (*Mucor* was grown at 22 °C, the other fungi at 30 °C, the bacteria at 37 °C, and the mammalian cells at animal body temperature) on chemical reaction rates, all of the measured rates of polypeptide elongation, including those for *Mucor*, fall within a rather narrow range of values. This indicates that the efficiency of the translational machinery does not differ very much among these diverse organisms. The limits within which the speed of translation is adjusted in *Mucor* make this fungal system one of the most versatile yet reported. It is perhaps the first system in which regulation of this molecular process has been reported to accompany morphogenesis. Not only is the rate of protein synthesis during *Mucor* morphogenesis regulated at the level of polypeptide chain elongation, but also by the percent of ribosomes actively engaged in translation. The percent of active ribosomes decreases at the same time the chain elongation rate increases. The observed rate of protein synthesis should represent the net result of these two opposing mechanisms. It is conceivable that the decreased number of active ribosomes found late in the morphogenesis could be a function of decreased rates of mRNA synthesis or decreased rates of nascent polypeptide chain initiation. Alternatively, these rates may remain static but become limiting in the face of the large increase in the polypeptide chain elongation rate. The present data, unfortunately, do not contain information about the polypeptide chain initiation rate.

Other work presently being carried out in this laboratory may ultimately uncover the molecular basis for the observed increased rate of ribosome movement along mRNA. The phosphorylation of a specific ribosomal protein is greatly elevated during the yeast-to-hyphae morphogenesis, coinciding with the increase in rate of protein synthesis and the altered step time of translation (Larsen and Sypherd, unpublished). At the same time intracellular concentrations of ATP (Orlowski, Dimmitt, and Sypherd, unpublished) and spermidine (Peters and Sypherd, unpublished) rise significantly and cAMP levels decrease (Larsen and Sypherd, 1974). Whether these measured parameters directly influence the efficiency of ribosome function during *Mucor* morphogenesis has yet to be determined.

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Antibodies to the Carboxyl-Terminal Fragment of Human Chorionic Gonadotropin β-Subunit: Characterization of Antibody Recognition Sites Using Synthetic Peptide Analogues†

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ABSTRACT: The immunochemical specificities of an antiserum (H-93) generated by immunization of a rabbit with a bovine serum albumin conjugate of a unique COOH-terminal tricosaglycopeptide fragment (residues 123–145) isolated after tryptic digestion of the S-carboxymethylated, desialylated β-subunit of human chorionic gonadotropin (hCG) were analyzed systematically with a radioimmunoassay system. Using [^{125}I]hCG as labeled antigen, a series of 32 synthetic peptides of various lengths, analogous to the polypeptide sequence of the native antigen, was evaluated for antigenic recognition by the antiserum. Immunological cross-reactivity with the dipeptide, Pro-Gln, but not with Gln or Pro-Glu was observed. The degree of immunoreactivity increased with increasing chain length and reached a plateau at the pentadecapeptide, which was equipotent with a highly purified hCG. The dose–response curves of hCG and the pentadecapeptide or longer synthetic peptides corresponding to the COOH-terminal peptide were superimposable. Thus, the antibody recognition was shown to reside at the last 15 amino acid residues of the COOH-terminal peptide of hCGβ. Evidence is also presented to indicate that a free COOH terminus is not essential for immunological cross-reactivity. Although cross-reactivity increased most significantly over the peptide sequence Arg-Leu-Pro-Gly (residues 133–136), two peptides containing this sequence, but lacking the last portion of COOH-terminal peptide, showed significantly lower (residues 125–137) or insignificant (residues 131–137) cross-reactivity. These results demonstrate that the Pro-Gln dipeptide segment is one of the important recognition units for the antibody and that the addition of the other 13 residues enhances the cross-reactivity by 5×10^4. Four more antisera produced in rabbits treated with the identical immunogen exhibited binding characteristics very similar to that of the H-93 antiserum. These studies describe the nature of the sites of antibody recognition and provide the basis for the high degree of specificity for hCG of the H-93 antiserum without cross-reactivity to structually similar hLH.

Human chorionic gonadotropin (hCG)[1] is a glycoprotein hormone of pregnancy, normally synthesized and secreted by the placenta and detectable in peripheral serum and urine coincident with implantation of the blastocyst 7–9 days after fertilization (Braunstein et al., 1973; Kosasa et al., 1973; Landsman and Saxena, 1976). Physiologically hCG stimulates enhanced and prolonged secretion of progesterone by the corpus luteum into early pregnancy and may stimulate development of fetal gonads during mid-gestation (Clements et al., 1976). HCG is measurable in serum and urine throughout pregnancy and, because of its long circulatory half-life, has been detected more than a week into the postpartum interval (Faiman et al., 1968; Jaffe et al., 1969). Except for the presence of hCG in the fluids and tissues of persons bearing neoplasms, trophoblastic or otherwise (Goldstein et al., 1974; Vaitukaitis et al., 1976), this glycoprotein had been regarded as a hormone exclusive to pregnancy. However, recent findings indicate the presence of an hCG-like substance in testicular tissue of normal men (Braunstein et al., 1975), in urinary and pituitary extracts of normal subjects (Chen et al., 1976b), as well as in media of bacterial populations (Livingston and Livingston, 1974; Cohen and Strampp, 1976). Accordingly, it is important to extend these new observations employing a specific hCG assay system that can discriminate hCG from hLH.

HCG consists of dissimilar α and β subunits (Canfield et al., 1971), much like other glycoprotein hormones, including hLH, hFSH, and hTSH. Although the α subunits of all these hormones have very similar primary structures, their respective β subunits are structurally different, thereby imparting expressions of specific hormonal activities (Pierce, 1971). However, the β subunits of hCG and hLH show remarkable similarity in amino acid sequence (Morgan et al., 1975). Even the nature of their biological activities is nearly analogous, in that they compete for the same receptors in both testicular (Catt et al., 1972) and ovarian tissues (Lee and Ryan, 1972;

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[1] Abbreviations used are: hCG, human chorionic gonadotropin; hLH, human luteinizing hormone; hTSH, human thyroid stimulating hormone; hFSH, human follicle stimulating hormone; RIA, radioimmunoassay; Boc-, *tert*-butyloxycarbonyl-; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; BSA, bovine serum albumin.