22 Kaplan, J.G., and Bona, C., Exp. Cell Res. *88* (1974) 388.
23 Vischer, T.L., Bretz, U., and Baggiolini, M., J. exp. Med. *144* (1976) 863.
24 Kast, R.E., Oncology *29* (1974) 249.
25 Vitetta, E.S., and Uhr, J.W., Immun. Rev. *37* (1977) 50.
26 Christensen, L.R., J. gen. Physiol. *30* (1946) 149.
27 Gordon, E.M., Ratnoff, O.D., and Goldsmith, H.G., J. Lab. clin. Med. *95* (1980) 507.

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Changes in protein synthesis in heat-treated and normally germinating conidia of *Neurospora crassa*

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Summary. The rate of protein synthesis, as measured by pulse-labeling with [^{3}H] leucine, and the level of polysomes were found to be reduced considerably in conidia of *Neurospora crassa* growing isometrically at 46 °C, when compared to those in conidia normally germinating at 25 °C.

Incubation of conidia of *Neurospora crassa* in a nutritional medium with 2% sucrose at 46 °C for 15 h prevents formation of their germ tubes and induces an isometric enlargement of spores, which is approximately twice the normal 'swelling' at 25 °C[2,3]. Protein synthesis is required for germ tube outgrowth from fungal spores[4]. It was therefore of interest to compare levels of protein synthesis and polysome formation in normally germinating conidia at 25 °C with those occurring in heat-treated, overswollen conidia.

Material and methods. Wild-type *Neurospora crassa,* strain Lindegren 354 A, was grown in Fernbach flasks containing solid nitrate minimal medium for 2 days in the dark at 25 °C and 4 days under constant aeration with humidified air in the light. Conidia were harvested with sterile distilled water, filtered to remove mycelium and washed by centrifugation. They were inoculated at a concentration of 5×10^6 per ml in 500 ml conical flasks containing 150 ml Vogel's medium[5] with 2% sucrose. Flasks were incubated in a water-bath for 15 h at 46 °C under reciprocal agitation. Control cultures were grown at 25 °C under the same conditions[3].

Total cellular protein was determined on aliquots after hydrolysis in 1 M NaOH by the method of Lowry et al.[6] using bovine serum albumin as standard. Dry weight measurements were made by filtering samples on preweighed filter papers, washing with acetone and drying to constant weight in a desiccator. For the measurements of the rate of protein synthesis, aliquots of 5 ml cultures were removed at intervals and incubated in a shaken water-bath for 15 min with 0.5 µCi/ml of L-[4,5-^{3}H] leucine (Radiochemical Centre, Amersham, England; sp. act. 53 Ci/mmole) at appropriate temperatures. 2 ml of 10% trichloroacetic acid was added to duplicate samples of 2 ml each, placed in a water-bath at 100 °C for 15 min and chilled. Suspensions were filtered on Whatman GF/C filters, washed with ice-cold 5% trichloroacetic acid, then with alcohol, dried and counted in a toluene-based scintillation solution in a Nuclear Chicago scintillation spectrometer. The linearity of incorporation was verified by analyzing samples at 5 and 10 min pulses.

Isolation of polysomes was made as described previously, at 4 °C using sterile glassware and solutions[7]. After harvesting cultures with cycloheximide (50 µg/ml) to prevent ribosomal run off[8], the conidia were broken for 180 sec on a Vortex mixer with glass beads and a high ionic strength homogenization buffer (pH 8.5, 50 mM Tris-HCl, 400 mM KCl, 50 mM Mg acetate, 200 mM sucrose). After centrifugation at 16,000×g for 15 min, the supernatant was layered on to a pad of 1 M sucrose in buffer B (pH 7.8, 50 mM Tris-HCl, 200 mM KCl, 10 mM Mg acetate) and centrifuged at 160,000×g for 4 h. The ribosomal pellet was resuspended in buffer B and centrifuged for 40 min at 190,000×g on a 10–40% (w/v) linear sucrose gradient made up in buffer B. Gradients were scanned at 260 nm and the areas under the polysome and monosome peaks were calculated to determine the percentage of ribosomes in polysomes. All results are representative of several experiments.

Results and discussion. During isometric growth of the conidia of *N. crassa,* owing to the elevated temperature of incubation (46 °C), the amount of total proteins and dry

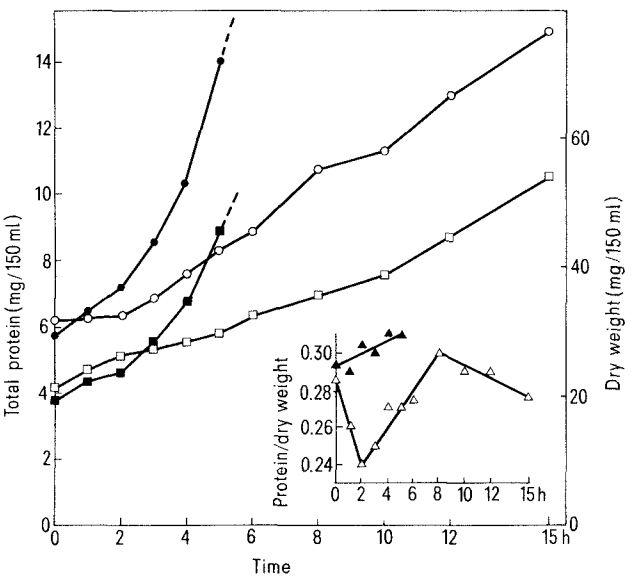

Figure 1. Changes in protein content and dry weight during the 1st h of germination at 25 °C and heat-treatment at 46 °C in shaken cultures of *N. crassa* in Vogel's medium at a concentration of 5×10^6 conidia/ml. Protein content of germinating conidia (●) and heat-treated conidia (○), and dry weight of germinating conidia (■) and heat-treated conidia (□). The inset shows the ratio protein (mg) per dry weight (mg) during germination (▲) and heat-treatment (△).

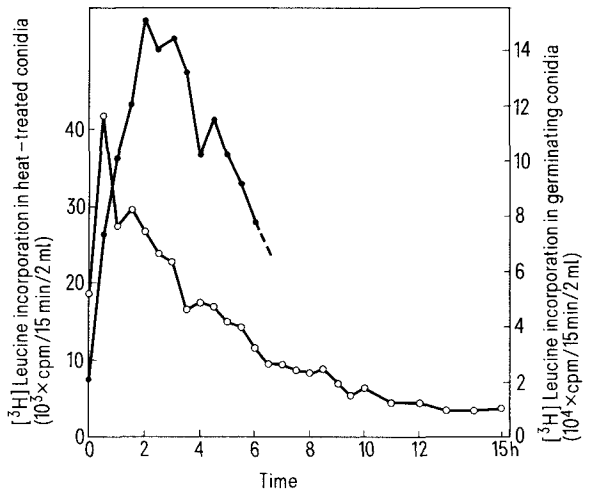

Figure 2. Rate of synthesis of total proteins measured by incorporation of L-[4,5-^{3}H] leucine into hot trichloroacetic acid-precipitable material during the 1st h of germination (●) at 25 °C and heat-treatment (○) at 46 °C of conidia of *N. crassa*. Conditions of culture as described in figure 1.

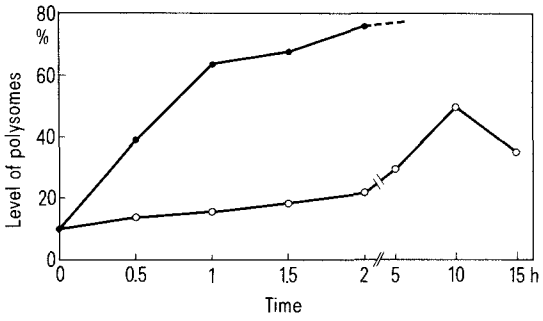

Figure 3. Comparison of the level of polysomes during the first 2 h of normal germination (●) at 25 °C and during the heat-treatment (○) at 46 °C of conidia of *N. crassa*.

weight increased at a slower rate than under normal conditions of germination at 25 °C (fig. 1). At 46 °C, the amount of protein per unit dry weight decreased rapidly during the first 2 h and then increased, becoming stable after 10 h of incubation (inset, fig. 1). This suggests that in the early period of heat-treatment the growth is unbalanced and the metabolism is oriented more towards the synthesis of cell wall polysaccharides than protein synthesis. Slow growth and excessive cell wall thickening has also been observed during spherical growth of *Aspergillus niger* at 44 °C by Smith et al.[9]. In germinating conidia, however, the ratio of protein to dry weight was more or less constant, indicating a more balanced synthesis of cellular material.

The rate of protein synthesis, as measured by [^{3}H] leucine incorporation into proteins, started to rise immediately after the inoculation of conidia at both temperatures of incubation (fig. 2). At 25 °C, it increased sharply up to 2 h, but declined subsequently. The initial rise in the rate of protein synthesis preceded the outgrowth of the germ tubes and then declined with the transformation of germ tubes into hyphae. At 46 °C, after an initial slight rise (up to 30 min), the rate of incorporation of [^{3}H] leucine into proteins declined constantly till the end of the heat-treatment. At the peak of incorporation, heat-treated conidia synthesized proteins at 70% reduced rate, compared to conidia incubated at 25 °C. The rapid increase in the rate of protein synthesis observed in the first 30 min at 46 °C might reflect an adjustment to the supraoptimal temperature. In fact, heat-shock proteins (70, 78 and 90 kdaltons) have been shown to be synthesized during the first 30 min of incubation at 46 °C[10].

The uptake of [^{3}H] leucine by the heat-treated cells was a reflection of the rate of protein synthesis. Our measurements of the specific activity of the endogenous pool of [^{3}H] leucine also showed a progressive decline. This means that the transport of leucine was slowed down, and only a small amount of leucine entered into the internal pool. The leucine taken up by the cells was rapidly equilibrated with endogenous leucine and used for protein synthesis.

The level of polysomes was found to be low in the 1st h of heat-treatment at 46 °C but increased slowly and steadily up to 10 h, by which time it represented 4.9 times the initial value of 10% (fig. 3). In contrast, germinating conidia at 25 °C showed a 6.4 times increase of polysomes in the 1st h of germination, a value close to that found by Mirkes[11]. In conclusion, the reduction of the rate of protein synthesis found in heat-treated, overswollen conidia compared to normally germinating conidia may be the consequence of both a lower level of polysomes and a reduced availability of the amino acid precursor. Active transport of amino acids and their incorporation into proteins being known to be energy-dependent processes[12], their lowered efficiency in overswollen, ungerminated conidia might well result from their heat-induced switch to the low energetic, cyanide-insensitive respiratory pathway[13].

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2 Cortat, M., and Turian, G., Archs Microbiol. *95* (1974) 305.
3 Grange, F., and Turian, G., Archs Microbiol. *119* (1978) 257.
4 Gottlieb, D., The germination of fungus spores. Meadowfield Press Ltd, Durham 1978.
5 Vogel, H.J., Am. Nat. *98* (1964) 435.
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
7 Grange, F., and Turian, G., FEMS Microbiol. Lett. *12* (1981) 283.
8 Alberghina, F.A.M., Sturani, E., and Gohlke, J.R., J. biol. Chem. *250* (1975) 4381.
9 Smith, J.E., Anderson, J.G., Kristiansen, B., Al-Rawi, A., and Yahya, A.G., in: The fungal spore: morphogenetic controls, p. 627. Eds G. Turian and H.R. Hohl. Academic Press, London 1981.
10 Van Tuinen, D., and Turian, G., Experientia *38* (1982) 1365, abstract.
11 Mirkes, P.E., J. Bact. *117* (1974) 196.
12 Lehninger, A.L., Principles of biochemistry. Worth Publ., New York 1982.
13 Michéa-Hamzehpour, M., Grange, F., Ton That, T.C., and Turian, G., Archs Microbiol. *125* (1980) 53.

0014-4754/83/101111-02$1.50+0.20/0