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THE EFFECTS OF HEAT SHOCK ON DEVELOPMENT  
AND PROTEIN SYNTHESIS OF THE MYXOMYCETE,  
*PHYSARUM POLYCEPHALUM*.

A thesis presented in partial fulfilment of the  
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## ABSTRACT

When *Drosophila* embryos and pupae, undergoing differentiation, are subjected to a brief heat shock at an elevated temperature, specific abnormalities are produced in the adult organism. The type of abnormality produced is dependant upon the stage in the differentiation process at which the heat shock is administered. In addition, *Drosophila* cells respond to heat shock with the rapid cessation of all pre-existing transcription and translation, and the simultaneous transcription of a specific set of heat shock genes. Heat shock mRNAs are subsequently translated preferentially into a novel set of heat shock proteins. This response of *Drosophila* is independant of the type of tissue and the developmental state. Many other organisms display a similar protein synthetic heat shock response.

*Physarum* plasmodia can be readily induced experimentally to undergo a process of differentiation leading to the development of mature sporangia. Heat shocks administered during this differentiation have been reported to result in the formation of abnormal sporangia. This thesis is concerned with the detailed investigation of normal sporangial development in *Physarum* and the effects on subsequent development of heat shocks administered at a number of different stages of development, using a combination of light microscopy, scanning electron microscopy and transmission electron microscopy. The more immediate response of *Physarum* protein synthesis to heat shock is also investigated, using radioactive labeling of proteins, SDS-polyacrylamide gel electrophoresis and fluorography.

Heat shocks administered during the early stages of *Physarum* sporangial development induce a delay in subsequent development but normal mature sporangia are produced. Heat shocks administered late in development induce the formation of grossly abnormal sporangia, with the type of abnormality induced being dependant upon the stage of development attained at the time of the heat shock. Heat shocks administered at a mid-point in development induce a complete, though not permanent, developmental arrest.

Heat shocks at a number of different stages of *Physarum* sporangial development induce a considerable

reduction in pre-existing protein synthesis, while the synthesis of a novel set of heat shock proteins is induced by each heat shock. The heat shock proteins of *Physarum* have approximate molecular weights of 85,000, 78,000, 75,000, 73,000, 69,000, 18,000, and 14,000 daltons, with the predominant heat shock protein being that of 69,000 daltons. *Physarum* plasmodia undergoing active growth synthesize the set of heat shock proteins typical of plasmodia undergoing development but, in this case, the pre-existing protein synthesis continues during the heat shock.

The effects of heat shock on both the development and protein synthesis of *Physarum* plasmodia are discussed and relationships between these two phenomena are proposed. Also discussed are the similarities between heat shock-induced abnormalities in *Drosophila* and *Physarum*, and the similarities between the protein synthetic heat shock responses of these two organisms. In both organisms, a similar molecular basis probably underlies abnormality production, while the major heat shock proteins of both organisms are remarkably similar in molecular weight.

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## SECTION 1. INTRODUCTION

It has long been established that the imposition of an environmental stress during the development of *Drosophila* can induce mutant-like abnormalities, described as phenocopies, in the mature adult. Thus a brief shift from the normal growth temperature to an elevated, but not lethal temperature (heat shock) at specific stages of embryological development or metamorphosis induces specific phenocopies (Goldschmidt, 1935, cited in Mitchell and Lipps, 1978). The cause of the abnormality has been suggested to be a heat shock-induced conformational change in specific proteins (Milkman, 1963). However, it has since become clear that phenocopy production results from the direct effect of heat shock on transcription of a gene that must be expressed in a specific time interval for normal development (Seybold *et al.*, 1975). Additional work on the induction of phenocopies in *Drosophila* led to the hypothesis that the transitory repression of a transcriptional activity is equivalent to a lack of function or an abnormal function in a mutant, and the time of phenocopy production corresponds to the time of expression of the normal allele of the mutant gene (Mitchell and Lipps, 1978). As well as affecting the transcription of structural genes directly, heat shock may also interfere in some general way with the action of regulatory elements in the development of *Drosophila* (Santamaria, 1979).

Heat shock is also effective in inducing developmental abnormalities in other organisms. When *Physarum polycephalum* plasmodia undergoing sporulation are heat shocked at specific stages of development, many abnormal sporangia are subsequently produced (Sauer *et al.*, 1969). Similarly, when *Naegleria gruberi* amoebae are heat shocked during differentiation into swimming flagellates, an increase occurs in the average number of flagella per cell from approximately 2 to either 4.5 (Dingle, 1970) or between 5 and 6 (Walsh, 1980).

During the normal development of the larval salivary gland of *Drosophila*, considerable changes occur in the pattern of puffing activity in the polytene chromosomes. Puffing at sites on these chromosomes represents a measure of transcriptional activity and thus changes in puffing

activity reflect changes in gene expression during development. However when salivary glands are heat shocked, a unique set of puffs is rapidly induced while most pre-existing puffs regress (Ritossa, 1962; Ashburner and Bonner, 1979). In addition, it has been possible to isolate a unique set of messenger RNAs that are induced by heat shock (Spradling *et al.*, 1977; Mirault *et al.*, 1978). In parallel with these changes in transcription, heat shock also redirects protein synthesis from the production of a broad spectrum of proteins which are characteristic of the various tissues and cells of *Drosophila*, to the production of a unique set of proteins, the heat shock proteins (hsp's), which are not tissue specific. The set of hsp's of *Drosophila* includes at least seven different proteins with apparent molecular weights of 82,000, 70,000, 68,000, 27,000, 26,000, 23,000, and 22,000 daltons (for review see Ashburner and Bonner, 1979). Of these, the 70,000 dalton protein (hsp 70) has been found to represent approximately 80% of the total proteins synthesized by *Drosophila* in response to heat shock (Velazquez *et al.*, 1980). The response to heat shock at the level of transcription is extremely rapid such that after only 60 seconds at the elevated temperature, 2.2% of the nuclear RNA is hsp 70 gene transcripts (Findly and Pederson, 1981). Within 8 to 12 minutes, heat shock mRNAs have been processed, transported to the cytoplasm, and translated into protein, while the synthesis of the normal complement of proteins and mRNAs is rapidly curtailed (Lindquist, 1980).

Similar protein-synthetic responses to heat shock have been reported for many other eukaryotic organisms including chicken embryo fibroblasts, mouse L cells, and baby hamster kidney cells (Kelley and Schlesinger, 1978); *Chironomus tentans* (Vincent and Tanguay, 1979); Chinese hamster ovary cells (Bouche *et al.*, 1979); *Saccharomyces cerevisiae* (McCalister and Finkelstein, 1979; Miller *et al.*, 1979); *Dictyostelium discoideum* (Loomis and Wheeler, 1980); *Polysphondilium pallidum* (Francis and Lin, 1980); *Naegleria gruberi* (Walsh, 1980); plant cells (Barnett *et al.*, 1980); *Tetrahymena pyriformis* (Fink and Zeuthen, 1980; Guttman *et al.*, 1980); *Tetrahymena thermophila* (Hauser and Levy-

Wilson, 1981); quail myoblasts (Atkinson, 1981); HeLa cells (Slater *et al.*, 1981); and developing sea urchin embryos (Roccheri *et al.*, 1981). The strict dependence of hsp synthesis on new transcription at the elevated temperature, as indicated by the inhibition of hsp synthesis by actinomycin D, has been demonstrated for *Drosophila* (Lewis *et al.*, 1975), *Dictyostelium* (Loomis and Wheeler, 1980), *Naegleria gruberi* (Walsh, 1980), *Tetrahymena pyriformis* (Fink and Zeuthen, 1980), HeLa cells (Slater *et al.*, 1981), and sea urchin embryos (Roccheri *et al.*, 1981). Although the sets of hsp's of a number of these organisms differ in molecular weight, hsp 70 or a protein of approximately the same molecular weight predominates in *Chironomus*, *Dictyostelium*, *T. pyriformis*, as it does in *Drosophila*. In addition, hsp 70 of *Drosophila* has been shown to co-migrate on SDS acylamide gels with the hsp 70 of *Dictyostelium* (Loomis *et al.*, unpublished results cited in Loomis and Wheeler, 1980, and Velazquez *et al.*, 1980) Such observations seem to suggest that the heat shock response is a truly universal eukaryotic phenomenon. *Escherichia coli* has also been reported to respond to an increase in temperature with a transient increase in the synthesis of a number of proteins (Lemaux *et al.*, 1978; Yamamori *et al.*, 1978) and this further suggests that prokaryotes may be subject to a similar type of response.

Although heat shock itself is the primary inducer of the heat shock response, the mechanisms at the molecular level by which the heat shock loci are specifically selected for expression remain to be elucidated. It has been possible to partially induce the heat shock puffs in individual *Drosophila* salivary gland nuclei by micro-injection of mitochondrial supernatant, obtained from mitochondria subjected to the elevated temperature, into the salivary gland cells (Sin, 1975). Similarly, incubation of isolated salivary gland polytene nuclei in cytoplasm prepared from *Drosophila* tissue culture cells which had been heat shocked before disruption, induces puffs at the heat shock loci (Compton and Bonner, 1978; Compton and McCarthy, 1978; Bonner, 1981; Craine and Kornberg, 1981). The active factor in the cytoplasmic extracts has been partially

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purified and characterized and found to be a protein (Craine and Kornberg, 1981). The observation that the heat shock loci are induced even in the absence of protein synthesis (Ashburner, 1970) suggests that proteins involved in gene activation must be components of cells during normal growth which are modified or translocated, or both, after heat shock. It remains a possibility that such proteins normally reside within mitochondria.

Additional controls in the heat shock response appear to operate at the level of translation. In parallel with the changes that occur in the patterns of proteins synthesized by *Drosophila* in response to heat shock, pre-existing polysomes disappear and are replaced by a population of polysomes containing RNA synthesized after the elevation in temperature (McKenzie *et al.*, 1975). When polysomal RNA is extracted from heat shocked *Drosophila* cells, it is found to direct the *in vitro* synthesis of heat shock proteins (McKenzie and Meselson, 1977). However mRNAs that are normally synthesized and translated remain in the cytoplasm of heat shocked cells and can be translated *in vitro* also (Mirault *et al.*, 1978). Furthermore, lysates prepared from heat shocked *Drosophila* cells preferentially translate the heat shock mRNAs, while the lysates prepared from normally growing *Drosophila* cells indiscriminately translate both normal mRNAs and heat shock mRNAs (Storti *et al.*, 1980; Krüger and Benecke, 1981; Scott and Pardue, 1981). *Drosophila* cells can therefore discriminate at the level of translation between two populations of mRNA which co-exist in the cell. The addition of crude ribosome fractions from normal cell lysates to lysates from heat shocked cells rescues translation of normal mRNA, suggesting that the discriminating elements are associated with ribosomes (Scott and Pardue, 1981). Upon the return of heat shocked cells to the normal temperature the normal proteins are again synthesized, initially from stored mRNAs (Lindquist, 1980; Storti *et al.*, 1980). The hsp's themselves are apparently not involved in translational control since *Drosophila* embryos, at a stage in development where they are unable to synthesize hsp's, still suffer an arrest or substantial reduction of normal protein synthesis (Dura, 1981).

On the basis of the heat shock-induced repression of normal protein synthesis, it has been proposed that phenocopy induction in *Drosophila* results from a non-coordinated recovery of the normal protein synthesis pattern following heat shock (Chomyn *et al.*, 1979). The process of differentiation in *Drosophila* pupae is accompanied by rapid changes in the patterns of synthesis of all the most abundant proteins and these changes, which are regulated at the level of transcription, can be related to phenocopy sensitivity on a temporal basis (Mitchell and Petersen, 1981). Although not a developmental abnormality in the sense of phenocopy induction, a delay in the onset of mitosis induced by heat shock occurs in a number of organisms. Often such delays can be related to morphological abnormalities within nuclei. In *Physarum polycephalum*, heat shock-induced mitotic delays with variable durations dependant upon the actual stage in the cell cycle at the time of the heat shock have been reported (Brewer and Rusch, 1968; Kauffman and Wille, 1975; Lomagin, 1978; Wright and Tollon, 1978; Tyson *et al.*, 1979; Wolf *et al.*, 1979). Similar cell cycle-dependant, heat shock-induced mitotic delays have been reported for *Tetrahymena* and *Schizosaccharomyces* (Zeuthen, 1974; Polanshek, 1977). In *Drosophila*, mitosis is blocked during heat shock and takes almost 3 hours at the normal incubation temperature before normal cell division occurs again (Arrigo *et al.*, 1980). There is some evidence to suggest that mitosis in both *Physarum* and *Schizosaccharomyces* is dependant upon protein synthesis at the sensitive stages of their cell cycles (Sachsenmaier *et al.*, 1972; Polanshek, 1977; Tyson *et al.*, 1979) and it is possible that the delays in mitosis onset are caused by the heat shock-induced repression of normal protein synthesis and therefore may have a molecular basis similar to that of phenocopy induction. It is interesting to note that division in *Escherichia coli* depends upon the accumulation of a specific protein throughout the cell cycle and that heat shock delays division increasingly with the age of the cell (Smith and Pardee, 1970).

While the physiological function of the heat shock response remains unclear, it is known that heat is not alone

in its ability to induce the transcriptional and translational changes associated with the heat shock response. Included amongst the many other effective inducing agents are anaerobiosis, uncouplers of oxidative phosphorylation, and inhibitors of various enzymes and other cellular functions (Ashburner and Bonner, 1979). Agents other than heat shock are also effective inducers of heat shock-type responses in a number of organisms besides *Drosophila*. Both deciliation and release from anoxia induce the same set of proteins in *Tetrahymena pyriformis* as does heat shock (Guttman *et al.*, 1980). Similarly, anaerobic treatment of maize seedlings results in the synthesis of a novel set of proteins, the major one being identified as alcohol dehydrogenase, an important enzyme for anaerobic survival (Sachs and Freeling, 1978).

Therefore it is likely that the heat shock response itself is but an example of a more general biological reaction to environmental stress. The proteins synthesized in response to that stress probably serve in some way to alleviate the effects of the stress on the organism, an hypothesis which is supported by a number of studies. When *Drosophila* embryos are heat shocked at any stage preceding the migration of the nuclei to the periphery of the egg, they fail to produce hsp's and stop cleaving, whereas embryos heat shocked at later stages do produce hsp's and continue developing (Graziosi *et al.*, 1980). Similarly, sea urchin eggs heat shocked prior to hatching fail to produce hsp's and do not survive the heat shock whereas those heat shocked after hatching do produce hsp's and survive (Roccheri *et al.*, 1981). *Dictyostelium* cells are protected from an otherwise lethal temperature following a pretreatment at a lower temperature which induces the heat shock response (Loomis and Wheeler, 1980). A mild heat shock pretreatment of *Drosophila* cells also greatly enhances survival and the recovery of protein synthesis after a higher temperature heat shock (Mitchell *et al.*, 1979; Petersen and Mitchell, 1981). In addition, pretreatment of *Drosophila* pupae leads to both increased survival of an otherwise lethal heat shock (Milkman, 1963; Mitchell *et al.*, 1979) and protection against phenocopy induction (Mitchell

*et al.*, 1979; Petersen and Mitchell, 1981). When *Drosophila* cells are subjected to a gradual rise in temperature, the temperature range of the heat shock response is greatly extended (Lindquist, 1980) in a manner resembling that of pretreatment. Clearly a relationship does exist between an effective heat shock response and both survival and protection from heat shock-induced developmental abnormalities.

The importance of hsp's can also be demonstrated by inhibiting their synthesis. Thus when the synthesis of hsp's by *Drosophila* cells is inhibited by the addition of cycloheximide to the cells prior to heat shock, normal transcription is strongly inhibited following the heat shock (Arrigo, 1980; DiDomencio *et al.*, cited in Velazquez *et al.*, 1980). Although the hsp's are not responsible for the repression of genes active before the heat shock, their synthesis is necessary for the resumption of RNA synthesis following the heat shock (Arrigo, 1980). The addition of cycloheximide to *Saccharomyces cerevisiae* cells prior to heat shock also prevented the recovery of the pre-heat shock mRNA pattern of these cells on subsequent incubation at the normal temperature (McAlister and Finkelstein, 1980). With *Dictyostelium* cells, the protective action of pretreatment was prevented if the cells were incubated with cycloheximide during the pretreatment (Loomis and Wheeler, 1980).

Therefore it is apparent that the hsp's do in some way protect the cell against the effects of the elevated temperature. A number of observations indicate that the actual site of this protective action is within the nucleus. Using cell fractionation procedures, it has been shown that some hsp's may become localized within nuclei (Mitchell and Lipps, 1975; Vincent and Tanguay, 1979). Further work using this technique has shown that approximately 80% of hsp's 22, 23, 26, and 27, and 30% of hsp's 68 and 70 become localized within nuclei of *Drosophila* cells following a one hour heat shock, whereas most of hsp 84 is found in the cytoplasm (Arrigo *et al.*, 1980). When heat shocked cells are returned to the normal temperature, most of the hsp's migrate from the nuclei into the cytoplasm (Arrigo *et al.*, 1980). Electron microscope autoradiography of *Drosophila* cells has

also demonstrated that a major portion of the proteins synthesized during heat shock is rapidly transported to the nucleus and very little appears in cytoplasmic vacuoles or in mitochondria; once inside the nucleus the hsp's become quantitatively associated with chromosomes (Velazquez *et al.*, 1980). A more detailed biochemical fractionation study has confirmed the association of hsp's with the nuclei of heat shocked *Drosophila* cells and has indicated that the hsp's become associated with an intranuclear scaffold rather than being directly associated with chromatin (Levinger and Varshavsky, 1981). Another study also locates a substantial fraction of the lower molecular weight hsp's within nuclei of heat shocked *Drosophila* cells and identifies the most prominent basic hsp as histone H2b (Sanders, 1981). One of the hsp's of *Tetrahymena pyriformis* also accumulates within the nucleus and may be associated with structural or scaffolding proteins in the nuclear matrix (Guttman *et al.*, 1980). Thus, although the actual molecular interactions remain unclear, the relationship between the hsp's and the nucleus must be an important one for cell survival at elevated temperatures.

In the study presented here, the myxomycete *Physarum polycephalum* is utilized to investigate the effects of heat shock on both development and protein synthesis. The life cycle of *Physarum* (Fig. 1) encompasses two vegetative phases of growth: uninucleate, haploid amebae, which grow and divide by binary fission, and a multinucleate plasmodium, which grows without cell division. In the presence of an adequate food supply, the plasmodium continues to grow indefinitely, undergoing synchronous mitoses. The entire plasmodium can also migrate around by means of protoplasmic streaming. When starved, plasmodia can undergo a reversible transformation to a resistant encysted form, the sclerotium. Starvation can also induce sporulation, for which a period of illumination is also necessary, and which involves a complex sequence of developmental changes. These changes include the morphogenesis of a fruiting-body (sporangium), the cleavage of the cytoplasm to form uninucleate cells, the hardening and darkening of spore walls around these cells, and both mitotic and meiotic divisions of the nuclei. In

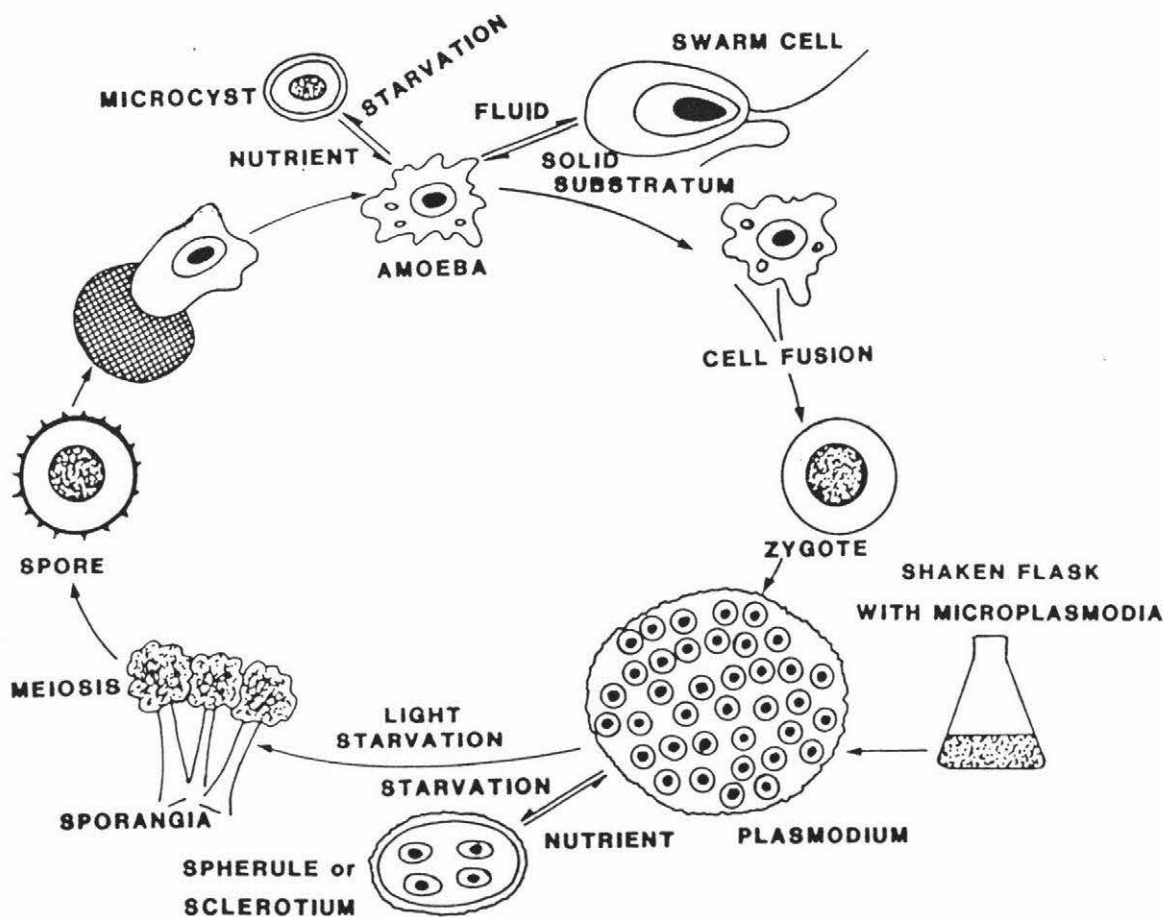


Figure 1. Diagrammatic representation of the life cycle of *Physarum polycephalum* (from Gorman and Wilkins, 1980).

moist conditions the spores germinate to release amoebae. When conditions become unfavourable, amoebae can also form a reversible encysted stage, the microcyst, and in very moist conditions they can form flagellated swarm cells. In heterothallic strains of *Physarum*, the transition from the amoebal to the plasmodial phase of the life cycle requires the fusion of two amoebae of different mating types, with the subsequent fusion of the two nuclei. However there are some strains, most notably the Colonia (CL) strain, in which the transition occurs apogamically within clones of amoebae to produce haploid plasmodia. The CL-2 strain, as used in the present study, is a diploid derivative of the CL strain produced by heat shocking CL plasmodia. (See Dee, 1975; Alexopoulos and Mims, 1979; Gorman and Wilkins, 1980). In the laboratory, the plasmodial phase is routinely cultured on a semidefined medium on a rotary shaker and under these conditions it breaks up into small pieces, the microplasmodia.

The biochemical, morphological, and ultrastructural changes that occur during the process of differentiation from plasmodia to mature spores are known in some detail (Guttes *et al.*, 1961; Sauer *et al.*, 1969; Rusch, 1970; Laane *et al.*, 1976; Goodman, 1980). In addition, it has been established that heat shocks at specific stages of this development result in subsequent developmental abnormalities (Sauer *et al.*, 1969). In the present study, these findings are extended by examining in greater detail the changes in the morphology and ultrastructure of the developing sporangia produced by heat shocks at specific stages of development.

To determine whether *Physarum* responds to heat shock with the changes in protein synthesis typical of *Drosophila* and, if so, whether this response is independent of developmental state, the changes in protein synthesis induced by heat shocks of sporulating plasmodia at different stages of development and of growing plasmodia are examined. A general suppression of translation in *Physarum* at an elevated temperature has been reported to occur (Bernstam, 1974; 1978). However it has yet to be established for *Physarum* whether this suppression of translation is

accompanied by the synthesis of specific hsp's. A heat shock induced repression of both normal transcription and translation, as has been established for *Drosophila*, could account for the developmental abnormalities reported to occur in *Physarum* (Sauer *et al.*, 1969). This possibility is investigated in this study by using heat shock as a probe of differences that occur in gene expression during sporulation.