Morphological study of fish fibroblast responded to heat shock treatment

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ABSTRACT

The present experiment describes the morphological change of BPS-4 cells, derived from the spleen of black porgy (*Acanthopagrus schlegeli*), after heat-shock treatment. The volume of BPS-4 cells increased with the increases of incubation temperature and duration of the heat treatment. The results on light and electron micrographs showed that the BPS-4 cells reacted strongly to 40°C /30min treatment. The stressed BPS-4 cells retained their normal fibroblastic morphology with a comparative round shape. Ultrastructural changes within the cytoplasm included (A) more electron-transparent, (B) increasing of disruption, vesiculation and fragmentation of endoplasmic reticulum, (C) increasing of fragmentation and swelling of mitochondria, (D) the disappearance of polysomes, (E) increasing numbers of disorganized organelles in the perinuclear region. Following heat-shocking, striking changes such as, distinct swelling of nuclear membrane, considerable alterations in the integrity of the nucleoli were found in the nucleus of cells.

Key words: black porgy, cell line, heat-shock, ultrastructure.

Introduction

Cell lines derived from fish, a poikilothermal species, are demonstrated to be able to grow within a wide range of temperature (Wolf and Mann, 1980; Wolf and Quimby, 1962; Mosser *et al.*, 1986). These characteristics enable fish cell to be a good system for the investigation of the heat-shock responses. Studies on the heat shock responses in fish cells have previously been performed on cultured RTG-2 cells derived from rainbow trout, *Salmo gairdnerii* (Kothary and Candido, 1982; Kothary *et al.*, 1984; Mosser *et al.*, 1986; 1987),CHSE-214 cells derived from Chinook salmon embryo cells (Heikkila

et al., 1982; Gedamu *et al.*, 1983), and TO-2 cells derived from *Tilapia* ovary (Chen *et al.*, 1988). These reports describe the synthesis and induction of the stress protein and the expression of stress genes in fish cell lines.

Studies on the morphological changes of mammalian cell lines in response to heat-shock, aiming to determine the function of stress protein are reported by Shyy *et al.* (1989) and Welch and Suhan (1985). However, little information have been available on the morphological changes of heat stressed fish cells except those at phase-contrast microscopic level (Wang *et al.*, 1989; Mosser *et al.*, 1986; 1987).

In the present study, the ultrastructural changes of fish fibroblast under heat shock treatment are described. In detailed observation is also described on the changes of cytoplasmic organelles, such as mitochondria, endoplasmic reticulum and polysomes. In addition, changes observed within the nucleus and nucleolus of cells after heat-shock treatment are also discussed.

Materials and Methods

Cells and culture conditions

A fibroblast-like cell line, BPS-4 cell line, derived from the spleen of juvenile black porgy, *Acanthopagrus schlegeli* (Tung *et al.*, 1991) was used for the present study. The cells were maintained in Leibovitz's L-15 medium (Hazlenton Biologies, Inc. USA) supplemented with 10% fetal calf serum (GIBCO, USA) and 0.15 M NaCl at 28 ℃ as described by Tung *et al.* (1991). The pH value of the culture media were around 7.4 during culturing and heat-stressing.

Heat-shock treatment

The heating for BPS-4 cells was carried out in precalibrated water baths. Monolayers of cells in parafilm-sealed petri dishes or flasks were submerged under water for acute heat shock treatments. The temperature of the water bath was adjusted to 40 ℃ ± 0.3 ℃ level unless those indicated separately.

Measurement of cell diameter and cell volume

The cell diameter and cell volume of trypsinized BPS-4 cell were measured with Coulter Counter (Coulter Electronic, England).

Light microscopy

Heat-shock cultured cells stained with or without Giemsa staining were observed and photographed using an Olympus IMT-2 inverted microscope.

Electron microscopy

Cells for scanning electron microscopic study were grown on acid washed coverslips. Both heat-shock and control cultured cells were washed with phosphate

buffered saline (PBS) buffer and fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer supplemented with 6.6% sucrose at room temperature for 60 min, followed by two changes of 0.1M cacodylate with 6.6% sucrose for 10 min, and post-fixed in 1% OsO in 0.1M cacodylate at 4 ℃ for 60 min. Then, cells were dehydrated in a graded series of ethanol, and dried in a Hitachi critical point drier. The cells were then coated with gold by using a ion sputter, and examined with an Hitachi S-520 Scanner operating at 20 KV.

For transmission electron microscopic study, the cells were grown on acid washed and Teflon coated coverslips as described by Chang (1971), and heated in monolayers at the exponentially growing phase. After treatment, cells were immediately washed in three changes of warmed PBS buffer. The samples were fixed as described above. After fixation, the samples were *en bloc* stained in saturated aqueous uranyl acetate for 30 min. The samples were washed in three changes of distilled water for 5 min and dehydrated as the above described and were embedded in Spurr's resin. Section were obtained with Sorvar MT-5000 ultramicrotome and doubly stained with uranyl acetate and lead citrate. Observations and photographs were made on a Hitachi H-600 electron microscope at 75 KV.

Results

The size of BPS-4 cells at different heat treatment

As shown in Table 1, under 35 ℃, 37

Table 1 The size of trypsinized BPS-4 cells treated under different heating

	control	35℃		37℃		40℃	
		1h	3h	1h	3h	15m	30m
diameter (um)	8.352	8.828	9.080	8.828	9.080	9.080	9.876
volume (um)	305.2	360.4	392.1	360.4	392.1	392.1	504.0
ratio *	1	1.18	1.29	1.18	1.29	1.29	1.65

After the heat treatment, the BPS-4 cells were trypsinized and their diameters were measured with coulter counter.
* The volume of control cell was defined as 1.

℃ and 40 ℃ for a period of time ranged from 15 min to 3 hr, the volumes of treated BPS-4 cells increased as prolong of incubation time or elevation of incubation temperature. The volume of cell treated with 40 ℃ for 30 min measured approximate 1.65 times of control cell.

Morphological changes after the heat treatment

When monolayer of BPS-4 cells endured at 40 ℃ for 30 min, the cells retained their normal fibroblast-like morphology and took on a more round attachment to the substratum surface. No additional morphological changes were observed after the heat-shock treatment (Fig. 1).

BPS-4 cells incubated at the normal temperature (i. e., 28 ℃) or after heat-shock treatment (30 min at 40 ℃) are shown in Figure 2. Control cells shows well-developed profiles of membranes of endoplasmic reticulum (ER), prominent nucleus, numerous mitochondria and other organelles. The central nucleus possesses a distinct and compact nucleolus with both the granular and fibrillar regions. Chromatin is mainly in the form of euchromatin with few patches of heterochromatin. The nuclear envelope of control cell consists a well-defined double unit membrane and nuclear pores are clearly observed (Fig. 2.A). Narrow cisternae with flatted sac or branched tubules distribute in ER (Figs. 2.A and C)

Mitochondria of normal BPS-4 cells are highly elongated and convoluted. The dense matrix, integrity of membranes and well-organized crests in mitochondria are also observed very clearly (Fig. 2.E). Polysomes are also found scattering throughout the cytoplasm of normal BPS-4 cell (Fig. 2.C).

After heat treatment, the deformities and irregularities in fine structure of BPS-4 cells were noted (Figs. 2.B, D and F). The cytoplasm revealed a more electron-transparent view when compared with the cells incubated in normal conditions. Many vesicular structure with dilation and vesiculation apparently derived from ER were frequently found (Figs. 2.B and D) in the heat treated cells. The mitochondrial vacuolation, fragmentation and degeneration in the treated cells were also observed in these cells. The enlarged intracrestal spaces and swollen inner membrane were observed in the treated cells (Fig. 2.F). Crests in most mitochondria disappeared, and crests in few mitochondria were still present (Fig. 2.F). In the heat-shock treated cells, an increase in the numbers of the mitochondrial and ER fragments and vesicularized membranes in the perinclear region were observed. Polysomes disappeared and only single ribosomes scattered throughout the cytoplasm were observed in the treated cells (Figs. 2.D and F). The changes of nucleus in the heat-treated

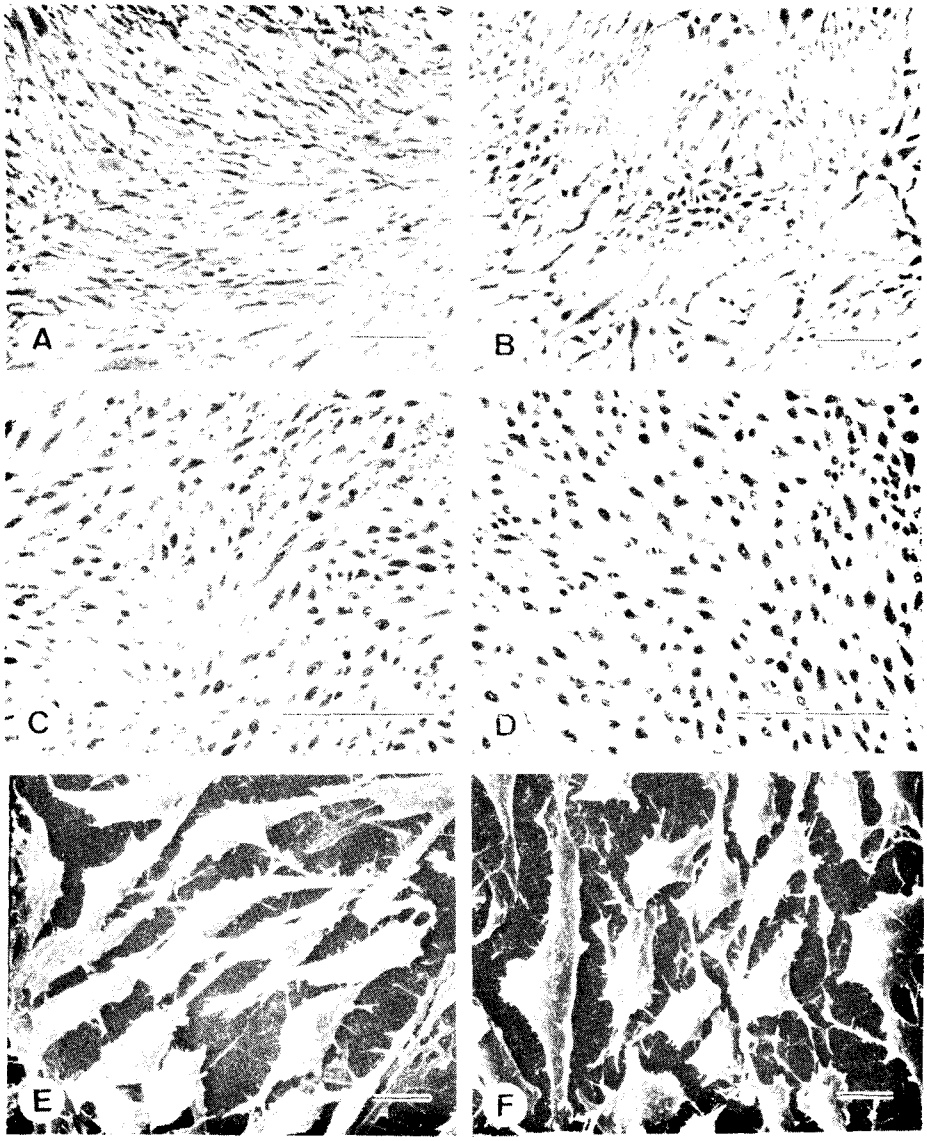

Fig. 1 The effects of heat treatment on BPS-4 cells maintained at 28 ℃ (A, C, E) and cells 40 ℃ /30min heat treatment (B, D, F). A and B, phase contrast micrograph. C and D, micrograph of May Grunwald-Giemsa stained cell. E and F, Scanning electron micrograph. Scales: 100um in A- >D and 10 um in E->F.

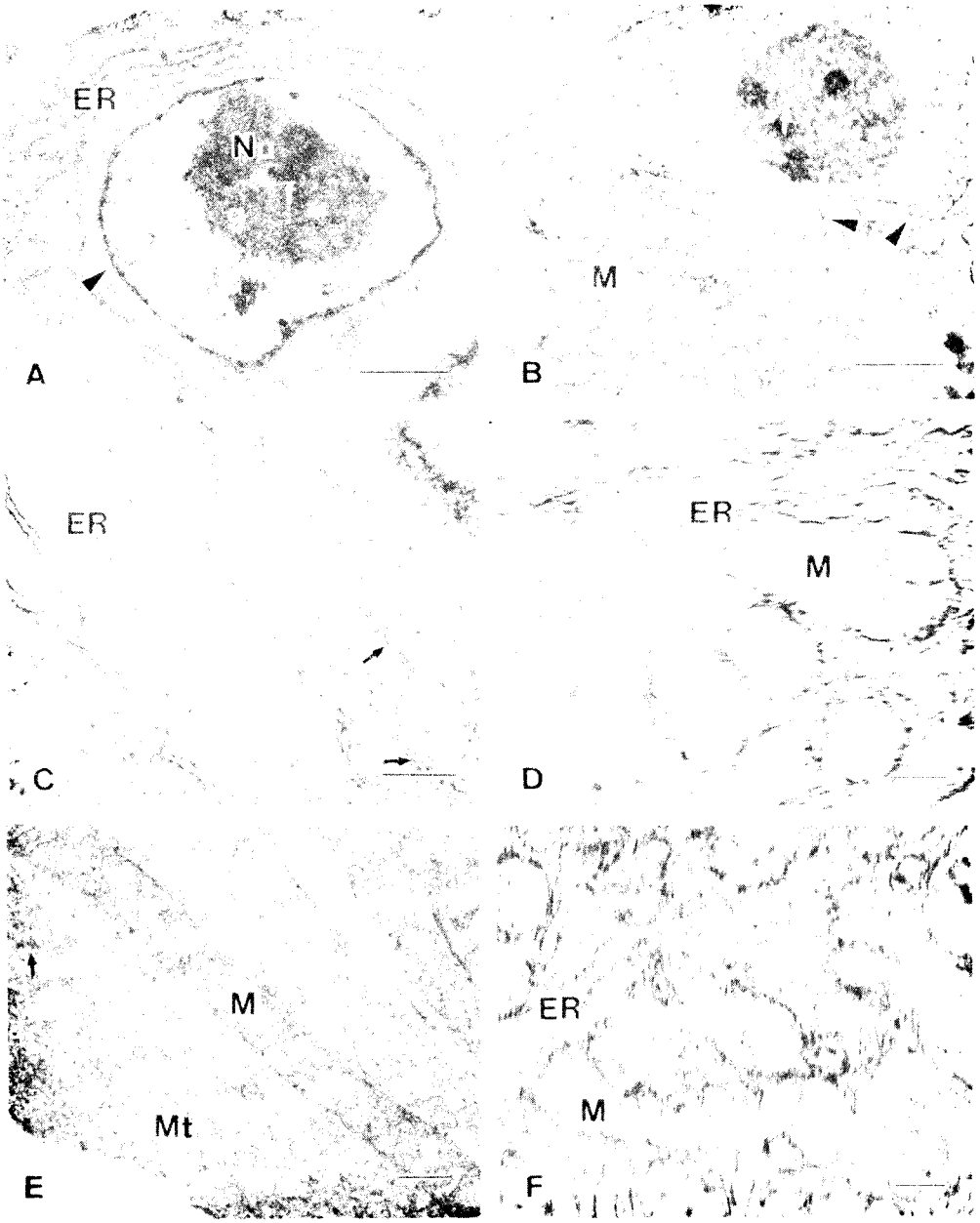

Fig. 2 Ultrastructural changes of BPS-4 cells under heat treatment. A, C and E were untreated cells; B, D and F were subjected to a 40 ℃ /30min heat shock. Arrows indicate polysomes and arrowheads indicate nuclear pores. N, Nucleus; M, Mitochondria, Mt, microtubules; Nu, Nucleolus; ER, Endoplasmic reticulum; f, fibrillar region of nucleolus; g, granular region of nucleolus. Scales, 1 um in A and B; 0.2 um in C->F. A and B, ultrastructure of whole BPS-4 cell; C and D, endoplasmic reticulum with polysomes in cytoplasm; E and F, mitochondria and its vicinity organelles. Note the lack of polysomes in D and F as compared to C and E.

cells consists: more electron-transparent for nucleoplasm (Fig. 2.B); swelling for the nuclear envelope; enlargement for the nuclear pore (Fig. 2.B); Amplification, segregation and degranulation for nucleoli (Fig. 2.B).

Discussion

It is generally known that the cells growing under tissue culture conditions are very sensitive to the changes of the environmental conditions. The cell under heat-shock showed the changes both in cell physiology and morphology (Welch *et al.*, 1991). This report described the morphological changes in fish cell lines after heat-shock treatment.

The increasing volume of treated BPS-4 cells was demonstrated related to temperature and duration of the heat treatment in the present study. Similar results were also reported in the JF cell (Wen *et al.*, 1990) and TO-2 cells (Wang *et al.*, 1989).

Under light microscope, the RTG-2 cells, grown routinely at 22 ℃, lost their normal fibroblast-like shapes and became more flatten and epithelial-like morphology at 28 ℃ for 24 h to 7 days (Mosser *et al.*, 1986; 1987). In contrary to RTG-2 cells, heat shocked BPS-4 cell retained their normal fibroblast-like morphology and showed more round-shaped morphology after 40 ℃ /30min heat treatment (Fig.1). It is suggested that BPS-4 cells could endure at high temperature for short time exposure. However, when monolayer of BPS-4 cells was transferred to 37 ℃ for 24 hr, the stressed cells became flattened. Moreover, the cells were degenerated at 37 ℃ for 72h incubation (data not showed).

Ultrastructural observations revealed that damageable membranes of stressed BPS-4 cells were the more metabolically active regions and deformities were, such as fragmentation, dilation, vesiculation and degeneration of ER, nuclear envelope and mitochondria. The phenomenona were similar to those obtained from rat embryo fibroblast exposed to heat-shock (Welch and Suhan, 1985) and Chang liver cells to low intensity microwave (Dwivedi *et al.*, 1989).

Many organelles in heat stressed BPS-4 cells were found to translocate toward the juxtanuclear area. The phenomenon was similar the those obtained from rat embryo fibroblast (Welch and Suhan, 1985) and mammary epithelial cells (Shyy *et al.*, 1989) exposed to abnormal heat treatment. Cytochemical studies, obtained from mammary epithelial cells by Shyy *et al.*, (1989) shows that within minutes after heat shock, the intermediate filaments are found to relocalize into a meshwork of filaments closely enveloping the nucleus. They conclude that the collapse of the intermediate filaments is

the relocalization of many organelles into the perinuclear region of the stressed cell. Heat shock also demonstrate to be able to inhibit nucleolar function, such as blocking of ribosome biogenesis and denaturation or aggregating of preribosomal particle (Welch *et al.*, 1991). The nucleoli of heat-treated BPS-4 cells showing less condense, segregation and degranulation might reflect a earlier stage of blockage for ribosome synthesis.

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References

Chang, J. P. 1971. A new technique for separation of coverglass substrate from Epoxy-embedded specimens for electron microscopy. J. Ultrastruct. Res. 37:370-377.

Chen, J. D., F. H. Yew, and G. C. Li 1988. Thermal adaptation and heat shock response of Tilapia ovary cells. J. Cell. Physiol. 134:189-199.

Dwivedi, R. S., U. Dwivedi, and B. Chiang 1989. Low intensity microwave radiation effects on the ultrastructure of Chang liver Cells. Exp. Cell Res. 180:253-265.

Gedamu, L., B. Culham and J. J. Heikkila 1983. Analysis of the temperature-dependent temporal pattern of heat-shock-protein synthesis in fish cells. The Biochemical Society. 647-658.

Heikkila, J. J., G. A. Schultz, K. Iatrou and L. Gedamu 1982. Expression of a set of fish genes following heat or metal ion exposure. J. Biol. Chem. 257(20):12000-12005.

Kothary, R. K., E. A. Burgess and E. P. M. Candido 1984. The heat-shock phenomenon in cultured cells of rainbow trout hsp 70 mRNA synthesis and turnover. Biochim. Biophys. Acta 783:137-143.

Kothary, R. K. and E. P. M. Candido 1982. Induction of a novel set of polypeptides by heat shock or sodium arsenite in cultured cells of rainbow trout, *Salmo gairdnerii*. Can. J. Biochem. 60:347-355.

Mosser, D. D., J. J. Heikkila and N. C. Bols 1986. Temperature ranges over which rainbow trout fibroblasts survive and synthesize heat-shock proteins. J. Cell. Physiol. 128: 432-440.

Mosser, D. D., J. V. Oostrom and N. C. Bols 1987. Induction and decay of thermotolerance in rainbow trout fibroblasts. J. Cell. Physiol. 132:155-160.

Shyy, T. T., B. B., Asch and H. L. Asch 1989. Concurrent collapse of keratin filaments, aggregation of organelles, and inhibition of protein synthesis during the heat shock response in mammary epithelial cells. J. Cell Biol. 108:997-1008.

Tung, L. C., S. N. Chen and G. H. Kou 1991. Three cell lines derived from spleen and kidney of black porgy (*Acanthopagrus schlegeli*). Fish Path., 26(3):109-117.

Wang, H. C., J. D. Chen, G. C. Li and F. H. Yew 1989. Characterization of a heat-resistant strain of Tilapia ovary cells. J. Cell Sci. 92:353-359.

Welch, W. J., H. S. Kang, R. P. Beckmann and L. A. Mizzen 1991. Response of mammalian cells to metabolic stress: Changes in cell physiology and structure/function of stress proteins. In "Heat shock proteins and immune response". S. H. E. Kaufmann (ed) Springer-Verlag Berlin pp31-55.

Welch, W. J. and J. P. Suhan 1985. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after hat-shock treatment. J. Cell Biol. 1198-1211.

Wen, C. -M., S. -N. Chen and G. -H. Kou 1990. A cell line derived from jarbua (*Therapan jarbua* FORSKAL) fin. COA Fisheries Series, 24. Fish Disease Research (X), 18-39. (in Chinese with English abstract).

Wolf, K. and J. A. Mann 1980. Poikilothermic vertebrate cell lines and viruses. A current listing for species. In Vitro 16:168-179.

Wolf, K. and M. C. Quimby 1962. Established eurythermic lines of fish cells in vitro. Science, 135:1065-1066.

魚纖維樣細胞對熱反應的形態學研究

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摘　要

黑鯛 *(Acanthopagrus schlegeli)* 脾臟細胞株(BPS-4)經熱處理後以光學顯微鏡、掃描及穿透式電子顯微鏡觀察細胞外形及其內部構造的變化。熱處理後會增加 BPS-4 細胞的體積，且在一定範圍內依熱處理時間的延長和溫度的升高而增加體積。在光學顯微鏡下 BPS-4 細胞仍保有纖維樣的細胞形狀，平鋪在基質上，細胞似乎立體些，細胞質變厚。在掃描電子顯微鏡下，除上述現象較明顯外，細胞上的微絨毛變多。在穿透式電子顯微鏡下，微細構造的變化有：細胞質內的內質網和粒線體膨脹、斷裂、泡化；粒線體的 intracrestal space 加寬，內、外膜較分開；多核糖體成為游離狀態；細胞核內的核仁分離、變鬆散、顆粒區成分變少，核膜的雙層膜分開；同時可見細胞質內的胞器往細胞核區集中。

關鍵字：黑鯛，細胞株，熱休克，微細構造。