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Acceleration of Starvation- and Glycerol-Induced Myxospore Formation by Prior Heat Shock in *Myxococcus xanthus*

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The effect of heat shock on *Myxococcus xanthus* was investigated during both glycerol- and starvation-induced development. Cells heat shocked at 40°C for 1 h prior to a development-inducing signal displayed an accelerated rate of myxospore formation at 30°C. Additionally, *M. xanthus* cells heat shocked prior to glycerol induction formed a greater total number of myxospores when sporulation was complete than did control cells maintained at 30°C. However, in starvation-induced fruiting cells the total number of myxospores in control and heat-shocked populations was about equal when fruiting body and myxospore formation was complete. When extended heat shock (3 h) was applied to cells prior to development, no acceleration of myxospore formation was observed. Heat shock elicited the premature expression of many developmentally regulated proteins. Cell fractionation and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography revealed the subcellular location and molecular weights of the 18 glycerol-induced and 9 starvation-induced developmental proteins. Comparison with previously identified *M. xanthus* heat shock proteins showed that nine of the developmental proteins found in glycerol-induced cells and three of the developmental proteins found in starvation-induced cells were heat shock proteins. Furthermore, heat shock increased the activity of alkaline phosphatase, a developmentally regulated enzyme, in vegetative cells, glycerol-induced cells, and starvation-induced cells.

All organisms respond to elevated temperatures by altering their pattern of growth and protein synthesis (16). Upon exposure to elevated temperatures, cells rapidly cease growth, repress the synthesis of most vegetative polypeptides, and coordinately synthesize a small number of different proteins (16). These heat shock proteins (HSPs) may also be induced by other environmental stresses, such as exposure to various alcohols, oxidants (16), and abnormal proteins (1).

In many diverse organisms, such as *Saccharomyces cerevisiae* (13), *Drosophila melanogaster* (22), and mice (12), some heat shock genes are expressed during particular stages of cellular development. The association of HSPs with certain periods of cellular differentiation suggests that these proteins may be involved in both normal developmental processes and stress responses.

Myxococcus xanthus is a gram-negative, rod-shaped, gliding bacterium (9). Although it is a procaryote, it exhibits a complex program of multicellular development. When subjected to starvation on a solid surface, cells migrate inwards towards localized aggregation centers to form raised mounds of cells. Within these mounds, individual cells differentiate to form round, environmentally resistant myxospores. These mounds of cells and spores are termed fruiting bodies (15). This process of fruiting body formation is cell density dependent, includes the temporal expression of proteins such as myxobacterial hemagglutinin (5) and proteins U (11) and S (8), and extends over a 48- to 72-h period (6). *M. xanthus* can also be induced to sporulate by the addition of glycerol (6), dimethyl sulfoxide, or phenethyl alcohol (6, 23). This developmental process differs from starvation-induced development in that the formation of glycerol-induced spores requires neither starvation nor a solid surface, is cell density independent, and lacks the expression of a variety of developmental proteins, including myxobacterial hemagglutinin and protein S (11), and glycerol-induced spores appear within 3 h (6).

The *M. xanthus* heat shock response has been characterized in vegetative, starvation-induced, and glycerol-induced cells (18). It is known that temperatures above 36°C elicit the expression of HSPs in this organism. Although HSP expression may endow *M. xanthus* cells with thermotolerance (18), it was not known whether HSPs had other functions. The intent of this investigation was to examine the effect of heat shock upon the development of *M. xanthus*. Hence, we demonstrate that heat shock of vegetative *M. xanthus* cells prior to the induction of either fruiting body and myxospore formation by starvation or spore formation by the addition of glycerol results in an acceleration of these developmental processes. Further, we find that heat shock induces the premature expression of both starvation- and glycerol-induced developmental proteins. Finally, we show that heat shock prematurely elevates the levels of alkaline phosphatase, a known developmental marker, during both glycerol- and starvation-induced myxospore formation. These findings suggest that heat shock and normal development are related.

MATERIALS AND METHODS

Cells and growth conditions. *M. xanthus* FB(DZF1) derived from DK101 (7) was used in all experiments. Vegetative cultures were grown in Casitone-yeast extract (CYE; Difco Laboratories, Detroit, Mich.) broth and maintained on CYE agar plates (3). For determination of the number of CFU per milliliter, samples (0.1 ml) were withdrawn from cultures and diluted aseptically into CYE broth, plated on CYE agar plates, and incubated at 28°C for 5 days. Cells grown to a density of 5×10^8/ml in CYE broth were prepared for glycerol induction by either of two methods. Either glycerol was added directly to the CYE broth to a final

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concentration of 0.5 M (6) or the cells were centrifuged (10,000 × *g*, 5 min), washed, and suspended in CT to the original density prior to the addition of 0.5 M glycerol (6). Cells were prepared for starvation by being grown to a density of 10^9/ml (300 Klett units, measured with a 560-nm filter), concentrated 10-fold by centrifugation (10,000 × *g*, 5 min), and suspended in CF (clone-fruiting) broth. Such cells were spotted onto CF agar to induce starvation (7).

Heat shock conditions. For glycerol experiments, exponentially growing *M. xanthus* cells at a density of 5×10^8/ml were heat shocked by transfer to 40°C for 1 h (18). For starvation experiments, cells at a density of 4,500 Klett units (560-nm filter) were heat shocked at 40°C for 1 h (18).

Determination of viable myxospores. Samples (1 ml) of the developing cell culture were periodically withdrawn, incubated at 53°C for 15 min, and pulse sonicated for 2 min. Both processes kill all vegetative cells while myxospores survive (19). Cell suspensions were diluted aseptically, spread plated on CYE agar, and incubated at 28°C for 5 days to determine the number of viable myxospores.

Isotope labeling conditions. Vegetative *M. xanthus* cells were prepared for labeling with [^{35}S]methionine by being grown at 30°C in A-1 medium (2) to a density of about 1.5×10^8/ml. The cells were centrifuged (10,000 × *g*, 5 min, 4°C), washed once with 10 mM Tris hydrochloride (pH 7.6)–8 mM $MgSO_4$ (TM buffer), and suspended to the same density in A-1 medium lacking methionine (18). Cells were divided into two aliquots. One culture was heat shocked, and the other was maintained at 30°C before the addition of glycerol (final concentration, 0.5 M). After incubation for 0, 30, 45, 60, or 90 min at 30°C in glycerol, the cells were labeled for 25 min with [^{35}S]methionine (10 μCi/ml; specific activity, 1.124 Ci/mmol; New England Nuclear Corp., Boston, Mass.).

Cells were prepared for starvation-induced labeling as follows. Vegetative *M. xanthus* cultures were concentrated to 3,000 or 4,500 Klett units (560-nm filter) and incubated at 30°C or heat shocked, respectively. Since a 1-h heat shock kills between one-third and one-half of the cell population, viable cell densities were equal. Both cultures were then spotted onto CF agar and incubated at 30°C to induce starvation and fruiting. Cells were harvested at various times and labeled for 20 min with [^{35}S]methionine (10 μCi/ml; specific activity, 1.017 Ci/mmol) as described previously (18).

Cell fractionation procedures. The procedures described by Nelson et al. (17) were used to obtain periplasmic, membrane, and cytoplasmic fractions of *M. xanthus* cells.

Electrophoretic methods. Polyacrylamide gel electrophoresis (PAGE) was carried out on 0.8-mm-thick 11% polyacrylamide slab gels with the buffer system of Laemmli (14). Protein samples for sodium dodecyl sulfate (SDS)-PAGE were concentrated by precipitation in 10% trichloroacetic acid. Equal amounts of radioactive material were loaded into each sample well. Polyacrylamide gels were fixed in a solution of 25% isopropanol and 10% acetic acid for 1 h before preparation for fluorography. Gels were prepared for fluorography by being soaked in Fluoro-Hance (Research Products International Corp., Mount Prospect, Ill.) in accordance with the instructions of the manufacturer.

Photomicroscopy. An Olympus C-35 camera mounted on an Olympus phase-contrast microscope (model BH-2) was used for photomicrographs. Pictures were taken with 35-mm Kodak Plus-X film and developed on Kodak paper.

Measurement of alkaline phosphatase. The following procedures were used to determine the activity of alkaline phosphatase during development. Cells (2×10^8/ml) induced by glycerol were allowed to develop at 30°C in CYE–0.5 M glycerol for 0, 1, 2, 3, 6, or 10 h. Samples (6 ml) were periodically withdrawn from the developing cell culture, concentrated threefold by centrifugation, and suspended in 10 mM Tris hydrochloride (pH 8.0). Cells were sonicated for 2 min with ice water cooling in the presence of a 0.5-ml volume of glass beads (diameter, 75 to 150 μm) to break cells and myxospores (20). Myxospore breakage was monitored by phase-contrast microscopy. Samples were then centrifuged at 5,000 × *g* for 5 min to remove debris from the cell extract, and supernatants (0.3-ml samples) were assayed for alkaline phosphatase (4). For determination of the alkaline phosphatase activity during starvation-induced cell development, exponential-phase cells were concentrated by centrifugation, suspended in CF broth to 3,000 or 4,500 Klett units, and incubated at 30°C or heat shocked, respectively. Cells were spotted on CF agar and allowed to develop for 0, 12, 24, or 36 h. At each time point cells were harvested from two CF agar plates, suspended in 1.5 ml of CF broth, sonicated for 2 min in the presence of a 0.4-ml volume of glass beads, and centrifuged and treated as described above to determine levels of alkaline phosphatase.

TABLE 1. Myxospore formation enhanced by heat shock prior to glycerol induction[a]

Time of development (h)	No. of viable glycerol-induced spores ± SEM (10^3 CFU/ml)[b] in:		Ratio of spores in heat-shocked cells to spores in control cells
	Control cells	Heat-shocked cells	
0	0	0	
1.5	0.01 ± 0.01	0.27 ± 0.06	21
2	0.21 ± 0.06	37 ± 8.6	174
3	37 ± 12	260 ± 113	7
4	61 ± 10	710 ± 212	12
5	890 ± 13	4,600 ± 733	5
10	1,400 ± 115	7,200 ± 924	5

[a] Heat shock and glycerol-induced spore formation of *M. xanthus* cells were performed as described in Materials and Methods.

[b] The number of viable myxospores was determiend as described in Materials and Methods. Each time point represents the mean of five separate experiments carried out in triplicate.

RESULTS

Effect of heat shock on myxospore formation in glycerol-induced *M. xanthus*. HSPs are expressed during normal development in many organisms. We examined whether the induction of HSPs by elevated temperatures affects sporulation at normal temperatures. Exponentially growing cells were divided into two aliquots. One culture was incubated at 30°C with 0.5 M glycerol. The other was heat shocked and then incubated at 30°C with 0.5 M glycerol. Samples were withdrawn from each aliquot after 0, 1.5, 2, 3, 4, 5, or 10 h of glycerol-induced development, and the number of viable myxospores was determined. The 1-h heat shock accelerated myxospore formation in glycerol-induced *M. xanthus* cells (Table 1). Despite the 50% decline in CFU that resulted from heat shock (18), the surviving cells formed myxospores sooner than did control cells. The acceleration of myxospore formation was most pronounced at 2 h, when the heat-shocked cell population had nearly 200-fold more myxospores than did the control cell population. The ratio of myxospores in the heat-shocked culture to myxospores in the control culture progressively decreased with time. However, even by 10 h, when sporulation was essentially complete, about five times more viable myxospores were found

TABLE 2. Effect of extended heat shock on the rate of glycerol-induced myxospore formation[a]

Time of development (h)	No. of viable glycerol-induced spores ± SEM (10^3 CFU/ml)[b] in:		Ratio of spores in heat-shocked cells to spores in control cells
	Control cells	Heat-shocked cells	
0	0	0	
1.5	0.09 ± 0.02	0.07 ± 0.02	0.78
2	0.36 ± 0.18	0.33 ± 0.15	0.92
3	53 ± 17	30 ± 20	0.57

[a] Heat shock and glycerol-induced spore formation of *M. xanthus* cells were performed as described in Materials and Methods except that cells were heat shocked at 40°C for 3 h instead of 1 h.
[b] See Table 1, footnote *b*.

TABLE 3. Acceleration of starvation-induced myxospore formation by prior heat shock[a]

Time of development (h)	No. of viable myxospores ± SEM (10^3 CFU/plate)[b] in:		Ratio of spores in heat-shocked cells to spores in control cells
	Control cells	Heat-shocked cells	
0	0	0	
18	4.8 ± 0.93	29 ± 8.7	6.0
24	98 ± 36	300 ± 176	3.1
30	270 ± 120	430 ± 133	1.6
36	600 ± 115	600 ± 57	1.0

[a] Heat shock and starvation-induced myxospore formation of *M. xanthus* cells were performed as described in Materials and Methods.
[b] See Table 1, footnote *b*.

in the heat-shocked culture than in the control culture. Thus, heat shock not only accelerated but also enhanced myxospore formation in glycerol-induced cells. The rate of myxospore formation and the rate at which cell shortening occurred were similarly accelerated by the 1-h heat shock (Fig. 1). The undifferentiated rods present in panels 2c and 2d are thought to be cells which died from the initial heat shock and did not sporulate. It should be pointed out that by 5 h, direct counts of phase-bright spores indicated that greater than 80% of the viable cells had sporulated in both heat-shocked and control cultures (data not shown). Thus, our data are in agreement with the results reported by Dworkin and Gibson (6). However, the data in Table 1 report the number of viable myxospores after treatments with heat and sonication to kill vegetative cells, and these data suggest that heat shock accelerates and enhances the entire sporulation process, including spore maturation, resulting in a consistently greater number of viable myxospores.

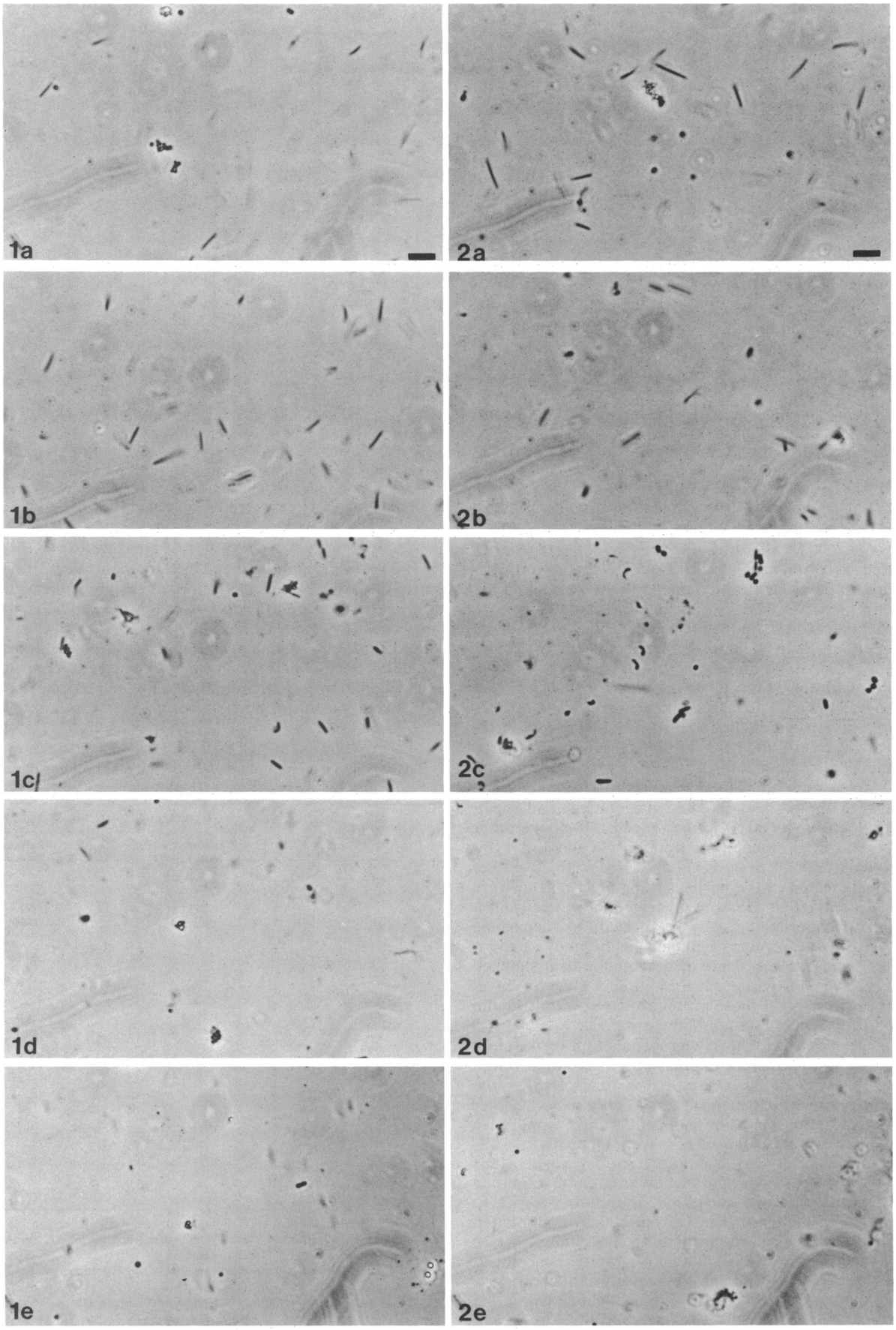
Since Dworkin and Gibson (6) had previously reported on the rate of glycerol-induced sporulation by *M. xanthus* in CT medium, we wanted to determine whether the acceleration of myxospore formation by prior heat shock also occurred in CT-glycerol. Cells were either incubated at 30°C or heat shocked prior to the addition of glycerol, and the number of viable myxospores was determined after 0, 1.5, 2, or 3 h of development in glycerol at 30°C. Prior heat shock accelerated the rate of myxospore formation in CT-glycerol to about the same extent as that reported above for cells in CYE-glycerol (data not shown).

In *Escherichia coli* it is known that the heat shock response wanes with prolonged exposure to elevated temperatures (16). If the active expression of HSP genes was responsible for the accelerated rate of myxospore formation observed above, then it can be predicted that the decreased expression of HSP genes resulting from an extended heat shock should cause no acceleration in the rate of myxospore formation. To test this prediction, we heat shocked *M. xanthus* cells at 40°C for 3 h prior to the addition of glycerol. Although heat shock for either 1 h or 3 h reduced the CFU by about 50% (18), extended heat shock did not accelerate the rate of myxospore formation (Table 2). In fact, at each of the time intervals tested, the control cell population had more myxospores than did the heat-shocked cell population.

Effect of heat shock on myxospore formation in starvation-induced *M. xanthus*. Since heat shock accelerated the rate of myxospore formation in glycerol-induced cells, we were interested in whether heat shock had the same effect on the rate of myxospore formation in starvation-induced fruiting cells. Preincubation at 40°C for 1 h accelerated the normal rate of myxospore formation in starvation-induced *M. xanthus* cells (Table 3). After 18 h of development on CF agar, the previously heat-shocked cells had sixfold more myxospores than did the control cells. However, as fruiting body formation proceeded, the differences between the numbers of myxospores in each population of cells grew progressively fewer. By 36 h of development on CF agar, there was an equal number of myxospores in the heat-shocked and control cell populations.

Premature expression of developmental proteins induced by heat shock in *M. xanthus*. The data presented above indicating that heat shock accelerated myxospore formation in both starvation- and glycerol-induced cells suggested the possibility that some HSPs of *M. xanthus* also possess developmental functions. That is, the preinduction by elevated temperatures of heat shock-developmental proteins could facilitate the rapid conversion of vegetative cells to myxospores. If so, heat shock would be expected to induce the premature expression of developmental proteins. The following experiment was performed to test this hypothesis. *M. xanthus* cells were divided into two aliquots. One culture was heat shocked, and the other was incubated at 30°C. Both cultures were then incubated at 30°C following the addition of 0.5 M glycerol. At 0, 30, 60, 90, or 120 min after the addition of glycerol, 1-ml samples were withdrawn from each aliquot and labeled with [^{35}S]methionine for 25 min. The cells were harvested and fractionated to yield periplasmic, membrane, and cytoplasmic fractions. The proteins in each fraction were analyzed by SDS-PAGE and fluorography. The fluorograms presented in Fig. 2 show the premature expression of glycerol-induced developmental proteins caused by heat shock. There was a marked difference in protein synthesis profiles at the onset of development. In general, proteins detected in the 0-min samples from the heat-shocked cells more closely resembled control cell proteins after 30 or 60 min of development than 0-min control cell proteins. This is not surprising, since development is

FIG. 1. Photomicrographs showing the effect of heat shock on glycerol-induced *M. xanthus*. Logarithmically growing cells were divided into two aliquots. One aliquot was incubated at 30°C for 1 h (control), and the other was incubated at 40°C for 1 h (heat shocked). Both aliquots were incubated at 30°C with 0.5 M glycerol to induce myxospore formation. Panels 1a to 1e show control cells at 0, 30, 60, 90, and 180 min, respectively, after the addition of glycerol. Panels 2a to 2e show heat-shocked cells at 0, 30, 60, 90, and 180 min, respectively, after the addition of glycerol. Bars, 5 μm.



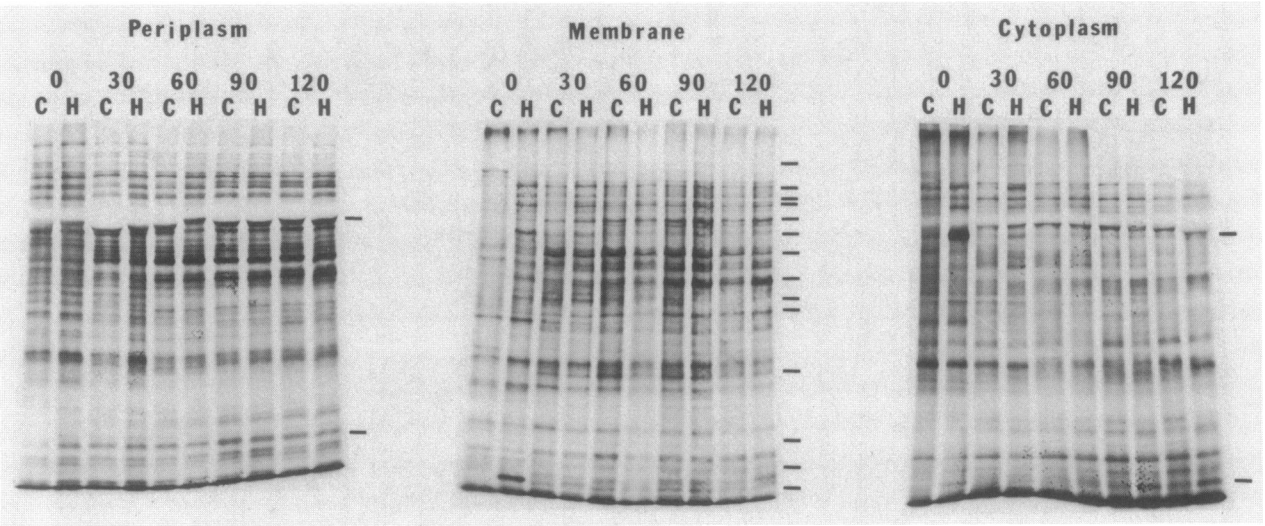


FIG. 2. Autoradiograms of developmental proteins induced prematurely by heat shock in glycerol-induced *M. xanthus* cells. *M. xanthus* cells were divided into two aliquots. The control aliquot (lanes C) was incubated at 30°C for 1 h, and the other aliquot was heat shocked at 40°C for 1 h (lanes H). Both aliquots were shifted to 30°C, and 0.5 M glycerol was added to induce myxospore formation. Samples (1 ml) were withdrawn from each aliquot at 0, 30, 60, 90, or 120 min (numbers above lanes) after the addition of glycerol and labeled with [^{35}S]methionine for 25 min. The labeled cells were treated to yield periplasmic, membrane, and cytoplasmic fractions, and the proteins in each fraction were analyzed by SDS-PAGE and fluorography. Lines to the right of each fluorogram represent prematurely induced HSPs.

accelerated by prior heat shock. Collectively, 18 proteins were found to be prematurely induced by heat shock in the three cell fractions. The periplasm and cytoplasm each contained 2 prematurely induced proteins and the membrane fraction contained 14 such proteins ranging in molecular mass from 14.5 to 130 kilodaltons (kDa).

The prematurely induced developmental proteins are characterized in Table 4. Half of these developmental proteins were previously detected in heat-shocked vegetative or heat-shocked glycerol-induced cells (18). Five of the prematurely induced developmental proteins corresponded in both molecular mass and subcellular fraction to HSPs identified previously in both heat-shocked vegetative and heat-shocked glycerol-induced *M. xanthus* cells (18). Two other proteins found in the membrane fraction (36 and 14.5 kDa) were previously found to be vegetative HSPs (18). Another two proteins located in the periplasm (63 and 18 kDa) were previously identified as HSPs in glycerol-induced cells (18).

A control experiment was performed to determine whether any of the proteins detected in the above experiment (Fig. 2 and Table 4) could be detected in heat-shocked cells returned to 30°C without the addition of glycerol. In all cases, the expression of these proteins ceased within 15 min of the return to 30°C (data not shown).

Since heat shock prior to glycerol induction of development induced the premature expression of several developmental proteins (including several that were also HSPs), the following experiment was carried out to determine whether heat shock prior to starvation-induced fruiting would also have similar effects. *M. xanthus* cells were incubated at 30°C or heat shocked prior to starvation. Both cell suspensions were spotted onto CF agar and incubated at 30°C to initiate development. Cells were harvested after 0.5, 1, or 2 h, labeled with [^{35}S]methionine in CF broth, and fractionated as described above. The proteins in each subcellular fraction were analyzed by SDS-PAGE and fluorography. Nine developmental proteins were either prematurely induced or expressed at higher levels in response to heat shock within the first 2 h of starvation-induced development (Fig. 3). Further, three of these proteins induced prematurely by heat shock in starvation-induced *M. xanthus* cells were previously identified as HSPs (Table 5).

Expression of alkaline phosphatase in developmental *M. xanthus*. Recently, R. A. Weinberg and D. R. Zusman (personal communication) found that alkaline phosphatase activity increases during both glycerol- and starvation-induced

TABLE 4. Glycerol-induced developmental proteins expressed prematurely by prior heat shock[a]

Cell fraction[b]	Mol mass (kDa)[c]	Expression detected in heat-shocked	
		Vegetative cells[d]	Glycerol-induced cells[d]
Periplasm	63	−	+
	18.5	−	+
Membrane	130	−	−
	97	−	−
	92	+	+
	85	+	+
	75	+	+
	69	−	−
	55	−	−
	48	−	−
	38	−	−
	36	+	−
	27	−	−
	20	−	−
	18	−	−
	14.5	+	−
Cytoplasm	75	+	+
	16	+	−

[a] The experimental protocol was as described in the legend to Fig. 2.
[b] Periplasmic, membrane, and cytoplasmic fractions of *M. xanthus* cells were obtained as previously described (17).
[c] Molecular masses of proteins were estimated by comparison to molecular mass standards separated on the same gel.
[d] HSPs previously identified by Nelson and Killeen (18).

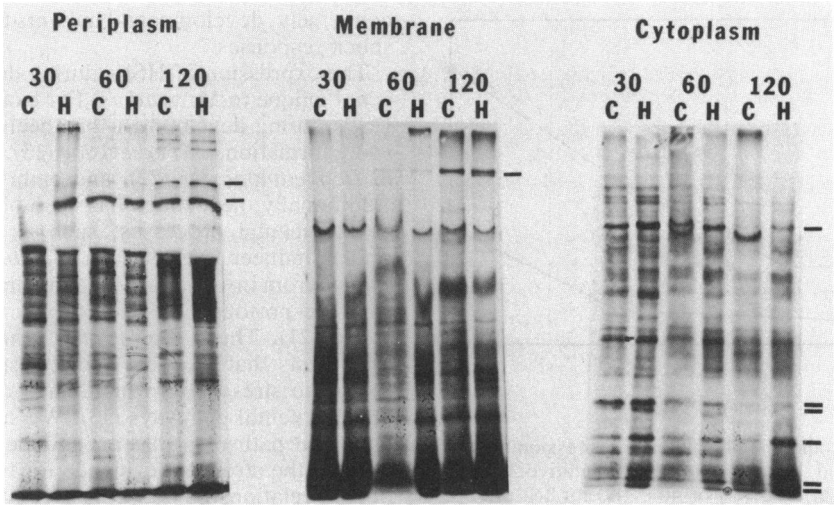


FIG. 3. Autoradiograms of developmental proteins induced prematurely by heat shock in starvation-induced *M. xanthus* cells. *M. xanthus* cells were incubated at 30°C (lanes C) or 40°C (lanes H) for 1 h and then spotted onto CF agar to induce starvation. Both cultures were allowed to develop for 0, 30, 60, or 120 min (numbers above lanes) at 30°C, harvested, and labeled with [^{35}S]methionine for 15 min. The labeled cells were treated to yield periplasmic, membrane, or cytoplasmic fractions, and the proteins in each fraction were analyzed by SDS-PAGE and fluorography. Lines to the right of each fluorogram represent prematurely induced HSPs.

development of *M. xanthus* and that it is a biochemical marker for myxospore formation. Since heat shock had induced the premature expression of some developmental proteins and had accelerated myxospore formation, we were interested in whether heat shock would affect the expression of alkaline phosphatase in the same manner.

Experiments were performed to compare alkaline phosphatase levels in developing *M. xanthus* cells. Glycerol-induced cells, with or without prior heat shock, were allowed to develop for 0, 1, 2, 3, 6, or 10 h before alkaline phosphatase levels were measured (Fig. 4). Despite an approximate 50% decline in viable cell numbers, the previously heat-shocked cells exhibited greater alkaline phosphatase activity than did the control cells at all times tested. The accelerated appearance of alkaline phosphatase activity was most evident after 1 h of glycerol induction, during the cell shortening period of cellular development. There was about threefold more enzyme activity in the previously heat-shocked cells than in the control cells. The ratio of alkaline phosphatase activity between the heat-shocked and control cells declined over the next 2 h and then increased from 6 to 10 h during mature myxospore formation.

Starvation-induced fruiting cells (with or without prior heat shock) were allowed to develop on CF agar at 30°C for 0, 12, 24, or 36 h before being harvested and before alkaline phosphatase levels were determined. The enzyme activity in the heat-shocked cells was higher than that in the control cells at all times of development (Fig. 5). As in glycerol-induced cells, the difference between the two levels of alkaline phosphatase activity was greatest at early times of development. From 0 to 24 h, there was about fivefold more alkaline phosphatase in the previously heat-shocked cells than in the control cells. By 36 h, this ratio had decreased to about twofold.

TABLE 5. Starvation-induced developmental proteins expressed prematurely by prior heat shock[a]

Cell fraction[b]	Mol mass (kDa)[c]	Expression detected in heat-shocked	
		Vegetatie cells[d]	Starvation-induced cells[d]
Periplasm	94	–	–
	85	–	–
Membrane	95	–	–
Cytoplasm	69	+	+
	24	–	–
	23	–	+
	19	–	–
	16	+	–
	14	–	–

[a] The experimental protocol was as described in the legend to Fig. 3.
[b] See Table 4, footnote *b*.
[c] See Table 4, footnote *c*.
[d] See Table 4, footnote *d*.

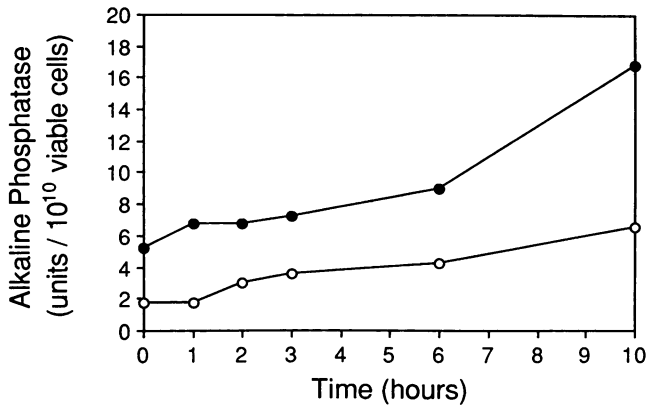


FIG. 4. Acceleration of alkaline phosphatase expression by heat shock in glycerol-induced *M. xanthus* cells. Vegetative *M. xanthus* cells were incubated at 30°C (○) or 40°C (●) for 1 h prior to the addition of 0.5 M glycerol. Both cultures were then incubated at 30°C and harvested at 0, 1, 2, 3, 6, or 10 h after the addition of glycerol. Cells were sonicated to yield cell extracts and assayed for alkaline phosphatase (units per 10^{10} viable cells) as described in Materials and Methods.

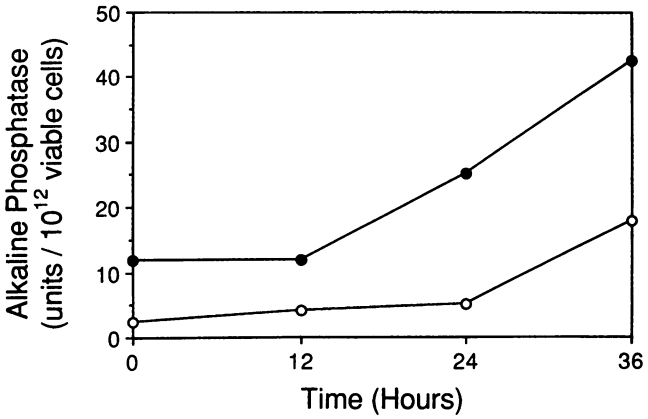


FIG. 5. Acceleration of alkaline phosphatase expression by heat shock in starvation-induced *M. xanthus* cells. Vegetative *M. xanthus* cells were incubated at 30°C (○) or 40°C (●) for 1 h prior to starvation on CF agar at 30°C. Cells were harvested after 0, 12, 24, or 36 h of development and assayed for alkaline phosphatase (units per 10^{12} viable cells) as described in Materials and Methods.

DISCUSSION

In a previous report (18), we characterized the heat-shock response of *M. xanthus* and demonstrated that the HSPs produced by vegetative and developmental cells were not completely identical. Some HSPs were unique to vegetative cells, and some were unique to developmental cells. This observation led us to question whether heat shock had any effect upon the developmental processes of *M. xanthus*. Here, we demonstrate that a 1-h heat shock applied to cells immediately prior to a development-inducing signal results in the acceleration of the rate of myxospore formation in both glycerol- and starvation-induced *M. xanthus* cells. Why does heat shock accelerate development in *M. xanthus*?

We hypothesized that there may be a common subset of proteins that are shared between the heat shock response and the developmental processes of *M. xanthus*. A comparison between the proteins produced by cells heat shocked prior to development and control cells (Fig. 2 and 3) revealed that some proteins are induced prematurely by prior heat shock. Half of the developmental proteins expressed prematurely during glycerol-induced myxospore formation were detected previously in either heat-shocked vegetative or heat-shocked glycerol-induced cells (Table 4). It is interesting to speculate that the mechanism behind glycerol-induced spore formation may be stress related. Perhaps osmotic stress is involved in this process. Similarly, three proteins expressed prematurely by starvation-induced fruiting cells were also detected previously in either heat-shocked vegetative or heat-shocked fruiting cells (Table 5).

Additionally, when we tested the effects of prior heat shock upon the expression of a development-specific enzyme activity, alkaline phosphatase, we found not only that the rate of expression was accelerated but also that alkaline phosphatase activity was induced by heat shock (Fig. 4 and 5). Thus, the effects of heat shock upon development in *M. xanthus* may be correlated at both the physiological level (myxospore formation) and the biochemical level (HSP expression and alkaline phosphatase activity). These observations allow us to predict that the relationships between heat shock and development in *M. xanthus* should extend to the genetic level. That is, it should be possible to select for heat shock mutants that are defective in development and, conversely, developmental mutants that are defective in heat shock responses.

The expression of HSPs during developmental processes is not unique to *M. xanthus*. For example, the expression of HSPs during development has been observed during ascospore formation in *S. cerevisiae* (13), oogenesis and pupation in *D. melanogaster* (22), and embryogenesis in mice (12). Additionally, heat shock has been observed to initiate some developmental processes, such as the production of the sexual inducer molecule in *Volvox carteri* (10) and the change from insect-adapted to mammal-adapted forms in the parasitic protozoans *Trypanosoma brucei* and *Leishmania major* (21). These reports and others have suggested the hypothesis that the heat shock response is an ancient response to stress from which many organisms have evolved developmental pathways (13). We suggest that those developmental pathways that are induced in response to stress provide the strongest evidence for this hypothesized evolutionary relationship between stress and development. Thus, our demonstration that in *M. xanthus* both the relatively complex, multicellular process of fruiting body formation and the simpler process of glycerol-induced spore formation are accelerated by heat shock and involve the expression of HSPs supports this hypothesis.

It will be most interesting to observe the regulatory processes that are shared by the heat shock response and development in *M. xanthus*. The observations reported here indicate that while heat shock can accelerate development, the initiation of development requires a specific signal. It is our eventual goal to learn how development is initiated in *M. xanthus* and how it is regulated. We believe that this study and future studies on the relationship between heat shock and development will provide a new paradigm for our understanding of developmental processes.

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