Growth-rate-dependent adjustment of ribosome function in the fungus *Mucor racemosus*

Michael ORLOWSKI

Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

(Received 27 August 1980/Accepted 14 January 1981)

The dimorphic fungus *Mucor racemosus* was grown at rates between 0.043 and 0.434 doubling/h while maintained as yeasts or at rates between 0.21 and 0.50 doubling/h while maintained as hyphae by altering the composition of the growth medium or the gaseous environment of the cells. Yeasts at the higher growth rates contained many more ribosomes than did yeasts at the lower growth rates. They also had a higher percentage of ribosomes active in protein synthesis and a faster rate of polypeptide-chain elongation than did the slower-growing cells. Hyphal cells at faster growth rates also contained many more ribosomes and showed a faster rate of polypeptide-chain elongation than did slower-growing cells. However, the faster-growing cells had a substantially lower proportion of ribosomes active in protein synthesis than did the slower-growing hyphae. Pulse-chase experiments failed to provide any evidence of protein turnover, which might otherwise invalidate the values calculated for the peptide-chain elongation rates.

There are essentially only two ways in which a cell can regulate the overall rate of protein synthesis: (i) adjustment of the rate of nascent polypeptide-chain elongation and (ii) adjustment of the number of nascent polypeptide chains. The rate of peptide-chain elongation as a function of cell growth rate has been studied extensively in bacteria. Most of the early work suggested a constant value for this parameter (Coffman *et al.*, 1971; Forchhammer & Lindahl, 1971; Dalbow & Young, 1975; Young & Bremer, 1976). This contention has been challenged, and a more pliable relationship between the rate of translation and the rate of cell growth, especially at slow growth rates, has been proposed (Forchhammer & Lindahl, 1971; Martin & Iandolo, 1975; Young & Bremer, 1976).

There has been relatively little work done relating peptide-chain elongation rates and cell growth rates of eukaryotic micro-organisms. What little has been published on the subject abounds with contradictions. Boehlke & Friesen (1975), using a method based on the recovery of active polyribosomes, determined that the peptide-chain elongation rate varied linearly with the growth rate over a 5-fold

range of doubling times in the fungus *Saccharomyces cerevisiae*. Bonven & Gulløv (1979) corroborated these findings in *S. cerevisiae*, using a method by Gausing (1972) based on the kinetics of radioactive labelling of nascent and completed polypeptides. In contrast, Waldron *et al.* (1977), using the same organism and the same method as Bonven & Gulløv (1979), concluded that the rate of amino acid addition to growing protein chains was constant. Alberghina & Sturani (1975) studied *Neurospora crassa*, using methods similar to those of Boehlke & Friesen (1975); however, they found little variance in the peptide-chain elongation rate over a 7-fold range of growth rates. Orłowski & Sypherd (1978a), using both types of methodology, reported a 4-fold increase in the rate of peptide-chain elongation during yeast-to-hypha conversion in the fungus *Mucor racemosus*. This morphogenetic process is accompanied by a 3-fold increase in the cellular growth rate.

Adjustment of the percentage of ribosomes actively engaged in translation is the major alternative mechanism for regulating the overall rate of protein synthesis. This parameter has also been studied as a function of growth rate in the above-mentioned fungal systems. Boehlke & Friesen (1975), as did Alberghina & Sturani (1975), found the percentage of active ribosomes to be constant over a wide range of growth rates. To the contrary,

Abbreviations used: TMK buffer, 50 mM-Tris/HCl (pH 7.25), 10 mM-magnesium acetate, 500 mM-KCl; TMKC buffer, TMK buffer containing 200 µg of cycloheximide/ml.

Waldron *et al.* (1977) and Bonven & Gulløv (1979) reported a greater percentage of active ribosomes in faster-growing cells than in slower-growing cells, whereas Orlowski & Sypherd (1978a) found a higher proportion of active ribosomes in the slower-growing than in the faster-growing cells.

In the present study I have grown the fungus *M. racemosus* in either the yeast or hyphal form exclusively over a wide range of growth rates by controlling the nutrients in the medium or the gaseous environment of the cells. I was thus able to study the regulation of the overall rates of protein synthesis in this organism uncoupled from cellular morphogenesis.

Materials and methods

Organism and growth conditions

Mucor racemosus (A.T.C.C. 1216B), a leucine auxotroph (*leu-2A*) derived from this strain (Peters & Sypherd, 1978), or a mutant strain (*coy*) that can grow only as a yeast in the absence of added methionine (Sypherd *et al.*, 1979), were used in these experiments. Several different growth media were used. YPG medium contained 0.3% (w/v) yeast extract, 1.0% (w/v) Bactopeptone (Difco, Detroit, MI, U.S.A.) and 2.0% (w/v) glucose. All of the defined media contained the following vitamins and basal salts (per litre): 1 mg of biotin, 1 mg of thiamin, 0.5 g of KH_2PO_4 , 0.5 g of Na_2HPO_4 , 0.5 g of MgSO_4 , 20 mg of MnSO_4 , 10 mg of CaCl_2 , 10 mg of ZnSO_4 , 10 mg of FeCl_3 and 10 mg of CuSO_4 . All yeast cultures were supplemented with 1.5 g of sodium glutamate, 1 g of $(\text{NH}_4)_2\text{SO}_4$ and 2 g of glucose (all per litre). Hyphal cultures were supplemented with 2 g of glucose or 2 g of mannitol as a carbon source and 1 g of $(\text{NH}_4)_2\text{SO}_4$ or 1 g of urea as a nitrogen source (all per litre). Cultures of the leucine auxotroph were supplemented with 100 μg of L-leucine/ml. All media were adjusted to pH 4.5 with conc. H_2SO_4 . All cultures were inoculated with 5×10^5 sporangiospores/ml. The spores were prepared as previously described (Orlowski, 1979) and washed with distilled water before inoculation. All cultures were grown at 22°C with shaking. Cultures were sparged with water-saturated air, 100% N_2 or 100% CO_2 as appropriate. Growth was monitored by measuring the A_{600} of the cultures. Concomitant with each absorbance measurement, samples of culture were collected and stored frozen for later measurement of protein and RNA. All cultures were allowed to grow to an A_{600} of 0.4–0.6 before use in any experiment. At such time all yeasts had multiple buds, and all hyphal cells had short germ tubes approx. 2–10 spore diameters in length.

Determination of active ribosomes

The procedure of Orlowski & Sypherd (1978a)

was used to measure the proportion of ribosomes active in protein synthesis. Cycloheximide (200 μg /ml final concn.) was added to the culture medium. After 2 min the cells were collected on a membrane filter (Millipore Corp., Type AA, pore size 0.8 μm), washed briefly with cold TMKC buffer and ground under liquid N_2 with a mortar and pestle. The broken cells were resuspended in TMKC buffer and centrifuged at 15000g for 10 min at 4°C. A volume of the supernatant fraction containing 4.0 A_{260} units was layered on a 10–40% (w/w) linear sucrose gradient (in TMK buffer, 11.0 ml) resting on a 60% (w/w) sucrose cushion (0.8 ml). Gradients were centrifuged in an SW41 rotor (Beckman) at 150000g for 80 min at 4°C. The gradients were scanned at 254 nm with an ISCO model 640 density-gradient fractionator. Previous analyses (Orlowski & Sypherd, 1978a) of the material on the gradients indicated properties diagnostic of polyribosomes, 80S, monoribosomes and 60S and 40S ribosomal subunits. Altering the centrifugal force (5000g to 27000g) in the preparatory step or treatment of the broken cell suspensions with Triton X-100 or deoxycholate did not change the ribosome profiles obtained on the gradients. Further extraction of the 15000g pellets with buffer or the aforementioned detergents liberated only small amounts of polyribosomes, which had the same size distribution as found in the initial supernatant fractions. Treatment of the broken cell suspensions with ribonuclease A, which converts all polyribosomes into 80S particles, did not change the observed subunit/active-ribosome ratios. Though the existence of a potential population of membrane-bound or very heavy polyribosomes not quantitatively recovered in these procedures is possible, the above observations minimize this possibility. The procedure of Forchhammer & Lindahl (1971) was previously used to show that all polyribosomes and all 80S monoribosomes in *Mucor* are active in translation (Orlowski & Sypherd, 1978a). The correct determination of active ribosomes thus includes the sum of all polyribosomes plus all 80S monoribosomes.

Protein and RNA measurements

Total cellular protein was measured by the procedure of Lowry *et al.* (1951). Total cellular RNA was measured by the method of Cheung *et al.* (1974). The proportion of total cellular RNA representing rRNA was determined by fractionating purified RNA on sucrose-density gradients by the method of Orlowski & Sypherd (1978b).

Cellular ribosome density

The number of ribosomes per g of total cellular

protein was calculated from the following relationship:

$$\frac{\text{RNA} \times \% \text{ of rRNA} \times \text{Avogadro's number}}{\text{Protein} \times \text{mol.wt. of rRNA}} = \text{no. of ribosomes/g of protein}$$

Protein, RNA and the % of rRNA were determined as described above. The combined molecular weights of rRNA species from *M. racemosus* as reported by Lovett & Haselby (1971) was 2.04×10^6 . Avogadro's number is 6.02×10^{23} .

Calculation of peptide-chain elongation rates

Peptide-chain elongation rates were calculated as described by Forchhammer & Lindahl (1971). The parameters measured were those necessary to complete the following relationship:

$$\frac{(\text{Protein}/120) \times \mu \times \ln 2}{(\text{rRNA}/\text{mol.wt. of rRNA}) \times \% \text{ of active ribosomes} \times 3600} = \text{no. of amino acids/s per ribosome}$$

where μ is the growth rate in doublings/h, $\ln 2$ is from the growth-rate equation, 3600 is the number of s/h and 120 represents the average molecular weight of the 20 naturally occurring L-amino acids. Total cellular protein, rRNA and the percentage of active ribosomes were measured as described above. The molecular weights of the rRNA species from *M. racemosus* were previously reported (Lovett & Haselby, 1971).

Transit-time determinations

For this, 200 ml of leucine-auxotroph cells in mid-exponential growth ($A_{600} = 0.4-0.6$) were collected on a membrane 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 medium was sparged with the appropriate gas (N_2 , CO_2 or air). Then $50 \mu\text{Ci}$ of L-[^3H]leucine was rapidly injected into the culture and 5 ml portions were drawn at intervals into 5 ml of an ice-cold solution of cycloheximide ($500 \mu\text{g}/\text{ml}$) and NaN_3 (10 mM). The labelled cells were collected on a membrane filter, washed with cold TMKC buffer and broken under liquid N_2 . The broken cells were resuspended in TMKC buffer and centrifuged at $15000g$ for 10 min at 4°C . The supernatant fluid (S-15) was recovered and a portion of it centrifuged at $150000g$ for 3 h at 4°C , which yielded a second supernatant fraction (S-150). This centrifugation step separated labelled nascent polypeptides (ribosome-bound, in the pellet) from labelled completed protein molecules (released into the S-150 fraction). Protein in the S-15 and S-150 fractions was precipitated in ice-cold 10% (w/w) trichloroacetic acid, collected on glass-fibre filters (Reeve-Angel, Clifton, NJ, U.S.A.), dried under a heat lamp and assayed for radioactivity by liquid-scintillation spectroscopy (Orlowski & Sypherd, 1977). The data

obtained were plotted and interpreted as described by Scornik (1974).

Assay for protein turnover

Portions (2 ml) of a culture were transferred to small test tubes (13 mm $\times$ 100 mm), where bubbling with the appropriate gas was continued. A radio-active amino acid (L-[^{14}C]valine, L-[^{14}C]proline or L-[^{14}C]leucine) was added to a final concentration of $2.5 \mu\text{Ci}/\text{ml}$. Portions ($100 \mu\text{l}$) of the culture were removed at intervals and placed into 1 ml of 10% (w/w) trichloroacetic acid. Samples were heated for 20 min at 90°C and then chilled for 30 min at 0°C .

The precipitates were collected on glass-fibre filters, washed with ice-cold 10% trichloroacetic acid, dried under a heat lamp and assayed for radioactivity by liquid-scintillation spectroscopy (Orlowski & Sypherd, 1977). After 20 min, a chase of 200-fold excess unlabelled amino acid was added to the culture and the assay of acid-insoluble radioactivity continued as above.

Chemicals

All biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All inorganic chemicals were purchased from Mallinckrodt Chemical Works, St. Louis, MO, U.S.A., and were of analytical grade. L-[3,4,5- ^3H]Leucine (118.3 Ci/mmol), L-[U- ^{14}C]leucine (289 mCi/mmol), L-[U- ^{14}C]valine (250 mCi/mmol) and L-[U- ^{14}C]proline (238 mCi/mmol) were obtained from New England Nuclear Corp., Boston, MA, U.S.A.

Results

Manipulation of cellular growth rate

Figs. 1(a) and 1(b) respectively show the growth response of *Mucor* yeasts and *Mucor* hyphae under a variety of nutritional and atmospheric conditions. Growth rates, expressed in mass doublings/h, were calculated from the slopes of the curves in Figs. 1(a) and 1(b) and are displayed alongside the appropriate growth curves. The yeasts showed a 10-fold variance in growth rate, whereas the hyphae exhibited only a 2.5-fold range for this parameter.

Cellular ribosome population and rate of peptide-chain elongation

Tables 1 and 2 respectively show how the growth rate of *Mucor* yeasts and *Mucor* hyphae correlate with the RNA/protein ratio and with several other

Table 1. *Polypeptide-chain elongation rate and other measured parameters as a function of growth rate in Mucor yeast cells*

For full details, see the Materials and methods section. 'YPG' is yeast-peptone-glucose medium (see the Materials and methods section). Glc-Glu-NH₃ medium contains vitamins and salts as described in the Materials and methods section, glucose (Glc) as carbon source, and glutamate (Glu) and (NH₄)₂SO₄ (NH₃) as nitrogen source.

Growth conditions		μ (doubling/h)	RNA/protein (w/w)	No. of ribosomes per g of protein	Percentage of active ribosomes	Polypeptide-chain elongation rate (amino acid molecules added/ s per ribosome)
Medium	Atmosphere					
YPG	N ₂	0.434	0.49	12.5×10^{16}	85.9	5.5
YPG	CO ₂	0.179	0.37	9.4×10^{16}	82.0	2.2
Glc-Glu-NH ₃	CO ₂	0.050	0.134	3.4×10^{16}	75.5	1.8
Glc-Glu-NH ₃	Air*	0.043	0.142	3.6×10^{16}	71.5	1.9

* Conditional yeast (*coy*) mutant grows as a yeast in air in the absence of methionine.

Table 2. *Polypeptide-chain elongation rate and other measured parameters as a function of growth rate in Mucor hyphae*

For full details, see the Materials and methods section. 'YPG' is yeast-peptone-glucose medium (see the Materials and methods section). The other media contain vitamins and salts as described in the Materials and methods section, glucose (Glc) or mannitol (Man) as carbon source, and (NH₄)₂SO₄ (NH₃) or urea as nitrogen source.

Growth conditions		μ (doubling/h)	RNA/protein (w/w)	No. of ribosomes per g of protein	Percentage of active ribosomes	Polypeptide chain elongation rate (amino acid molecules added/ s per ribosome)
Medium	Atmosphere					
YPG	Air	0.50	0.47	12.0×10^{16}	60.8	6.7
YPG	N ₂	0.43	0.47	12.0×10^{16}	45.7	7.7
Glc-NH ₃	Air	0.33	0.34	8.6×10^{16}	86.0	4.3
Glc-urea	Air	0.24	0.33	8.3×10^{16}	84.1	3.3
Man-NH ₃	Air	0.21	0.22	5.6×10^{16}	75.3	4.8

parameters each having the potential for altering the overall rate of protein synthesis. Examples of the quality of RNA resolution and ribosomal resolution achieved in the present systems have been published (Orlowski & Sypherd, 1978a,b). The percentage of rRNA was a relatively constant $85.7 \pm 2.3\%$ (mean $\pm$ S.E.M., $n = 8$) over a wide range of growth conditions. Cells of relatively fast-growing yeasts and hyphae contained more ribosomes and had faster rates of peptide-chain elongation than did relatively slow-growing cells. When these parameters were plotted versus growth rate, a linear relationship was observed in both cases (Figs. 2a and 2b). The percentage of ribosomes active in protein synthesis did not relate to growth rate in such a straightforward manner. In yeasts there was an increase, though not a linear one, in the proportion of ribosomes active at increasing growth rates (Table 1). However, in hyphae nearly the converse was true. Some slower-growing cells had a higher fraction of ribosomes active in protein synthesis than faster growing cells (Table 2).

Transit time of mRNA translation

The transit time is the period of time required for a

ribosome to bind to an mRNA molecule, complete translation and release a finished polypeptide. The kinetics of transfer of radioactive amino acids from nascent peptide chains to completed proteins are directly related to the transit time (Haschemeyer, 1969; Fan & Penman, 1970; Scornik, 1974). Centrifugation at $150000g$ was used to separate broken cells into a ribosome fraction, where labelled nascent peptides were located, and a supernatant fraction, where labelled completed proteins were found (see the Materials and methods section). The distribution of label between these two fractions after a pulse of L-[³H]leucine was measured over short time intervals and plotted as the fraction of radioactivity in nascent protein (N/T). Transit times were calculated for *Mucor* yeasts and hyphae at several growth rates from the decay kinetics of N/T (Fig. 3) as described by Scornik (1974). The calculated transit-time values are presented in Table 3 (column 5). Assuming that there is no major change in the molecular-weight distribution of proteins made at the different growth rates, the peptide-elongation rates and transit-time values should change in a strict relationship with one another, expressed in the following equation:

$$\text{Transit time} \times \text{peptide-elongation rate} \times 120 = \text{average protein molecular weight}$$

Fig. 1. Growth kinetics of *Mucor* yeasts and hyphae under various nutritional and gaseous environments. Growth rates (μ), expressed in mass doublings/h, were calculated from the slopes of the growth curves and are displayed alongside the appropriate curves. (a) Yeasts. Symbols: $\circ$, YPG medium, N_2 atmosphere; $\square$, YPG medium, CO_2 atmosphere; $\bullet$, defined medium (see the Materials and methods section) with glucose, glutamate and $(NH_4)_2SO_4$, CO_2 atmosphere; $\blacksquare$, defined medium as above, air atmosphere, *cory* mutant strain (all other experiments performed with strain 1216B). (b) Hyphae. Symbols: $\circ$, YPG medium, air atmosphere; $\square$, YPG medium, N_2 atmosphere; $\blacksquare$, defined medium with glucose and $(NH_4)_2SO_4$, air atmosphere; $\blacktriangle$, defined medium with glucose and urea, air atmosphere; $\bullet$, defined medium with mannitol and $(NH_4)_2SO_4$, air atmosphere. All experiments were performed with strain 1216B.

measurements accurate. Such calculations have been made by using the appropriate values of the peptide-elongation rate (column 6 of Table 3) taken from Tables 1 and 2. The calculated values appear in column 7 of Table 3 and are relatively constant. Thus the data from the two methods correlate quite well.

Protein turnover

One important assumption made, which must be true in order for the calculated peptide-elongation rates to be valid, is that protein turnover does not occur to any significant degree. *Mucor* cells pulse-labelled with a radioactive amino acid under a wide variety of different growth conditions never released a significant amount of radioactivity after a chase with unlabelled amino acid. Figs. 4(a) and 4(b) show representative results for hyphal cells growing in various media under air with L-[^{14}C]valine or L-[^{14}C]proline respectively as the label. Identical results were obtained for yeast cells under CO_2 or N_2 with these amino acids or L-[^{14}C]leucine or L-[^{14}C]lysine as the label. These and other previously published experiments (Orlowski & Sypherd, 1977) strongly suggest that little or no protein turnover occurs in actively growing *Mucor* cells.

Discussion

The present data indicate that *Mucor* yeasts and hyphae can effect regulation of the overall rate of protein synthesis by adjusting both the number of nascent polypeptide chains and the rate of nascent-polypeptide-chain elongation. The number of nascent peptide chains in the cell is the product of the number of cellular ribosomes and the percentage of ribosomes active in translation. Changing either of the latter two parameters, of course, alters the first. Both ribosome numbers and percentage of active ribosomes were measured directly in this study. The velocity of translation was determined in two different ways. The number of cellular ribosomes, the proportion of ribosomes active in protein synthesis and the velocity of ribosome movement along the mRNA all varied with the cellular growth rate. All of these parameters appeared to change independently of one another, with the net rate of protein synthesis apparently representing a synergy of all three mechanisms. The results of this work agree more with the findings of Bonven & Gulløv (1979) than with any of the other studies cited in the introduction. Bonven & Gulløv (1979) found that both the velocity of translation and the percentage of ribosomes active in translation varied with the growth rate. As mentioned above, the other studies using fungal material reported a change in only one (Boehlke & Friesen, 1975; Waldron *et al.*, 1977) or none (Alberghina & Sturani, 1975) of these parameters as the cellular growth rate changed.

Since the calculated average protein molecular weights are a product of the two measured parameters, they should not vary significantly from one another if the methods used are reliable and the

Fig. 2. Polypeptide-chain elongation rate (a) and cellular ribosome density (b) as a function of growth rate. Data are taken from Tables 1 and 2.

Table 3. Calculated transit times of mRNA translation in *Mucor* yeasts and hyphae at various growth rates

For full details, see the Materials and methods section. 'YPG' is yeast-peptone-glucose medium. The other media contain vitamins and salts as described in the Materials and methods section, glucose (Glc) as carbon source, and glutamate (Glu) and $(\text{NH}_4)_2\text{SO}_4$ (NH_3), or urea, as nitrogen source.

Cell morphology	Growth conditions		μ (doubling/h)	Transit time (s)	Peptide elongation rate (amino acid molecules added/s per ribosome)	Average protein mol.wt.
	Medium	Atmosphere				
Yeasts	YPG	N_2	0.434	150	5.5	9.9×10^4
Yeasts	YPG	CO_2	0.179	366	2.2	9.6×10^4
Yeasts	Glc-Glu- NH_3	CO_2	0.050	450	1.8	9.7×10^4
Hyphae	YPG	Air	0.500	114	6.7	9.2×10^4
Hyphae	Glc-urea	Air	0.240	228	3.3	9.2×10^4

Alberghina & Sturani (1975) observed a consistently low (50%) proportion of active ribosomes in *N. crassa*, whereas Boehlke & Friesen (1975) reported a constant high percentage (90%) of active ribosomes in *S. cerevisiae*. The latter authors used lysed spheroplasts to make this determination, a condition leading to spuriously high values, as demonstrated by Bonven & Gulløv (1979). The *Neurospora* study (Alberghina & Sturani, 1975) was the only work other than studies on *Mucor* that used a filamentous fungus. Physiologically and morphologically normal cells were broken under liquid N_2 in each case. Previous studies with *Mucor* have shown that the percentage of active ribosomes in hyphal cells can be as low as reported for *N. crassa*, but the values are closely correlated with the stage of hyphal development (Orlowski & Sypherd, 1978a,c). The general pattern was for the fastest-growing hyphae with well-developed germ tubes to have the lowest percentage of active ribosomes. This is why cell morphology was so rigorously controlled

in the present study. Whether it was even considered in the *Neurospora* study is not known.

The relationship between growth rate and the active ribosome population in *Mucor* yeasts was qualitatively similar to that reported for *Saccharomyces* yeasts; however, the very low values reported in slow-growing cells (Waldron *et al.*, 1977; Bonven & Gulløv, 1979) were never seen. Bonven & Gulløv (1979) reported 36% active ribosomes in *S. cerevisiae* growing at 0.17 doubling/h, and Waldron *et al.* (1977) indicated 50% active ribosomes in the same organism growing at 0.3 doubling/h. *Mucor* yeasts growing at much slower rates had a substantially higher percentage of active ribosomes (Table 1).

Though the Bonven & Gulløv (1979) study agrees with the present findings on an adjustable rate of peptide-chain elongation, the work of Waldron *et al.* (1977) does not. Both groups would like to suggest that slow-growing yeasts, as previously reported for bacteria (Koch, 1971), have a large excess of

Fig. 3. Kinetics of flow of radioactive amino acids from nascent polypeptides to completed and released protein molecules

Results are represented as the rate of decay of the fraction of total incorporated label occurring in the ribosome-bound (nascent protein) fraction (N/T). Symbols: ●, yeasts grown under CO_2 in YPG medium; ○, yeasts grown under CO_2 in the defined medium with glucose as carbon source and glutamate plus $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source; ■, yeasts grown under N_2 in YPG medium; □, hyphae grown under air in the defined medium with glucose as carbon source and urea as nitrogen source; ▲, hyphae grown under air in YPG medium. Strain *leu-2A* was used in all these experiments.

Fig. 4. Pulse-chase assay for protein turnover

Results for hyphae grown aerobically in various media are shown. The pulse with a radioactive amino acid was begun at zero time. The chase with unlabelled amino acid was commenced at 20 min (arrows). (a) L-[^{14}C]valine label; (b) L-[^{14}C]proline label. Symbols: ○, YPG medium; □, defined medium (see the Materials and methods section) with glucose and $(\text{NH}_4)_2\text{SO}_4$; △, defined medium with glucose and urea; ●, defined medium with mannitol and $(\text{NH}_4)_2\text{SO}_4$. Strain 1216B was used in all experiments.

inactive ribosomes present in a 'standby' status until pressed into immediate service by a sudden improvement in the environment. The immediate mobilization of these excess ribosomes, rather than an increase in the speed of translation, would initiate the increased rate of protein synthesis upon a shift-up. The support for such a notion is not very impressive for *Mucor* yeasts, and non-existent for *Mucor* hyphae. One priority for future work with the *Mucor* system will be nutritional shift-up experiments. If previous CO_2 -to-air shifts (with accompanying morphogenesis) (Orlowski & Sypher, 1978a) prove to be the equivalent of a nutritional shift-up, the organism will respond to the improved conditions by immediately increasing the peptide-chain elongation rate, but only gradually increasing the number of cellular ribosomes. The percentage of active ribosomes may not change very suddenly or very much. Certainly much remains to be done before a complete understanding of the mechanisms controlling protein synthesis in eukaryotes, or merely in fungi, is possible. Indeed, it still remains to be determined whether different fungi use alter-

native strategies or one universal mechanism to regulate the rate of protein synthesis.

References

- Alberghina, F. A. M. & Sturani, E. (1975) *J. Biol. Chem.* **250**, 4381-4388

- Boehlke, K. W. & Friesen, J. D. (1975) *J. Bacteriol.* **121**, 429–433
- Bonven, B. & Gulløv, K. (1979) *Mol. Gen. Genet.* **170**, 225–230
- Cheung, S. C., Kobayashi, G. S., Schlessinger, D. & Medoff, G. (1974) *J. Gen. Microbiol.* **82**, 301–307
- Coffman, R. L., Norris, T. E. & Koch, A. L. (1971) *J. Mol. Biol.* **60**, 1–19
- Dalbow, D. G. & Young, R. (1975) *Biochem. J.* **150**, 13–20
- Fan, H. & Penman, S. (1970) *J. Mol. Biol.* **50**, 655–670
- Forchhammer, J. & Lindahl, L. (1971) *J. Mol. Biol.* **55**, 563–568
- Gausing, K. (1972) *J. Mol. Biol.* **71**, 529–545
- Haschemeyer, A. E. V. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 128–135
- Koch, A. L. (1971) *Adv. Microb. Physiol.* **6**, 147–217
- Lovett, J. S. & Haselby, J. A. (1971) *Arch. Microbiol.* **80**, 191–204
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Martin, S. E. & Iandolo, J. J. (1975) *J. Bacteriol.* **122**, 1136–1143
- Orlowski, M. (1979) *Biochem. J.* **182**, 547–554
- Orlowski, M. & Sypherd, P. S. (1977) *J. Bacteriol.* **132**, 209–218
- Orlowski, M. & Sypherd, P. S. (1978a) *Biochemistry* **17**, 569–575
- Orlowski, M. & Sypherd, P. S. (1978b) *Arch. Microbiol.* **119**, 145–152
- Orlowski, M. & Sypherd, P. S. (1978c) *J. Bacteriol.* **134**, 76–83
- Peters, J. & Sypherd, P. S. (1978) *J. Gen. Microbiol.* **105**, 77–81
- Scornik, O. A. (1974) *J. Biol. Chem.* **249**, 3876–3883
- Sypherd, P. S., Orlowski, M. & Peters, J. (1979) in *Microbiology 1979* (Schlessinger, D., ed.), pp. 224–227, American Society for Microbiology, Washington, D.C.
- Waldron, C., Jund, R. & Lacroute, F. (1977) *Biochem. J.* **168**, 409–415
- Young, R. & Bremer, H. (1976) *Biochem. J.* **160**, 185–194