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DYNAMIC CHANGES IN THE LOCALIZATION AND QUANTITY OF HEAT SHOCK PROTEINS IN A CULTURED FIN CELL LINE OF COLOR CARP *CYPRINUS CARPIO*

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Chen-Chun Ku, Shiu-Nan Chen and Guang-Hsiung Kou (1992) Dynamic changes in the localization and quantity of heat shock protein in a cultured fin cell line of color carp *Cyprinus carpio. Bull. Inst. Zool., Academia Sinica* **31**(4): 276-289. Immunoblotting and indirect immunofluorescence were used to study the dynamic changes in quantity and intracellular distribution of major heat shock proteins *hsp*87, 70, 33, and 27 in cultured fin cells of color carp, *Cyprinus carpio* (CCF), subjected to heat shock at 40°C and allowed to recover at 31°C.

*Hsp*87 was enhanced immediately following heat shock; however, cellular *hsp*87 did not increase noticeable until 12 h following heat shock. Through indirect immunofluorescence, *hsp*87 was observed localizing in the cytoplasm before and after heat shock. During heat shock, significant amounts of *hsp*70 were immediately synthesized and moved from the cytoplasm to both the nucleolus and the nucleus; it then "overflowed" into the perinuclear region. Intracellular levels of *hsp*33 increased significantly (as quickly as *hsp*70) following heat shock, but disappeared completely 12-24 h later. Furthermore, during heat shock *hsp*33 appeared first at the nucleus; it was then found exclusively in the cytoplasm before disappearing. *Hsp*27 was detected in nostressed cells; after reaching maximum level 6 h following heat shock,a steady-state level was maintained for the remainder of the experimental period. Also by indirect immunofluorescence, *hsp*27 was detected in the nuclei of about half of the tested unstressed CCF cells. During heat shock, cells with nuclear staining appeared diminished, and little or no fluorescence was observed in the CCF cells. Subsequently, filament staining within the perinucleus began to increase, then intense *hsp*27 cytoplasmic staining appeared.

CCF cells were heated to 40°C for 10 h, then cooled to 31°C. Indirect immunofluorescence showed *hsp*87, 70, and 33 levels returned to preheat-shock levels 36 h after recovery. However, intense *hsp*27 cytoplasmic staining remained even after the 36-h recovery period. These results suggest heat shock proteins are degraded in the cytoplasm of *C. carpio*.

Key words: Heat shock proteins, localization, fish cell line

Heat shock proteins are produced when a cell is stressed by various environmental insults including high temperatures, heavy metals, amino acid analogues, and exposure to a variety of chemical compounds (Lee and Hahn, 1988). Although these factors do not affect various species equally, the heat shock

response has been shown to occur in many different systems-from bacteria and plants up to all higher mammalian tissue and a number of eukaryotic cell lines (Craig, 1985; Lindquist, 1986). Although this response has been observed in every species examined (Craig, 1985), very few studies have addressed the dynamics of changes in the localization of heat shock proteins in cells.

Numerous studies of *Drosophila* and mammalian cultured cells have shown that the subcellular locations of heat shock proteins are dependent on the stressed state of the cell. Upon experiencing stress, *hsp*70 is concentrated in the nucleus or nucleoli; during recovery, *hsp*70 returns to the cytoplasm until another stress factor is encountered (Arrigo *et al.*, 1988; Collier and Schlesinger, 1986; Lewis and Pelham, 1985; Welch and Feramico, 1984). Similar cytoplasmic and nuclear concentrations of large (80-90 KDa) (Berbers *et al.*, 1988) and small (20-30 KDa) (Caltabiano *et al.*, 1986, 1988; Arrigo *et al.*, 1980, 1987, 1988; Rossi and Lindquist, 1989) heat shock proteins have also been reported in *Drosophila, mammalian*, and plant cells.

Studies of heat shock response in fish cell lines have previously been performed primarily on cultured RTG-2 and RTH cells of rainbow trout (*Salmo gairdnerii*) (Kothary and Candido, 1982; Kothary *et al.*, 1984a; Mosser *et al.*, 1986; Misra *et al.*, 1989), CHSE-214 cells derived from Chinook salmon embryo cells (Heikkila *et al.*, 1982; Gedamu *et al.*, 1983), or TO-2 cells derived from the ovaries of adult tilapia hybrids of *T. mossambica* and *T. nilotica* (Chen *et al.*, 1988). Fish cell line responses to heat, sodium arsenite, or metal ions are very similar to those observed in *Drosophila* (Lewis *et al.*, 1975). At the molecular level, the cDNA of trout *hsp*70 has been cloned and sequenced; it shows extensive homology with the *hsp*70 genes of both *Drosophila* and yeast (Kothary *et al.*, 1984b).

Although the function of *hsp*70 proteins made in cells is that of a chaperone (Schlesinger, 1990). The function and mechanisms of *hsp*70 proteins are limited to acting as a chaperone (Hightower , 1991). To study the function of heat shock proteins, it is important to know their localization in cells. The present paper describes heat shock response in a cultured fin cell line of the color carp, *Cyprinus carpio*. Via immunoblotting and indirect immunofluorescence assays, polyclonal antisera against fish heat shock proteins were used to investigate the dynamic changes in quantity and intracellular localization of heat shock proteins in CCF cells during heat shock.

MATERIALS AND METHODS

Cell culture and stress conditions

CCF cell line derived from color carp fin (Ku and Chen, 1992) was used in all experiments. Cells were maintained in Leibovitz's L_{15} medium (Hazleton) supplemented with 10% fetal calf serum (Sera-Lab Ltd., England), 100 U/ml penicillin-streptomycin, and 25 μg/ml amphotericin B (Boehringer Mannheim GmbH, West Germany). Cultures were incubated at 31°C. The pH value of the culture media was maintained at approximately 7.0 during culturing and heating. For heat treatment, cells were placed in a water bath equipped with a circulating Thermomix 810 (Hotech).

Cell labelling and gel electrophoresis

Cells were labelled in methionine-free medium with 20 μCi/ml ^{35}S-methionine (specific activity 1,209.3 Ci/mmol) for 2 h immediately following the various treatments.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze protein patterns according to procedures described by Laemmli (1970). Cells were rinsed with cold phosphate-buffered

saline (PBS) and lysed in an equal volume of SDS-PAGE sample buffer (0.07 M Tris, pH 6.8, 11.2% glycerol, 3% SDS, 0.01% bromophenol blue, and 5% β-mercaptoethanol). Cell lysates were boiled for 5 min then analyzed on a 10% polyacrylamide gel with a 5% polyacrylamide stacking gel in a buffer system of 0.025 M Tris (pH 8.8), 0.192 M glycine, 0.1% SDS, and 0.002 M EDTA. After electrophoresis, gels were fixed with 30% methanol and 10% acetic acid for 1 h, then dried and exposed to Dupont cronex X-ray film at –70°C for six days. Autoradiographs were developed in a Dupont cronex X-ray developer. In order to compare protein band intensities, equal sample volumes were loaded into the gel wells.

Immunoblot assay

Following electrophoresis, proteins were transferred with a Hoeffer electrotransphor apparatus to Immobilon PVDF Transfer Membranes (Millipore) for 1 h at 1 A, then blocked with 3% skim milk (Difco) in TBS (20 mM Tris, 500 mM NaCl, pH 7.5) for 1 h at room temperature. Rabbit anti-CCT *hsp*87, anti-CCT *hsp*72 (prepared from a color carp testis cell line, CCT), rabbit anti-CCG *hsp*33 or anti-CCG *hsp*27 (prepared from color a carp gill cell line, CCG) antibodies were used at a dilution of 1:100 in 3% skim milk-TBS; these were rinsed three times in TBS containing 0.05% Tween 20 (10 min per wash), incubated for 1 h with a 1:10,000 dilution of peroxidase-conjugated goat anti-rabbit antibody (Jackson Immunoresearch), rinsed three times as before, then reacted with 4-chloro-1-naphtol (Merck).

Indirect immunofluorescence

Approximately 10^5 cells were plated directly onto a 35×10 mm petri dish and allowed to adhere for 24 h. Cells were fixed in 3.7% paraformaldehyde in PBS for 10 min, followed by immersion in 0.2% Triton X-100 in PBS for 2 min plus a PBS wash. Rabbit anti-hsp antibody was used at a dilution of 1:500 in 3% BSA and incubated with cells for 1 h at room temperature. Cells were rinsed with PBST (containing 0.05% Tween 20) and incubated for 1 h with a 1:20 dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (Jackson Immunoresearch). The dishes were then rinsed, mounted in phosphate-buffered glycerol, and observed with a microscope equipped with epifluorescence optics. Samples were photographed with Kodak 35-mm Tri-X film shot at 400 ASA for 50 or 60 seconds.

RESULTS

Protein synthesis of CCF cells after various heat treatments

When CCF cells were exposed to high temperatures, the protein synthesis pattern changed-as visualized by SDS-PAGE analysis of radiolabelled cellular proteins. Four major heat shock proteins (*hsp*87, 70, 33, and 27) plus a minor one (*hsp*97) were induced. Figure 1 shows that CCF cells began to synthesize heat shock proteins at 37-43°C. The cells were able to synthesize all five heat shock proteins at 40°C or 43°C, but at 37°C only heat shock proteins with high molecular weights were synthesized.

Kinetics of *hsp* synthesis after or during heat shock at 40°C

Figure 2 shows the kinetics of hsp synthesis following various periods of heat shock at 40°C. *Hsp*87, 70, and 33 were enhanced after 5 min of treatment, and reached their maximum synthetic rate after 60 min of treatment. *Hsp*27 synthesis was the slowest among the five heat shock proteins, and was enhanced after 15 min of treatment.

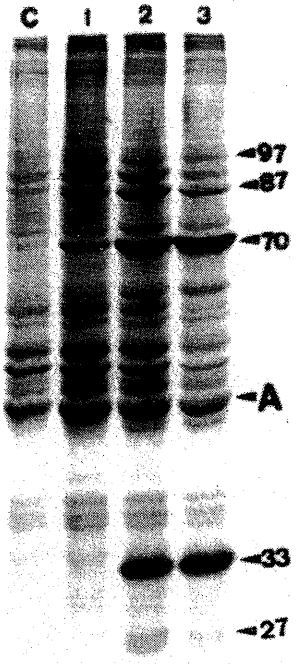

Fig. 1 Autoradiographs showing the enhanced synthesis of heat shock proteins following different temperature elevations. CCF cells were heated at (C) 31°C; (1) 37°C, 1 h; (2) 40°C, 1 h; (3) 43°C, 15 min. They were labelled with ^{35}S-methionine for 2 h at 31°C.

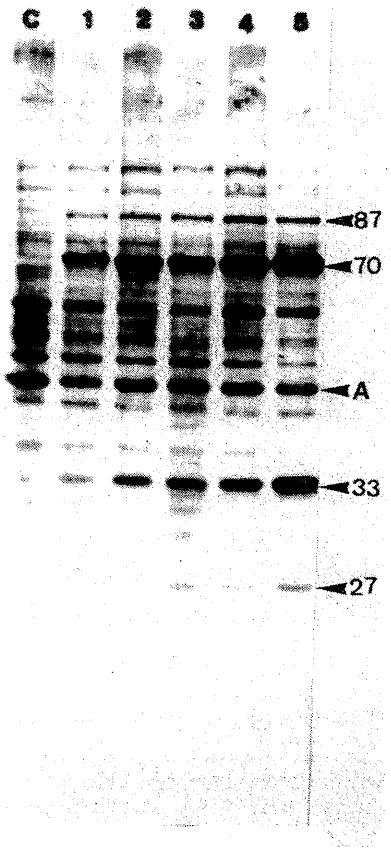

Fig. 2 Autoradiographs showing the enhanced synthesis of heat shock proteins after heating to 40°C. CCF cells were maintained at 40°C for (C) 0, (1) 5, (2) 10, (3) 15, (4) 30, (5) 60 min, then labelled with ^{35}S-methionine for 2 h at 31°C. After heating at 40°C for 30 min, cells were labelled during the (7) 4-6 or (8) 8-10 h recovery period at 31°C.

The kinetics of *hsp* synthesis during heat shock at 40°C were also investigated (Fig. 3). The synthesis of *hsp*97, 87, 70, and 27 was continuously enhanced during the experimental period; however, the synthesis of *hsp*33 ceased after 6 h. *Hsp*97 synthesis was enhanced from 2-4 h and reached its maximum synthetic rate between 8-10 h. *Hsp*87 reached its maximum rate between 2-4 h and maintained a steady-state synthetic rate until 12 h. The apparent synthesis of *hsp*70 and 33 were observed before 8 h and 6 h respectively. *Hsp*70 levels declined abruptly after 8 h, and *hsp*33 levels returned to control levels by 6 h. *Hsp*27 was enhanced and reached its maximum synthetic rate between 2-4 h, then declined after 10 h.

Pulse-chase test

*Hsp*27 synthesis appeared after *hsp*33 synthesis decreased, so it was necessary to distinguish the relationship between the *hsp*33 and 27. Therefore we performed the following pulse-chase experiment: after heating at 40°C for 2 h in the presence of ^{35}S-methionine, cells were washed with PBS and fresh medium added; incubation then continued for another 10 h at 40°C. Samples were collected every 2 h after fresh medium was added and analyzed by SDS-PAGE. Figure 4

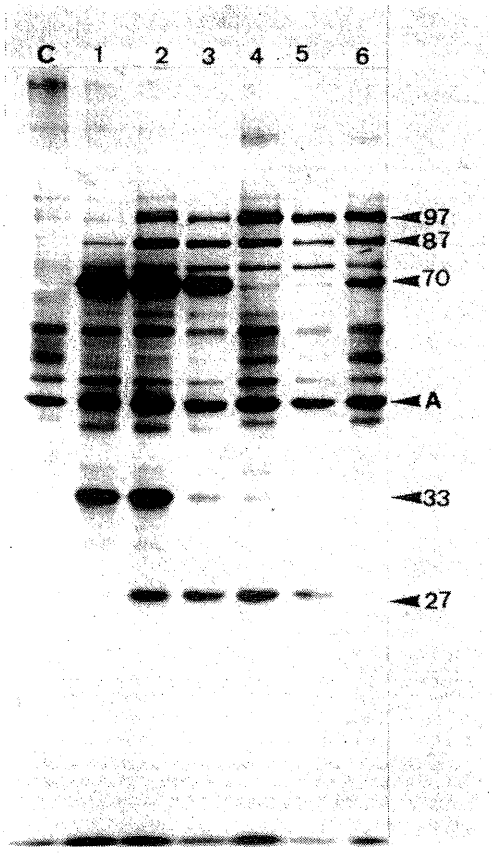

Fig. 3 Autoradiographs showing the enhanced synthesis of heat shock proteins during heating at 40°C. CCF cells were heated continuously at (C) 31°C and labelled for 2 h, or at 40°C and labelled from (1) 0-2, (2) 2-4, (3) 4-6, (4) 6-8, (5) 8-10, (6) 10-12 hours.

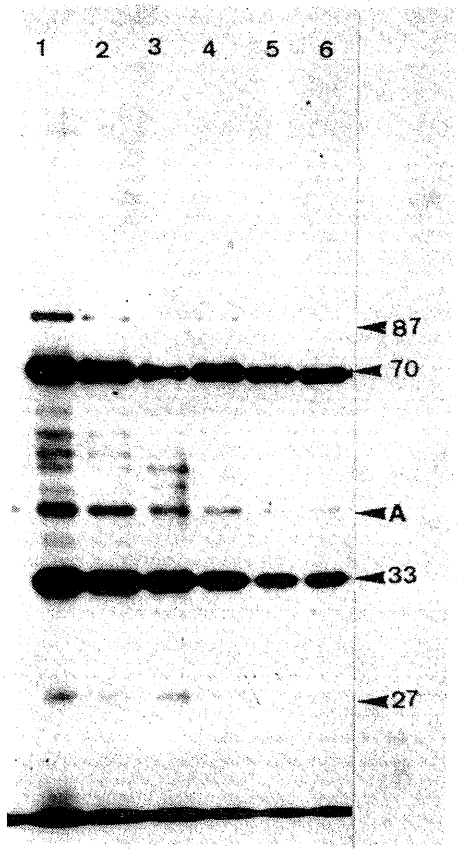

Fig. 4 Pulse-chase test. After being heated at 40°C for 2 h in the presence of ^{35}S-methionine, CCF cells were washed with PBS, after which fresh medium was added; the cells were then incubated at 40°C. Samples were collected (1) 0, (2) 2, (3) 4, (4) 6, (5) 8, (6) 10 h after the fresh medium was added, then determined by 10% SDS-PAGE.

shows that the amount of *hsp*27 did not increase, indicating that *hsp*27 was not degraded by *hsp*33.

Quantification of heat shock proteins by immunoblot analysis

Levels of heat shock proteins expression in CCF cells heated at 40°C were observed immunologically with antibodies specific to *hsp*87, 70, 33, or 27. Results of the immunoblot assay show that these antibodies cross-reacted strongly with the *hsp*87, 70, 33, and 27 in the CCF cells. The *hsp*70 and 33 were not detectable at normal growth temperatures (Fig. 5).

The accumulation of heat shock proteins in CCF cells was analyzed during prolonged heat shock at 40°C. Levels of *hsp*87 evidently did not increase until 12 h, and was thereafter maintained during the experimental period. However, *hsp*70 levels were very high after 1 h of heat shock, and thereafter maintained a steady-state level during the experimental period. *Hsp*33 reached its maximum levels by 1 h, sustained steady-state levels until 12 h, then returned to control levels between 12-24 h. Levels of *hsp*27 increased progressively after 1 h and reached

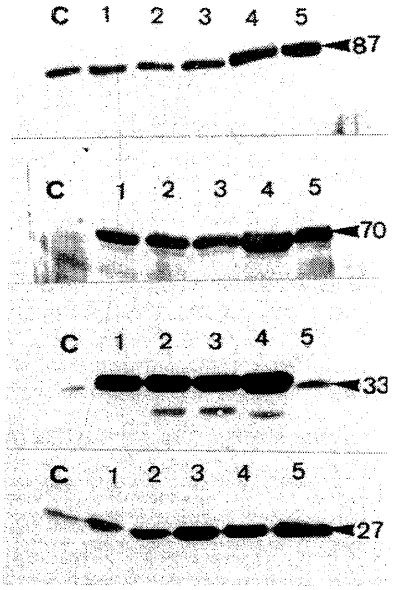

Fig. 5 Western blot analysis of induced heat shock proteins. CCF cells were heated at 40°C for various time periods. Cells were harvested and proteins analyzed on a 7% (*hsp*87 and 70) or 12.5% SDS-PAGE (*hsp*33 and 27). They were then transferred to membranes and subsequently hybridized to antibodies of CCT *hsp*87, CCT *hsp*72, CCG *hsp*33 and CCG *hsp*27 as described in Materials and Methods section. (C) 0; (1)1; (2) 3; (3) 6; (4) 12; (5) 24 h.

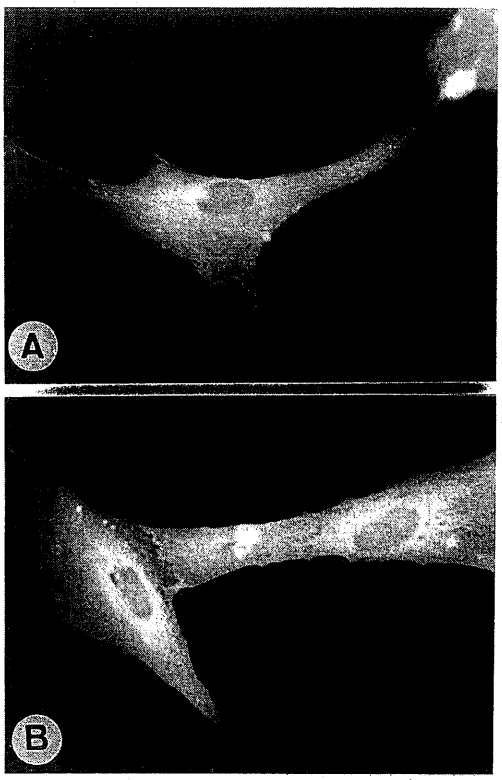

Fig. 6 Dynamic distribution of *hsp*87 during heat shock at 40°C. CCF cells were heated at 40°C for (A) 0, (B) 24 h. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCT *hsp*87 antibody. X 370.

a steadystate level at 6 h; these level was thereafter maintained during the experimental period.

Dynamic distribution of heat shock proteins during heat shock at 40°C

For researching the final approach toward characterizing the dynamic distribution of heat shock proteins as a function of heat shock treatment, CCF cells were incubated at 40°C for varying lengths of time, then fixed and examined. The intracellular distribution of each protein was determined by indirect immunofluorescence analysis. Results showed that the distribution of *hsp*87 was exclusively cytoplasmic before and during heat shock (Fig. 6) as well as after recovery (Fig. 10A-2, -3, -4).

*Hsp*70 was absent from CCF cells grown at 31°C (Fig. 7-A). Following heat-shock treatment for 2 h (Fig.7-C), intense nuclear staining (with prominent staining of the nucleoli and a slight increase in cytoplasmic staining) began to appear in the vast majority of cells. By 5 h (Fig. 7-E) or 6 h (Fig. 7-F), the nuclear staining was observed to be strong in the nucleoli, and we observed intense cytoplasmic staining around the nucleus. These cell staining conditions were displayed 24 h (Fig. 7-G) or 48 h (Fig. 7-H) after heat shock treatment. However a few cells displayed identical conditions at 31°C (figure not shown).

*Hsp*33 was also undetected in CCF cells grown at 31°C (Fig. 8-A). Following heat shock treatment for 1 h or 6 h (Fig. 8-B, -C), intense nuclear staining with diffuse cytoplasmic staining was observed. However,

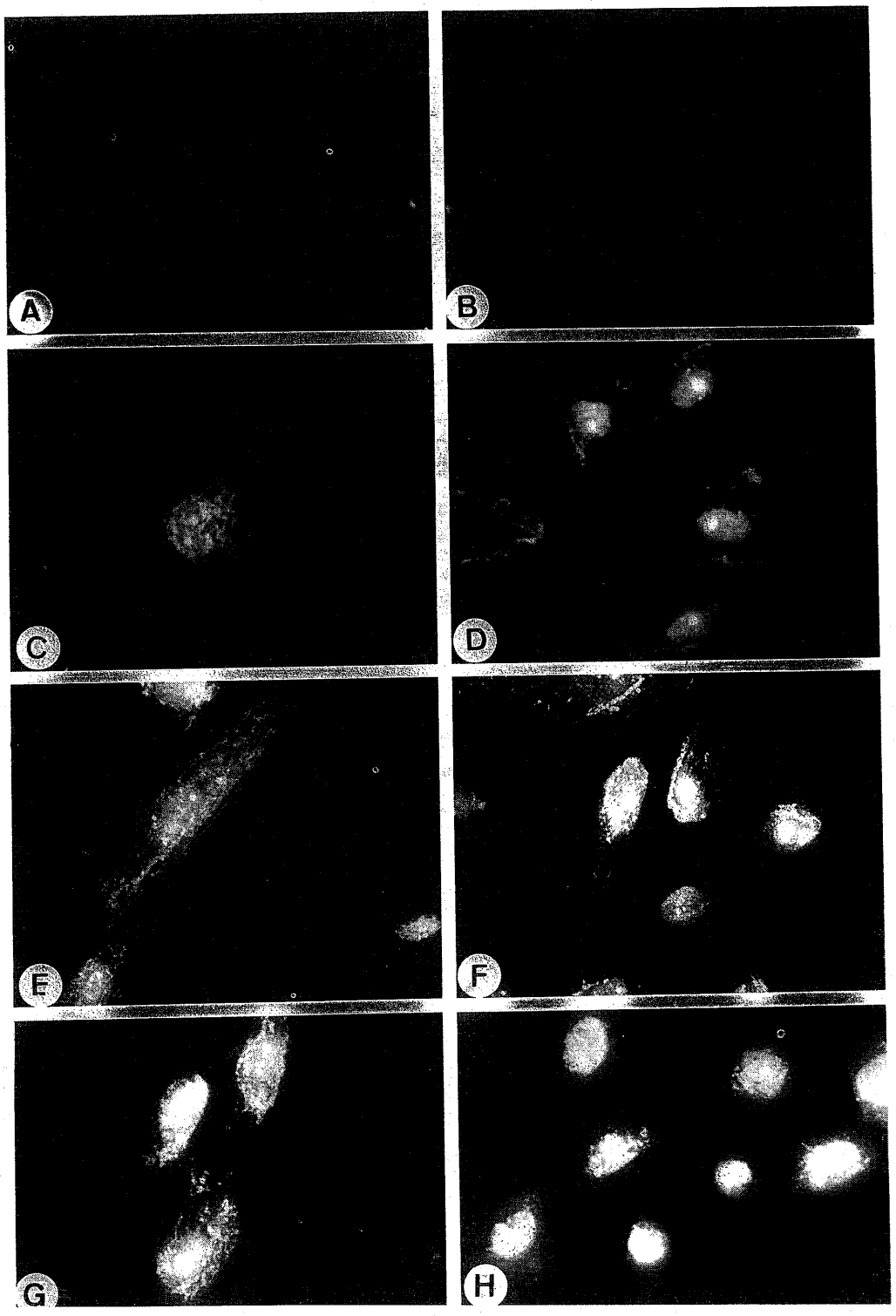

Fig. 7 Dynamic distribution of *hsp*70 during heat shock at 40°C. CCF cells were heated at 40°C for (A) 0, (B) 1, (C) 2, (D) 4, (E) 5, (F) 6, (G) 24, (H) 48 h. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCT *hsp*72 antibody. X 370.

twelve hours after heat-shock treatment (Fig. 8-D), we found exclusive *hsp*33 cytoplasmic staining. Furthermore, by 24 h (Fig. 8-E) or 48 h (Fig. 8-F)-except for very few cells containing *hsp*33 cytoplasmic aggregates-the CCF cells lost their *hsp*33 staining.

*Hsp*27 could be detected in the nuclei of approximately half of the tested unstressed CCF cells; the other cells showed very diffuse staining in their cytoplasm (Fig. 9-A). After heat shock treatment for 1 h (Fig. 9-B), cells with nuclear staining appeared diminished, and little or no fluorescence was observed in the CCF cells. By 3 h (Fig. 9-C), 5 h (Fig. 9-D) or 6 h (Fig. 9-E), filament staining within the region of the perinucleus began to increase. After 7 h of heat shock treatment (Fig. 9-F), intense *hsp*27 cytoplasmic staining appeared; this staining persisted to 24 h (Fig. 9-G), and in some case even up to 48 h (Fig. 9-H), after heat shock.

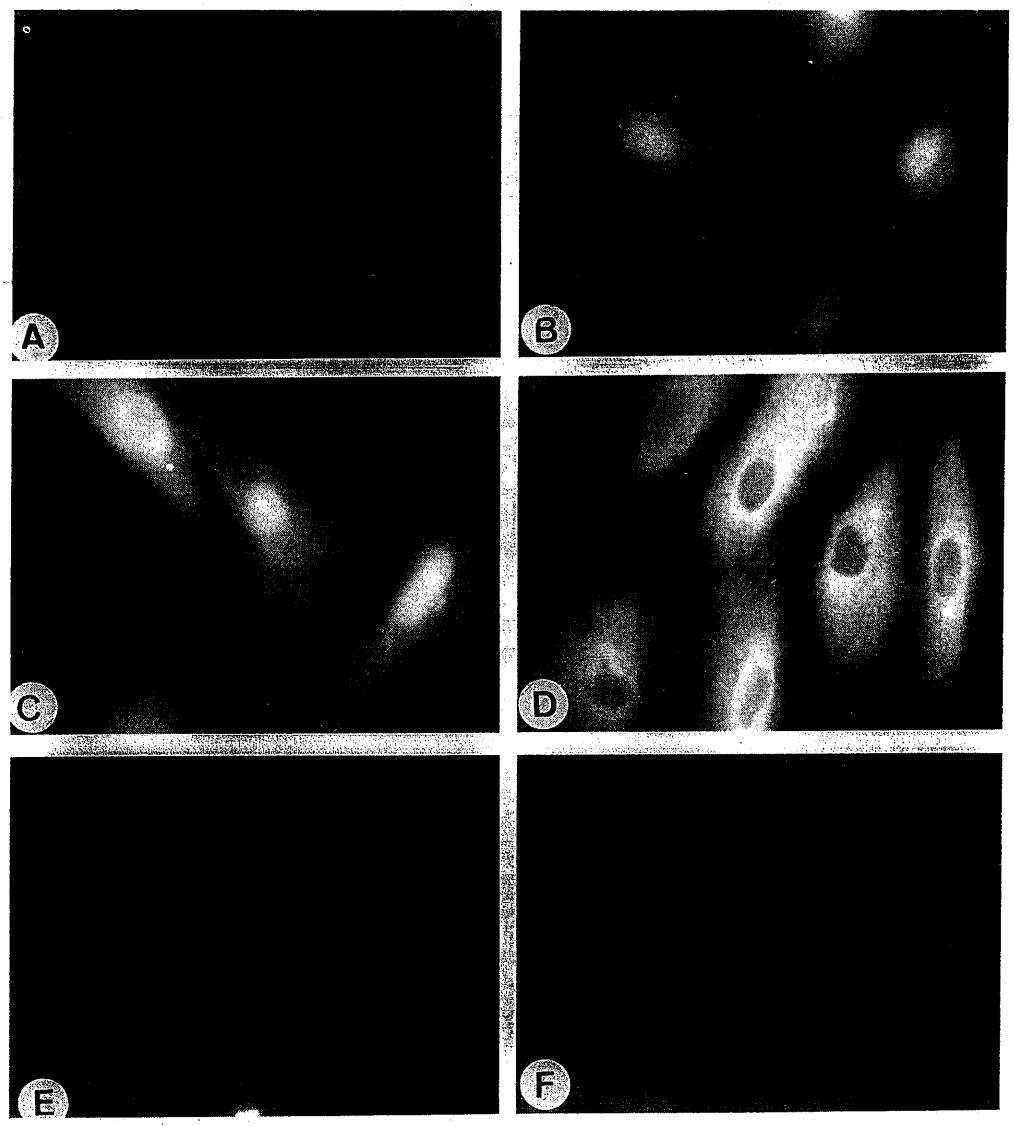

Fig. 8 Dynamic distribution of *hsp*33 during heat shock at 40°C. CCF cells were heated at 40°C for (A) 0, (B)1, (C) 2, (D) 3, (E) 12, (F) 24 h. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCG *hsp*33 antibody. X 370.

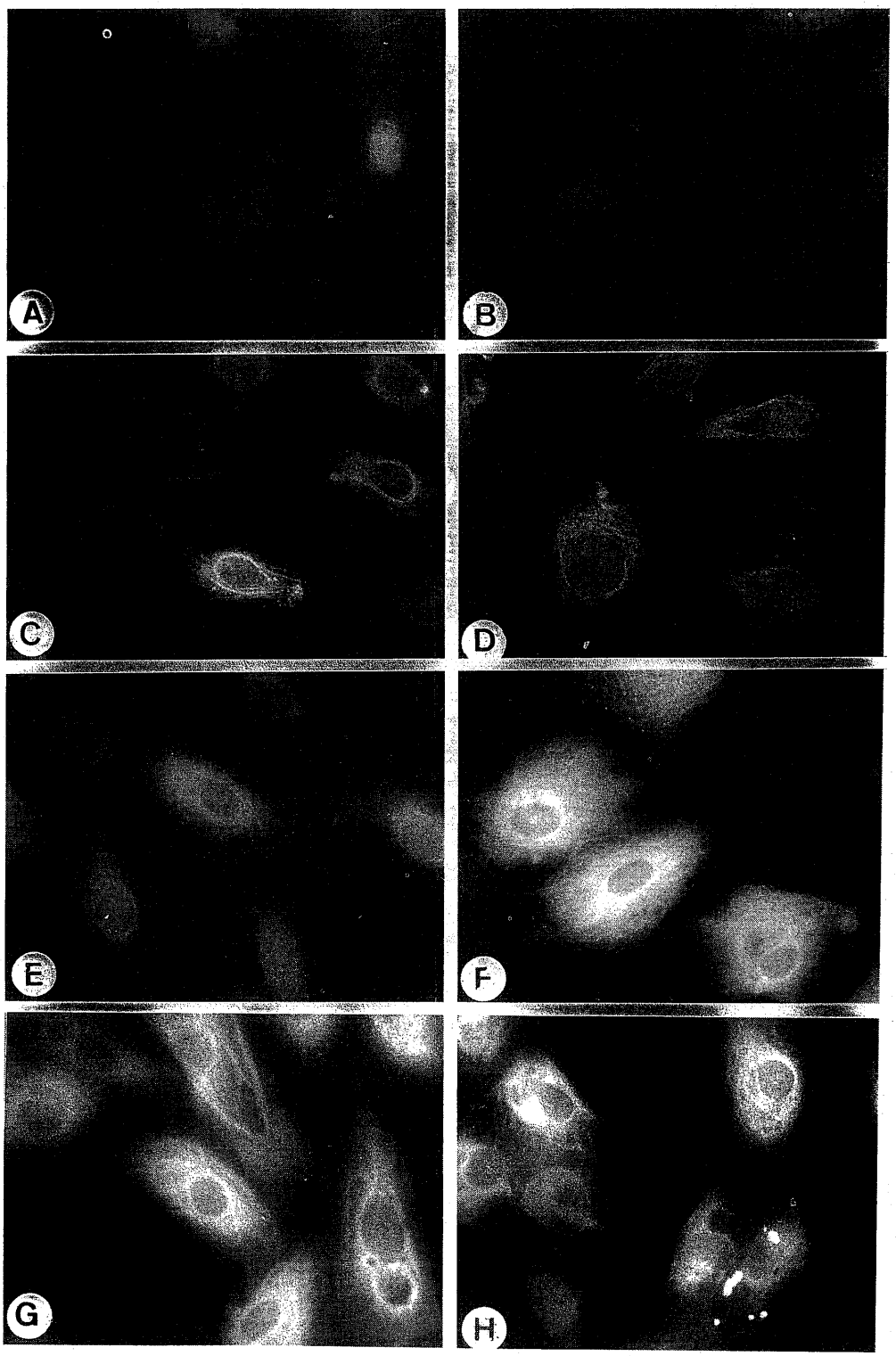

Fig. 9 Dynamic distribution of *hsp*27 during heat shock at 40°C. CCF cells were heated at 40°C for (A) 0, (B) 1, (C) 3, (D) 5, (E) 6, (F) 7, (G) 24, (H) 48 h. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCG *hsp*27 antibody. X 370.

Localization changes of heat shock proteins after recovery at 31°C from heat shock at 40°C

CCF cells were heated at 40°C for 10 h then cooled to 31°C. After recovery for 0, 14, 24 or 36 h, immunofluorescent stains revealed that levels of *hsp*87, 70, and 33 returned to preheat shock levels within 36 h of recovery, but that intense *hsp*27 cytoplasmic staining remained after 36 h of recovery. *Hsp*70 was absent from CCF cells grown at 31°C (Fig. 10B). Following heat shock treatment at 40°C for 10 h, intense nuclear staining-with a slight increase in cytoplasmic staining-appeared in all cells (Fig. 10B-1). By 14 h after cells were cooled to 31°C (Fig. 10B-2), the staining intensity of *hsp*70 diffused into the cytoplasm. By 36 h, the staining intensity returned to pre-heat shock levels (Fig. 10B-4).

*Hsp*33 levels were very low in nonstressed CCF cells (Fig. 10C). Following heat shock treatment at 40°C for 10 h, we observed intense cytoplasmic staining with prominent granules (Fig. 10C-1). During a 14 h recovery period at 31°C, the intensity of cytoplasmic staining dramatically decreased, but intensely stained cytoplasmic aggregates were still observable (Fig. 10C-2). After recovery for 36 h, we found that the cytoplasmic staining had disappeared; only a few cells containing cytoplasmic aggregates were observed (Fig. 10C-4).

*Hsp*27 was detected in the nuclei of about half of the tested nonstressed CCF cells; the other cells showed very diffuse staining in the cytoplasm (Fig. 10D). However, after heat shock at 40°C for 10 h, bright cytoplasmic staining was observed (Fig. 10D-1). When cells were allowed to recover at 31°C for 14, 24, or 36 h after heat stress, the intensity of cytoplasmic staining remained prominent (Fig. 10D-2, -3, -4).

DISCUSSION

The present study looked closely at the changes in quantity and localization of heat shock proteins in a cultured fin cell line (CCF) of the color carp, *Cyprinus carpio*. Our results were very similar to those found in gill cells (CCG) of the same species (Ku and Chen, 1991). Our comparison of changes in the localization of four heat shock proteins in response to heat shock showed that each *hsp* appeared to be independently and directly regulated by CCF cells. Similar results were obtained by Johnston *et al.* (1980), who showed -using tryptic peptidemapping procedures-that four heat shock proteins in chick embryo cells are unrelated.

Our experiments with *hsp*70 suggest that the synthesis of *hsp*70 is self-regulated, that is, after *hsp*70 levels reached their maximums in heated CCF cells (Fig. 4), *hsp*70 synthesis declined, then continued at a low but steady rate (Fig. 3). Similar mechanisms have been proposed for *E. coli* (Tilly *et al.*, 1983) and *Drosophila* (Didomenico *et al.*, 1982), indicating that repression of *hsp*70 synthesis requires a critical level of *hsp*70. Additionally, Stone and Craig (1990) indicated that this cause-and-effect relationship is at least partially mediated at the transcriptional level; they also suggested that there is an element-either close to or overlapping heat shock element 2- that confers sensitivity to the SSA1 protein (yeast *hsp*70). As to localization, *hsp*70 levels in CCF initially relocated to nuclear component(s) during heat shock; they then moved to nucleoli and "overflowed" from the nucleus to the cytoplasm-at last with a predominantly perinuclear distribution. Similar results were reported by Lewis and Pelham (1985). The accumulation of *hsp*70 in the nucleoli suggests that *hsp*70 participates in RNA proc-

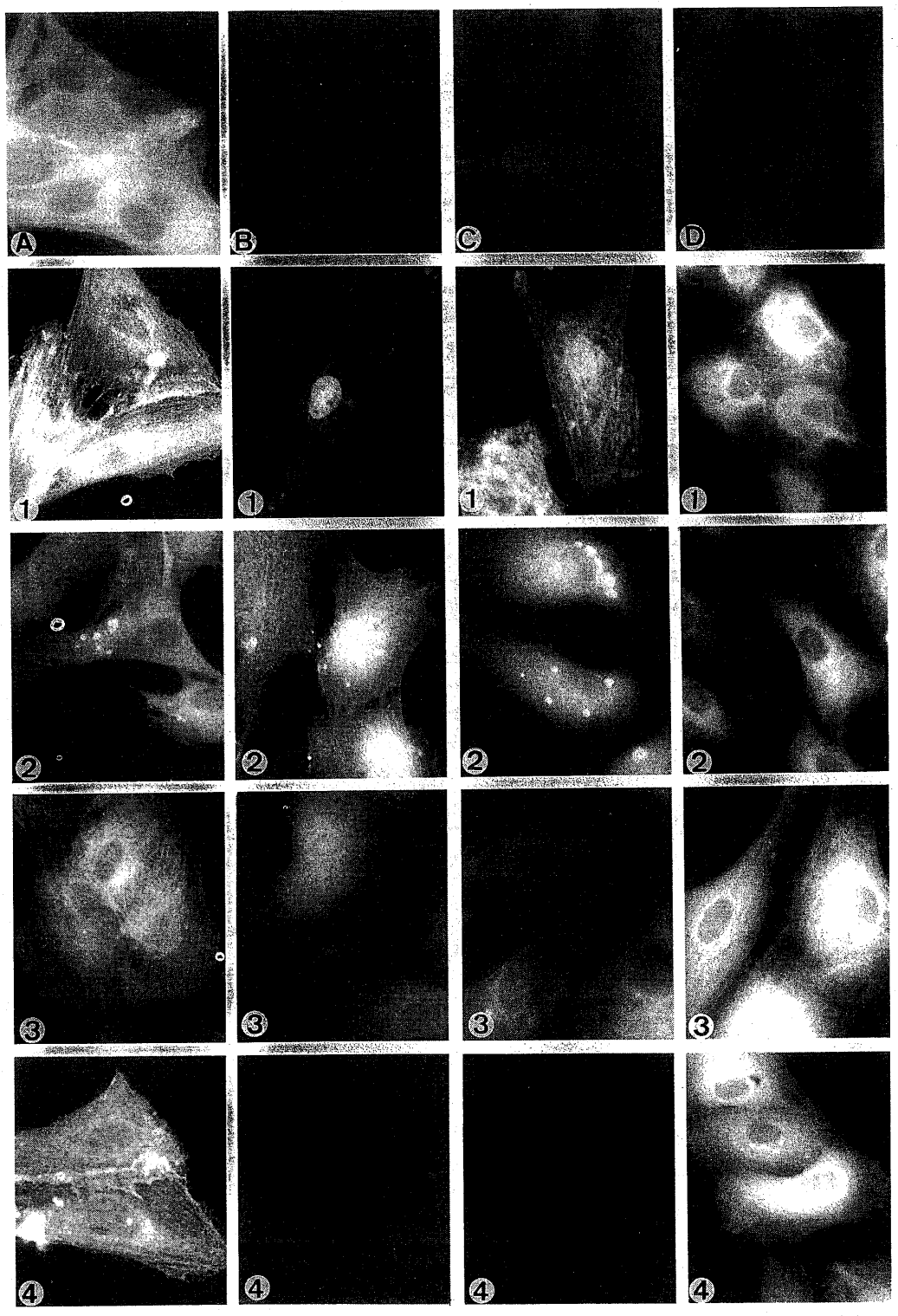

Fig. 10 Localization change of heat shock proteins after recovery at 31°C from heat shock at 40°C. Cells were held at 31°C (A, B, C or D), then heated at 40°C for (1) 10 h and allowed to recover at 31°C for (2) 14, (3) 24 ,(3) 36 or (4) 48 h. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100, and stained with rabbit anti-CCT *hsp*87 (column A), anti-CCT *hsp*72 (column B), anti-CCG *hsp*33 (column C), or anti-CCG *hsp*27 antibody (column D); this was followed by staining with FITC-conjugated anti-rabbit IgG antibody. X 370.

essing and ribosomal assembly (Welch and Feramisco, 1984, 1985; Lewis and Pelham, 1985). The significance of this nucleolar distribution of *hsp*70 in heat shock-stressed cells is not clear at this time. However, through a mutational analysis of human *hsp*70, Milarski and Morimoto (1989) showed that sequences in the carboxyl termius of *hsp*70 are both important for nucleolar localization and associated with nuclear structure.

*Hsp*33 is perhaps the most interesting *hsp* in CCF cells. It was not present during normal growth temperatures; however, during heat shock it was synthesized at a maximum rate, then ceased synthesis just after or at the same time that the synthesis of *hsp*27 reached its maximum rate. However, both an immunoblot assay and pulse-chase test showed that *hsp*33 is not a precursor of *hsp*27, nor is there any cross-reaction between them. When observed in the immunoblot assay, *hsp*33 was synthesized at high levels, but it degraded more quickly than the other heat shock proteins during high-temperature heat shock (Fig. 3 and 6).

Antibodies directed against *hsp*33 were also used in our immunofluorescence study of CCF. After heat shock, staining was first concentrated in the nucleus, then found exclusively in the cytoplasm, and finally disappeared completely. During the return of heat-shock-treated cells (10 h) to normal temperature, *hsp*33 staining had disappeared from the cytoplasm, although cytoplasmic granules of *hsp*33 were still observable after a 24-36 h recovery time. The characteristics of a short half-livese and unique change in the localization of *hsp*33 in cells were only observed in CCF and CCG cells (Ku and Chen, 1991); they were not observed in the testis cell line of the same species (unpublished data) nor in other organisms (Arrigo and Pauli, 1988; Arrigo *et al.*, 1988). Low molecular weight heat shock proteins in these cells localize only in cytoplasm (Caltabians *et al.*, 1988; Collier and Schles-inger, 1986) or nuclei (Rossi and Lindquist, 1989) and have similar half-lives to high molecular weight heat shock proteins. We are now attempting to identify other cellular proteins in CCF cells with which *hsp*33 may associate, looking for indications as to its function.

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錦鯉鰭細胞株之熱休克蛋白質量和位置之動力學表現

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本文主要利用免疫轉印和螢光顯微技術觀察錦鯉鰭細胞株(CCF)內的四種主要熱休克蛋白質，*hsp*87、70、33 和 27，在細胞被持續 40℃熱緊迫及放回 31℃復元條件後，其內蛋白質量及位置的動力改變。

*Hsp*87 在 CCF 細胞受熱後立即生產，但其在細胞內的總量必須在受熱 12 小時後才會明顯增加，然不論受熱前或受熱後其分布位置均明顯的座落在細胞質上。*Hsp*70 則在細胞受熱後即大量的生產並依序自細胞質進入核仁，細胞核並推積到細胞核四周的細胞質。和 *hsp*70 一樣，*hsp*33 的量在細胞受熱後明顯大量的增加，然而卻在受熱 12 到 24 小時之間退化消失，而此時 *hsp*87、70 和 27 則依舊大量存在。*Hsp*33 在細胞受熱後先大量的出現在細胞核內，然後轉移至細胞質。至於 *hsp*27，在常溫即存在著，當細胞受熱 6 小時後其合成率達最大量並以一定量保持於細胞內。相應蛋白質量的變化，約有半數實驗細胞的細胞核在常溫時會出現明顯的抗 *hsp*27 螢光，細胞受熱後此位於細胞核的螢光會逐漸消失，整個細胞也少有螢光存在。然而緊隨著 *hsp*27 合成率的遞增，*hsp*27 會以絲狀螢光出現在細胞核四周，最後表現在整個細胞質。

CCF 細胞在 40℃受 10 小時熱緊迫後，放回 31℃生長溫度復元並以螢光顯微鏡觀察，發現 *hsp*87、70 和 33 在 31℃，36 小時後已恢復至未受熱前的狀況，然而 *hsp*27 則大量存在在細胞質內。這些結果推測細胞質應為熱休克蛋白質自我退化的場所。

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SEASONAL SPAWNING OF SERGEANT MAJOR DAMSELFISH *ABUDEFDUF VAIGIENSIS* IN THE SUBTROPICAL WATERS OF TAIWAN[1]

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Rong-Quen Jan and Rupert F.G. Ormond (1992) Seasonal spawning of sergeant major damselfish *Abudefduf vaigiensis* in the subtropical waters of Taiwan. Bull. Inst. Zool. Academia Sinica **31**(4): 290-311. A survey of the spawning activity of the sergeant major damselfish, *Abudefduf vaigiensis*, was undertaken off the northern coast of Taiwan (121°41'E, 25°12'N) from April to October, 1986. Temporal variation in spawning intensity and its correlation with environmental factors were analyzed. Spawning was observed in 58 of the 169 days between May 1 (the date on which the first nests were found) and October 16 (the date the last nests were found). In total, 966 nests were located during this period. Spawning occurred in the warm season of the year, but the correlation between daily spawning intensity (assessed as number of new nests found) and water temperature was not significant. Within the spawning period, peak spawning occurred twice, once in May and again in July. We found no indication of a significant lunar component or long-term periodicity in spawning activity within the spawning period. We did find that the spawning period was highly correlated with higher zooplankton production. Though the overall spawning period occurred entirely within the typhoon season, the first of the two peak spawning periods occurred outside the main typhoon season. The overall spawning period did not appear to correlate with any trend in inshore current speed, even though it occurred during the period when off-shore currents were strong. In general, the spawning timing of *A. vaigiensis* seems most likely to be associated with higher food availability for offspring. In addition, it seems quite possible that the timing of spawning may have been selected in order to retain larvae in their natal habitat.

Key words: *Abudefduf vaigiensis*, Environmental factors, Spawning season, Seasonal spawning, Sergeant major

Seasonal spawning has been observed in many reef fishes over a wide range of latitudes (Erdman, 1977; Russell *et al.*, 1977; Johannes, 1978; Walsh, 1987). For example, even though many fishes in the northeastern Caribbean spawn year-round, they nevertheless show a seasonal peak once or twice per year (Munro *et al.*, 1977). Studies on the spawning patterns of reef fishes have also shown that in some demersal species there is a pronounced lunar periodicity of reproductive activity (Doherty, 1983; Foster, 1987).

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