JOURNAL OF BACTERIOLOGY, July 1986, p. 124–129
0021-9193/86/070124-06$02.00/0

Vol. 167, No. 1

Heat Shock Protects Germinating Conidiospores of *Neurospora crassa* against Freezing Injury

C. L. GUY,† NORA PLESOFSKY-VIG, AND ROBERT BRAMBL*

Department of Plant Pathology, The University of Minnesota, Saint Paul, Minnesota 55108

Received 8 November 1985/Accepted 9 April 1986

Germinating conidiospores of *Neurospora crassa* that were exposed to 45°C, a temperature that induces a heat shock response, were protected from injury caused by freezing in liquid nitrogen and subsequent thawing at 0°C. Whereas up to 90% of the control spores were killed by this freezing and slow thawing, a prior heat shock increased cell survival four- to fivefold. Survival was determined by three assays: the extent of spore germination in liquid medium, the number of colonies that grew on solid medium, and dry-weight accumulation during exponential growth in liquid culture. The heat shock-induced protection against freezing injury was transient. Spores transferred to normal growth temperature after exposure to heat shock and before freezing lost the heat shock-induced protection within 30 min. Spores subjected to freezing and thawing stress synthesized small amounts of the heat shock proteins that are synthesized in large quantities by cells exposed to 45°C. Pulse-labeling studies demonstrated that neither chilling the spores to 10°C or 0°C in the absence of freezing nor warming the spores from 0°C to 30°C induced heat shock protein synthesis. The presence of the protein synthesis inhibitor cycloheximide during spore exposure to 45°C did not abolish the protection against freezing injury induced by heat shock. Treatment of the cells with cycloheximide before freezing, without exposure to heat shock, itself increased spore survival.

Diverse organisms show a common response to the stress of a heat shock. When exposed to high temperature, they synthesize large amounts of a characteristic group of proteins that have been evolutionarily conserved (28). This response has been observed in *Drosophila melanogaster* (1), yeast (23, 24), mammals (7, 16), chicken cells (12), plants (13), bacteria (35), and filamentous fungi (10, 26, 29). It has been demonstrated that exposure of cells and tissues to temperatures that induce a heat shock response confers protection against higher, otherwise lethal temperatures (16, 25). In several instances, protein synthesis has been shown to be required for the development of protection that results from heat shock (19, 22, 26, 36).

The heat shock proteins may be general stress proteins since their synthesis is induced by conditions other than exposure to high temperature. Ethanol, respiratory poisons, amino acid analogs, and anoxia have all been found to induce the synthesis of heat shock proteins (1, 9, 11, 31), although the change in protein synthesis is usually less dramatic than that caused by heat shock. It has been reported that administration of arsenite to soybean seedlings induced the synthesis of heat shock proteins and made the seedlings resistant to injury from lethally high temperatures (17) and that prior exposure to ethanol similarly made yeast cells thermotolerant (27). Conversely, *Drosophila* larvae that were exposed to heat shock were protected against the toxic effects of anoxia (30), and heat-treated yeast cells developed an increased tolerance for ethanol (33). However, it was not demonstrated that these examples of induced cross-protection required protein synthesis.

We have reported (26) that germinating conidiospores of *Neurospora crassa*, normally incubated at 30°C, synthesize large amounts of heat shock proteins during exposure to 45°C and that exposure to this heat shock temperature protects them from injury at 50°C, which otherwise is lethal. Heat shock protein synthesis appears to be necessary for this protection, since the cells were killed at 50°C if they had been treated with the protein synthesis inhibitor cycloheximide during exposure to 45°C (26). In the present study, we wished to determine whether the heat shock response protected germinating *Neurospora* spores against other types of stress and thus functioned as a general type of stress protection. Since germinating fungal spores are very susceptible to freezing injury (15), we examined the effect of a prior heat shock on cellular resistance to damage from rapid freezing and slow thawing, a regimen that produces maximal injury due to intracellular ice formation (15). We found that a prior heat shock does protect *Neurospora* cells from low-temperature injury, but this protection does not apparently require the synthesis of heat shock proteins.

MATERIALS AND METHODS

Cell growth, temperature treatments, and viability determination. Conidiospores of *N. crassa* 74-OR23-1A (Fungal Genetics Stock Center, Arcata, Calif.) were harvested with a paraffinic hydrocarbon (4) to prevent spore hydration and activation, and the spores were stored at −60°C until use. Spores (300 mg) were suspended in 30 ml of minimal medium containing 2% sucrose (32) and incubated at 30°C in a gyratory (150 rpm) water bath. Heat shock treatment was initiated by transfer of the culture to a second gyratory water bath at 45°C. The temperature of the spore suspensions reached the temperature of the second water bath within 5 min after transfer from 30 to 45°C or from 45 to 30°C. A 60-min exposure to 45°C was the heat shock treatment used for all experiments. Cycloheximide was added to spore suspension cultures at a final concentration of 150 μg/ml.

Spores were prepared for freezing by transferring a 1-ml portion (10 mg of spores) of the culture to a sterile, screw-cap polypropylene tube that was centrifuged for 1 min at 12,000 × g_{avg}. The supernatant fluid was decanted, and the

* Corresponding author.
† Present address: Ornamental Horticulture Department, The University of Florida, Gainesville, FL 32611.

TABLE 1. Heat shock protection against freezing injury of germinating conidiospores

Temp treatment[a]	% Germination	Colony counts (% control)	Dry wt (mg)
0°C (control)	93	100	120
−196°C	13	11	8
45°C → −196°C	65	46	38
−196°C → 0°C → 45°C[b]	7	1	4
45°C → 0°C	92	101	95

[a] All spores were incubated at 30°C for 2 h before treatment and were kept at 0°C for 1 h after treatment.
[b] Spores were thawed at 0°C for 1 h before transfer to 45°C.

pelleted cells were frozen immediately in the closed tube by direct immersion in liquid nitrogen. The cells reached the temperature of liquid nitrogen (−196°C) within 3 min. As determined with a thermocouple and an electronic thermometer, the average rate of temperature decline from 30 to 0°C was approximately 130°C/min; from 0 to −30°C it was 225°C/min; and from −30 to −100°C it was 260°C/min. The cells were kept in liquid nitrogen for 10 min and then transferred directly to an ice bath (0°C) to thaw for 1 h. The average thawing rate from −196°C (liquid nitrogen) to −100°C was about 65°C/min; from −100 to −30°C it was 45°C/min; and from −30 to 0°C it was 2°C/min. After thawing, the spore sample (10 mg) was resuspended in 30 ml of minimal medium and incubated in a gyratory water bath at 30°C for viability determinations.

Conidiospore survival was determined by the ability of spores to produce germ tubes, by formation of discrete colonies on a sorbose-containing agar medium (5), and by dry-weight accumulation of spore cultures during the early stages of exponential growth. Germination kinetics were determined by counting hourly for 7 h the number of spores (at least 300 spores per sample) that developed germ tubes longer than one spore diameter during incubation at 30°C. Survival of cells was determined by colony formation on a nutrient agar medium containing sorbose as described previously (26), except that the spore suspensions were diluted 100,000-fold before plating. Dry-weight accumulation was measured after the spores had been resuspended in minimal medium and incubated at 30°C for 10 h.

Radiolabeling techniques and gel electrophoresis. L-[^{35}S]methionine (specific activity, 1,460 Ci/mmol) was added at a final concentration of 1 μCi/ml to 30-ml spore suspensions containing 1 mg spores per ml. The cells were radiolabeled for 30 min at 30 and 45°C, but they were labeled for 2 h at 0 and 10°C. Unlabeled DL-methionine (final concentration of 650 μM) was added to the culture medium as a chase 2 min before the radiolabeled spores were collected by filtration onto a 0.22-μm (pore size) Millipore filter. The filtered cells were washed with cold distilled water and immediately transferred to a chilled (0°C) MSK homogenization tube (Braun Scientific Co.) containing 2.7 g of glass beads (1 mm) and 1 ml of protein extraction buffer (60 mM Tris hydrochloride [pH 6.8], 1% dithiothreitol, 1% sodium dodecyl sulfate, 10% glycerol), in which the cells were frozen for storage at −60°C. The spores were disrupted and protein extracts were prepared as previously described (26). Radiolabeled proteins in cell extracts were separated by one-dimensional sodium dodecyl sulfate gel electrophoresis in polyacrylamide slab gels (14). Proteins were separated at a constant current of 25 mA and were fixed and stained with Coomassie blue. Gels were dried onto filter paper and exposed to Kodak XAR-5 film at −70°C.

RESULTS

Heat shock protection and freezing stress. Under normal conditions of incubation, *Neurospora* conidia begin to develop germ tubes at 90 to 120 min, and by 300 min more than 90% of them have rapidly elongating germ tubes. Spores that initiated germination for 2 h at 30°C were susceptible to injury when subjected to rapid freezing and slow thawing; only 13% completed germination, while over 90% of the unfrozen spores germinated (Table 1). However, if the spores were given a 1-h heat shock at 45°C before freezing, 65% were able to complete germination. The heat shock treatment alone did not affect germination (Table 1). A comparison of the kinetics of germination of these spores is shown in Fig. 1. That the germinating spores remained viable is also indicated by a comparison of the colony counts which show that a heat shock prior to freezing increased cell survival four- to fivefold from about 9 to 40% of the number of colonies produced by unfrozen spores (Tables 1 and 2).

The time at which the heat shock was administered to the cells strongly influenced cell survival. A heat shock given after freezing, rather than before, caused more injury to cells than freezing alone; only 7% of the spores germinated, and only 1% formed colonies compared with the unfrozen,

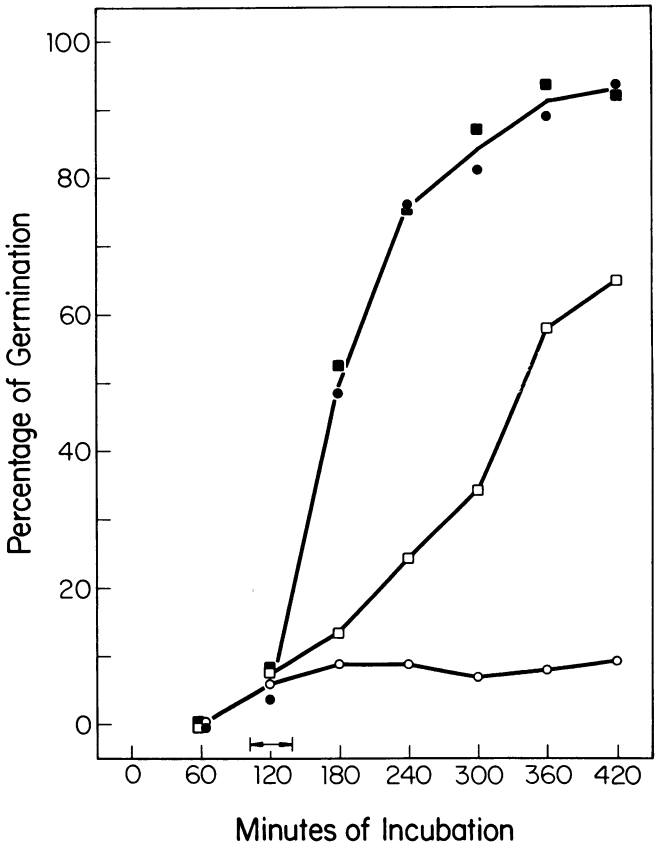

FIG. 1. Germination kinetics of *Neurospora* conidiospores subjected to heat shock, freezing, or chilling. All spores were incubated for 2 h at 30°C and were then chilled at 0°C (●), heat shocked at 45°C (■), frozen in liquid nitrogen (○), heat shocked at 45°C and frozen in liquid nitrogen (□). The double arrow at 120 min indicates both the time at which spores were withdrawn for experimental treatment and the time at which they were resuspended in incubation medium after experimental treatment. Germination at 120 min of incubation was measured immediately after spore resuspension.

TABLE 2. Loss of heat-shock-induced protection against freezing injury

Time at 30°C after heat shock and before freezing	% Germination	Colony counts (% control)	Dry wt (mg)
Unfrozen control	95	100	50
0	54	36	16
5	63	34	14
15	30	28	12
30	17	6	7
45	14	6	4
60	18	8	4

control spores (Table 1). On the other hand, a prior, protective heat shock rapidly became ineffective if the spores were returned to 30°C between the heat shock treatment and subsequent freezing (Table 2). Although there was little loss in protection during the first 5 min of incubation at 30°C, between 5 and 15 min after transfer from 45°C there was a substantial decrease in the protective effect of the heat shock (20 to 50%), and by 30 min of incubation at 30°C the protection against freezing injury was abolished.

Heat shock protein synthesis. As shown previously (26), exposure of *N. crassa* spores to 45°C induces the synthesis of large amounts of three radiolabeled heat shock proteins, with M_rs of 98,000, 83,000, and 67,000, and during the first hour of exposure to 45°C normal protein synthesis is strongly suppressed (Fig. 2). Cells exposed to 30°C do not synthesize detectable amounts of the heat shock proteins (Fig. 2). Since stresses other than high temperature have been shown to induce the synthesis of heat shock proteins in several organisms, we examined the possibility that exposure to cold temperature or freezing and thawing could induce the synthesis of heat shock proteins by *N. crassa*. Protein synthesis patterns of spores radiolabeled with [^{35}S]methionine at 10 and 0°C and at 30°C following rapid freezing-rapid thawing and rapid freezing-slow thawing are shown in Fig. 2. Chilling spores to either 10 or 0°C without freezing, or warming chilled spores rapidly from 0 to 30°C (Fig. 3) did not alter the normal pattern of protein synthesis. In contrast, subjecting the spores to freezing and thawing did induce the synthesis of low amounts of the heat shock proteins (Fig. 2). Spores rapidly frozen and rapidly thawed at 30°C were not severely injured and were able to incorporate more radiolabeled amino acid into proteins (including heat shock proteins) than spores rapidly frozen and slowly thawed at 0°C. Normal protein synthesis was not discontinued during this synthesis of heat shock proteins by spores that were rapidly frozen and thawed.

Our previous study showed that the protection against lethally high temperatures that develops in *N. crassa* spores during heat shock depends on protein synthesis (26). Therefore, we tested the possibility that synthesis of heat shock proteins at 45°C also contributed to spore protection against injury caused by freezing stress. Spores that had germinated for 2 h were exposed to 45°C in the presence of cycloheximide to block protein synthesis before immersion in liquid nitrogen. Surprisingly, the protective effect of a prior heat

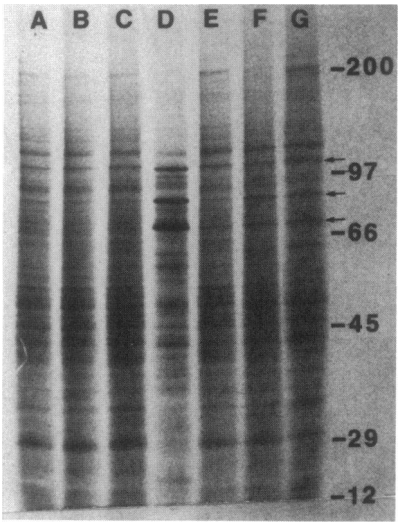

FIG. 2. Autoradiogram of cellular proteins separated electrophoretically in a sodium dodecyl sulfate-12% polyacrylamide gel. Spores that had initiated germination for 2 h at 30°C were pulse-labeled with L-[^{35}S]methionine during subsequent exposure to 30°C (A), 10°C (B), 0°C (C), 45°C (D), 30°C followed by rapid freezing and slow thawing (E), 30°C after rapid freezing and slow thawing (F), and 30°C after rapid freezing and rapid thawing (G). Arrows indicate heat shock proteins. All samples contained equal amounts of acid-precipitable radioactivity (31,000 dpm). Specific incorporations of radioactivity (10^6 dpm/mg of spores) in each treatment were 1.65 (A), 1.55 (B), 0.45 (C), 1.38 (D), 1.27 (E), 0.05 (F), and 0.57 (G).

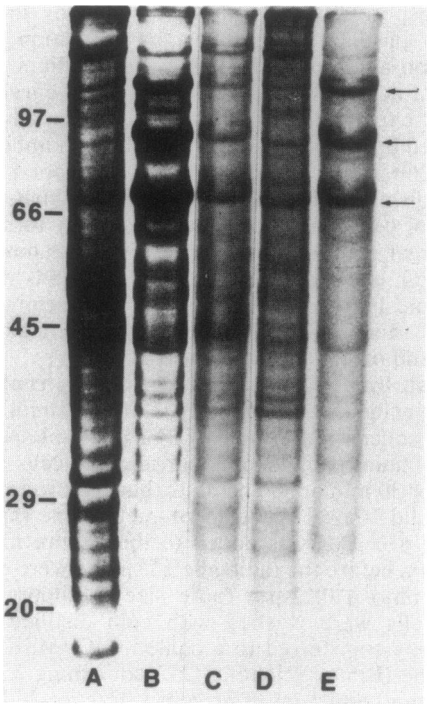

FIG. 3. Autoradiogram of a sodium dodecyl sulfate-15% polyacrylamide gel showing cellular proteins from (2 h, 30°C) conidiospores that were pulse-labeled with L-[^{35}S]methionine at 30°C immediately after exposure at 0°C (A), 45°C (B), 30°C after freezing and thawing (C), 30°C after exposure to 45°C, followed by freezing and thawing (D), and 30°C after exposure to 45°C with cycloheximide, followed by freezing and thawing (E). All samples contained equal amounts of acid-precipitable radioactivity (52,000 dpm). Specific incorporations of radioactivity (10^6 dpm/mg spores) in each treatment were 1.67 (A), 1.4 (B), 0.04 (C), 0.32 (D), and 0.09 (E).

TABLE 3. Effect of cycloheximide on heat-shock-induced protection against freezing injury

Temp and CHI treatment[a]	Colony counts	
	No. of viable spores	% Control
0°C (control)	113 ± 8	100
−196°C	19 ± 4	17
$30°C_{CHI} \rightarrow -196°C$[b]	41 ± 6	36
45°C → −196°C	71 ± 6	63
$45°C_{CHI} \rightarrow -196°C$[c]	64 ± 10	57
$45°C_{CHI} \rightarrow$ −196°C to 0°C to $30°C_{CHI}$[d]	66 ± 2	58
$45°C_{CHI} \rightarrow 0°C$[c]	117 ± 7	104

[a] All spores were incubated at 30°C for 2 h before treatment and were kept at 0°C for 1 h after treatment. CHI, Cycloheximide.

[b] Spores were incubated for 1 h at 30°C in the presence of cycloheximide (150 μg/ml) before freezing.

[c] Cycloheximide was added 10 min before spores were transferred to 45 or 0°C.

[d] Cycloheximide was included in the post-thaw resuspension medium (1 h), as well as during heat shock.

shock was not diminished by the presence of cycloheximide (Table 3). Colony counts indicate that the survival of spores frozen after a heat shock was similar, regardless of whether cycloheximide was added during the 45°C incubation. In contrast, we found that, under identical experimental conditions, induced thermotolerance was reduced more than 90% by the addition of cycloheximide, as determined by the same colony plating assays (26; data not shown). Protection against freezing in these experiments with cycloheximide may not be wholly attributable to the heat treatment, however, since the addition of cycloheximide, in the absence of a heat shock, confers a small increase (two- to threefold) in resistance to freezing injury (Table 3). Nevertheless, there is no indication that the synthesis of heat shock proteins would increase induced resistance to freezing injury. The addition of a 10-fold-lower concentration of cycloheximide (15 μg/ml) during heat shock did not increase freezing tolerance, although it was as effective as the higher concentration of cycloheximide in increasing the freezing resistance of non-heat-shocked spores (data not shown). The survival of unfrozen spores was not affected by cycloheximide (Table 3).

Neurospora spores that were exposed to a heat shock in the presence of cycloheximide synthesized large amounts of the heat shock proteins upon reinoculation into cycloheximide-free medium and incubation at 30°C (Fig. 3). This synthesis was apparent following freezing and thawing of the spores more than 1 h after the heat shock conditions had been removed. Blocking this delayed synthesis of heat shock proteins by the addition of cycloheximide to the resuspension medium for 1 h also did not diminish the resistance against freezing injury conferred by a prior heat shock.

DISCUSSION

When germinating conidiospores of *N. crassa* were subjected to rapid freezing by immersion in liquid nitrogen and slow thawing on ice, about 90% were killed. Such a regimen of rapid freezing followed by slow thawing injures cells (2, 3, 34) by the formation of intracellular ice (15). Most of the injury occurs between −20 and 0°C (20) and is caused by recrystallization of ice during thawing (21). The sensitivity of germinating spores of *N. crassa* to freezing injury (8, 15, 34) makes these cells experimentally useful for testing the effectiveness of heat shock in protection against a type of stress other than high temperature.

When germinating spores were given a heat shock (45°C for 1 h) immediately prior to freezing, survival was sharply increased. This increase in survival was demonstrated by germination kinetics, by ability to form colonies on solid nutrient medium, and by dry-weight accumulation during exponential growth. A heat shock given after freezing and thawing, however, decreased cell survival, compounding the injury resulting from freezing and thawing alone. This result contrasts with the activating effect of a brief heat treatment on the frozen spores of *Puccinia graminis* (18) and indicates that the lack of growth of *Neurospora* spores following freezing is likely not due to a freezing-induced dormancy, such as occurs in *P. graminis* (6). Freezing appears to make the surviving *Neurospora* cells intolerant of a 45°C heat shock.

Exposure of *Neurospora* cells to 45°C radically changes the pattern of protein synthesis, resulting in the production of a discrete group of heat shock proteins and the suppression of normal protein synthesis (26). When germinating *Neurospora* spores were subjected to freezing stress, protein synthesis was also altered and small amounts of the heat shock proteins were produced upon thawing. This change in protein synthesis was not evident when the cells were merely chilled or chilled and rapidly warmed and suggests that the synthesis of heat shock proteins is dependent on the imposition of a physical stress or cellular injury. Stresses other than heat shock are known to induce heat shock protein synthesis in other organisms (1, 9, 11, 17, 27, 31).

Since protein synthesis is required in order for heat shock to protect *Neurospora* spores against lethally high temperatures (26), we tested the hypothesis that heat shock proteins also helped to confer the protection against freezing injury. Cycloheximide blocks the synthesis of heat shock proteins by *N. crassa* at 45°C and prevents the induction of thermotolerance (26). However, the presence of cycloheximide during heat shock did not diminish the protection heat shock afforded against freezing injury. This surprising result indicates that exposure to high temperature must produce another response, other than synthesis of heat shock proteins, that increases the resistance of cells to freezing injury. The lack of involvement of heat shock proteins is also indicated by the lability of the heat shock protection when cells were returned to 30°C before being frozen. The protection against freezing injury was reduced by 15 min and abolished by 30 min of incubation at 30°C. In contrast, the protection against lethally high temperatures conferred on cells by exposure to heat shock was stable at 30°C for 1 h before decreasing by 60% during each of the next 2 h (26).

Germinating spores of *N. crassa* that have been rapidly frozen synthesize small amounts of heat shock proteins upon thawing and resuspension in culture medium, and spores that have received a prior heat shock in the presence of cycloheximide synthesize large quantities of these heat shock proteins after the freeze-thaw treatment. The possibility that heat shock proteins perform their protective function after thawing and that the delayed synthesis of heat shock proteins following cycloheximide treatment is sufficient to rescue cells from freezing injury is unlikely, however, since addition of cycloheximide to the post-thaw resuspension medium for 1 h did not alter the protection conferred by a prior heat shock.

Within the first hour of exposure to 45°C, *Neurospora* cells discontinue synthesis of normal proteins and respiration is severely depressed (26). It is likely that other metabolic processes also are slowed by this treatment, since the cells grow very slowly during extended exposure to 45°C

(26). It has been reported that freezing sensitivity is a function of metabolic activity (15), and it is possible that the heat shock protection against freezing injury derives mainly from this induced quiescence. This possibility is supported by two observations. The heat shock protection against freezing injury declines rapidly between 15 and 30 min of incubation at 30°C; we found earlier that within 30 min after temperature downshift from 45 to 30°C heat shock protein synthesis was discontinued and normal protein synthesis was resumed (26). Presumably, at this time there is a resumption of normal cellular metabolism as well. In addition, we found in the present study that treatment with cycloheximide itself, before freezing, increased the resistance of germinating spores to freezing injury. This small increase may be due to the cessation of protein synthesis and interruption of cell metabolism caused by cycloheximide. Low levels of cycloheximide were reported to inhibit DNA replication in mammalian cells and thereby protect chromosomes from damage caused by heat shock (S. W. Sherwood, A. S. Daggett, and R. T. Schimke, Abstr. Meet. on Heat Shock 1985, Cold Spring Harbor Laboratory, p. 79).

Exposure to 45°C protects germinating *Neurospora* spores against extremes of both heat and cold. However, the basis for this protection against the two stresses differs. Resistance to high temperature depends on heat shock protein synthesis, whereas the quiescent metabolism induced by a heat shock appears to be sufficient to increase cellular resistance to freezing injury. These two different effects of heat shock, heat shock protein synthesis and interruption of normal metabolism, may protect cells under different conditions, and the relationship between these two responses to thermal stress should be explored. This study also shows that although non-heat-shock stresses, including freezing, may induce the synthesis of heat shock proteins, these proteins may not be useful to the cells during or after exposure to the inducing stress.

ACKNOWLEDGMENT

This research was supported by Public Health Service research grant GM-19398 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in *Drosophila* by heat shock. Cell **17**:241–254.
2. **Barnhart, E. R., and C. E. Terry.** 1971. Cryobiology of *Neurospora crassa*. I. Freeze response of *Neurospora crassa* conidia. Cryobiology **8**:323–327.
3. **Barnhart, E. R., and C. E. Terry.** 1971. Cryobiology of *Neurospora crassa*. II. Alteration in freeze response of *Neurospora crassa* conidia by additives. Cryobiology **8**:328–332.
4. **Bonnen, A., and R. Brambl.** 1983. Germination physiology of *Neurospora crassa* conidia. Exp. Mycol. **7**:197–207.
5. **Brockman, H. E., and F. J. de Serres.** 1963. Sorbose toxicity in *Neurospora*. Am. J. Bot. **50**:709–714.
6. **Bromfield, K. R.** 1964. Cold-induced dormancy and its reversal in uredospores of *Puccinia graminis* var. *tritici*. Phytopathology **54**:68–74.
7. **Cosgrove, J. W., and I. R. Brown.** 1983. Heat shock protein in mammalian brain and other organs after a physiologically relevant increase in body temperature induced by D-lysergic acid diethylamide. Proc. Natl. Acad. Sci. USA **80**:569–573.
8. **Faull, A. F.** 1930. On the resistance of *Neurospora crassa*. Mycologia **22**:288–303.
9. **Guttman, S. D., C. V. C. Glover, C. D. Allis, and M. A. Gorovsky.** 1980. Heat shock, deciliation and release from anoxia induce the synthesis of the same set of polypeptides in starved *T. pyriformis*. Cell **22**:299–307.
10. **Kapoor, M.** 1983. A study of the heat shock response in *Neurospora crassa*. Int. J. Biochem. **15**:639–649.
11. **Kelley, P. M., and M. J. Schlesinger.** 1978. The effect of amino acid analogues and heat shock on gene expression in chicken embryo fibroblasts. Cell **15**:1277–1286.
12. **Kelley, P. M., and M. J. Schlesinger.** 1982. Antibodies to two major chicken heat shock proteins cross-react with similar proteins in widely divergent species. Mol. Cell. Biol. **2**:267–274.
13. **Key, J. L., C.-Y. Lin, and Y. M. Chen.** 1981. Heat shock proteins of higher plants. Proc. Natl. Acad. Sci. USA **78**: 3526–3530.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Leef, J. C., and P. Mazur.** 1978. Physiological response of *Neurospora* conidia to freezing in dehydrated, hydrated or germinated state. Appl. Environ. Microbiol. **35**:72–83.
16. **Li, G. C., and Z. Werb.** 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc. Natl. Acad. Sci. USA **79**:3218–3222.
17. **Lin, C.-Y., J. K. Roberts, and J. L. Key.** 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol. **74**:152–160.
18. **Loegering, W. Q., and D. L. Harmon.** 1962. Effect of thawing temperature on urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen. Plant Dis. Rep. **46**:299–302.
19. **Loomis, W. F., and S. Wheeler.** 1980. Heat shock response of *Dictyostelium*. Dev. Biol. **79**:399–408.
20. **Mazur, P.** 1956. Studies on the effects of subzero temperatures on the viability of spores of *Aspergillis flavus*. I. The effect of rate of warming. J. Gen. Physiol. **39**:869–888.
21. **Mazur, P., and J. J. Schmidt.** 1968. Interactions of cooling velocity, temperature, and warming velocity on the survival of frozen and thawed yeast. Cryobiology **5**:1–17.
22. **McAllister, L., and D. B. Finkelstein.** 1980. Heat shock proteins and thermal resistance in yeast. Biochem. Biophys. Res. Commun. **93**:819–824.
23. **McAllister, L., S. Strausberg, A. Kulaga, and D. B. Finkelstein.** 1979. Altered patterns of protein synthesis induced by heat shock of yeast. Curr. Genet. **1**:63–74.
24. **Miller, M. J., N.-H. Xuong, and E. P. Geiduschek.** 1979. A response of protein synthesis to temperature shift in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **76**:5222–5225.
25. **Mitchell, H. K., G. Moller, N. S. Petersen, and L. Lipps-Sarmiento.** 1979. Specific protection from phenocopy induction by heat shock. Dev. Genet. **1**:181–192.
26. **Plesofsky-Vig, N., and R. Brambl.** 1985. The heat shock response of *Neurospora crassa*: protein synthesis and induced thermotolerance. J. Bacteriol. **162**:1083–1091.
27. **Plesset, J., C. Palm, and C. S. McLaughlin.** 1982. Induction of heat shock proteins and thermotolerance by ethanol in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **108**:1340–1345.
28. **Schlesinger, M. J., M. Ashburner, and A. Tissières (ed.).** 1982. Heat shock, from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
29. **Silver, J. C., D. R. Andrews, and D. Pekkala.** 1983. Effect of heat shock on synthesis and phosphorylation of nuclear and cytoplasmic proteins in the fungus *Achlya*. Can. J. Biochem. Cell Biol. **61**:447–455.
30. **Velazquez, J. M., and S. Lindquist.** 1984. Hsp 70: nuclear concentration during environmental stress and cytoplasmic storage during recovery. Cell **36**:655–662.
31. **Vincent, M., and R. M. Tanguay.** 1982. Different intracellular distributions of heat-shock and arsenite-induced proteins in *Drosophila* Kc cells: possible relation with the phosphorylation and translocation of a major cytoskeletal protein. J. Mol. Biol. **162**:365–378.
32. **Vogel, H. J.** 1956. A convenient growth medium for *Neuros-*

pora. Microb. Genet. Bull. **13**:42.

33. **Watson, K., G. Dunlop, and R. Cavicchioli.** 1984. Mitochondrial and cytoplasmic protein syntheses are not required for heat shock acquisition of ethanol and thermotolerance in yeast. FEBS Lett. **172**:299–302.
34. **Wellman, A. M., and L. Pendyala.** 1979. Permeability changes in membranes of *Neurospora crassa* after freezing and thawing. Cryobiology **16**:184–195.
35. **Yamamori, T., and T. Yura.** 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **79**:860–864.
36. **Yura, T., T. Tobe, K. Ito, and T. Osawa.** 1984. Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperature but is dispensable at low temperature. Proc. Natl. Acad. Sci. USA **81**:6803–6807.