



# Biochemical studies on the enhancement of suspension cells and protoplasts proliferation in rice (*Oryza sativa* L.) by heat shock treatment

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**Abstract.** Heat shock treatment at 45°C (8 min) and then in ice water (10 seconds) resulted in an enhancement of the growth rate and viability of rice (*Oryza sativa* L.) cells or protoplasts. The plating efficiency of 45°C-treated protoplasts was 1.8-fold higher than that of the 28°C control, but there was no difference between the plating efficiency of 40°C-treated protoplast and that of the 28°C control. Heat shock treatment also caused an enhancement of the incorporation of <sup>35</sup>S-methionine into proteins and changed the protein synthetic patterns. Comparison of protein synthetic patterns in 40°C- and 45°C-heat shock treatments showed that three protein bands, 59 (5 spots in 2-D gel), 51 and 34 (1 spot in 2-D) were unique under 45°C, and two protein bands in a molecular weight of 70 KD (4 spots in 2-D gel) and 37 KD were much more abundant than at 40°C. When protoplasts were treated at 45°C for 5 min rather than 8 min, the plating efficiency was similar to that of the 28°C control. The protein synthetic patterns in 45°C HS-5 min-treated protoplasts were also similar to those of the 28°C control. Those experiments indicate that four groups of heat shock proteins (70, 59, 51, 34 KD) may play a role in the enhancement of cell division and protoplast proliferation.

**Key words:** Heat shock; Heat shock proteins; Gel electrophoresis; Proliferation; Rice (*Oryza sativa* L.); Suspension cell protoplasts.

## Introduction

Plant regeneration from protoplasts offers a unique tool for genetic manipulation of higher plants. The genetic transformation of higher plants can be achieved by *Agrobacterium* mediated transformation (Zambryski *et al.*, 1980), by direct DNA uptake into protoplasts (Paszkowski *et al.*, 1984) and by several other methods. In the Gramineae family, which

includes the most important crop species, direct DNA transfer into the protoplasts is the most commonly used method at present (Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Fromm *et al.*, 1986). But in only a few cases have rice plants been regenerated from protoplasts. Thompson *et al.* (1986) described a reproducible method for rice protoplast culture by means of agarose-solidified media. Later Abdullah *et al.* (1986) used this method to achieve efficient plant regeneration from protoplasts of rice. A high frequency of plant regeneration from rice protoplasts by novel culture methods using nurse cells was also reported by Kyojuka *et al.* (1987).

When a wide variety of organisms, from bacteria to man, are briefly grown at a temperature 10 to 15°C higher than normal growth conditions (heat shock con-

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<sup>2</sup>**Abbreviations:** 2-D, two-dimension; FDA, fluorescein diacetate; HS, heat shock; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Tris, tris (hydromethyl) amino methane.

dition), heat shock proteins (HSPs) are translated from a new set of mRNAs induced by the heat shock (HS) (Key *et al.*, 1981). A strong positive correlation between the accumulation of HSPs and the acquisition of thermotolerance has been reported (Li and Werb, 1982; Lin *et al.*, 1984; Lin *et al.*, 1985; Lindquist, 1986; Chou *et al.*, 1989). Thompson *et al.* (1987) reported that a brief heat shock (45°C for 5 min), as described by Shillito *et al.* (1983), enhanced protoplast division in rice suspension cultures.

In this paper, we report the effect of heat shock treatment of 45°C for 8 min on the proliferation of rice suspension cells or protoplasts and a correlation to change in protein synthetic patterns. We propose that three classes of heat shock proteins in the molecular weight ranges of 34, 57, and 70 KD may play important roles in the enhancement of protoplast proliferation in rice.

## Materials and Methods

### *Initiation of Callus and Suspension Culture*

The suspension culture of rice (*Oryza sativa* L. var. Tainan No. 5) used in this study was obtained from Dr. L. F. Liu (Department of Agronomy, National Taiwan University, Taiwan, ROC). Callus developed from rice immature embryos was used for initiation of this suspension culture. The suspension cultures were placed in a 250 ml flask containing 60 ml of R2 medium (Ohira *et al.*, 1973), and the flask was shaken in a rotary shaker at 120 rpm. The R2 medium contained the following components: 150 mg/l CaCl<sub>2</sub> · 2H<sub>2</sub>O, 335 mg/l NH<sub>4</sub>NO<sub>3</sub>, 4,000 mg/l KNO<sub>3</sub>, 3,272.7 mg/l KH<sub>2</sub>PO<sub>4</sub>, 250 mg/l MgSO<sub>4</sub> · 7H<sub>2</sub>O, 3 mg/l H<sub>3</sub>BO<sub>3</sub>, 0.125 mg/l Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O, 17 mg/l MnSO<sub>4</sub> · 4H<sub>2</sub>O, 0.05 mg/l CuSO<sub>4</sub> · 5H<sub>2</sub>O, 2 mg/l ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 16.4 mg/l Na<sub>2</sub>-EDTA, 1 mg/l thiamine, 20 g/l sucrose, and 2 mg/l 2,4-D. The medium was adjusted to pH 6.0 before used.

### *Measurement of Cell Growth*

Two different methods were used for the measurement of cell growth: (1). The volume of settled cells method: Suspension culture cells were allowed to settle or 20 min in the graduated side arm of a 250 ml culture flask, and the volume of settled cells was measured. (2). Dry weight method: A measured volume of suspension culture cells was filtered with Whatman No. 1 filter paper and then dried at 70°C for 4 h. The dry weight of

cells was then measured.

### *Protoplast Isolation and Culture*

Protoplasts were isolated from a cell suspension culture maintained in amino acid (AA) medium for 4 to 5 weeks according to the method of Thompson *et al.* (1986) with slight modification. Five grams of cultured cells were placed in a 10 cm plastic plate containing 20 ml of an enzyme mixture consisting of 1% (w/v) pectolyase (Seishin Pharmaceutical Co. Ltd, Japan), 0.5% (w/v) macerzyme (Yakult Co. Ltd, Japan) in CPWM13 medium. The plate was shaken in a rotary shaker at 20 rpm for 6 h and then at 60 rpm for 30 min. After incubation, the enzyme mixture was passed through a 64 μm and then a 40 μm nylon mesh. The filtrate was centrifuged at 80 xg for 5 min, and the pelleted protoplasts were collected and washed three times with CPWM13, CPWM13 + KPR (1:1), and KPR medium, respectively. The isolated protoplasts at a concentration of 4 × 10<sup>5</sup> cells/ml were cultured in KPR medium. Protoplasts were cultured by using the agarose-bead method (Shillito *et al.*, 1983). One ml of the protoplast suspension was mixed with an equal volume of molten agarose (2.5% in KPR medium) in a 6 cm plastic plate. The solidified agarose containing the protoplasts was cut into blocks and transferred to a 6 cm plate containing 5 ml of the KPR medium and incubated at 27°C. The percentage of plated protoplasts remaining intact was determined after 5 days of culture using a haemocytometer under a light microscope. Protoplast division frequency was calculated as the percentage of plated protoplasts dividing after 14 days of culture. Plating efficiency was based on macroscopic colony formation from plated protoplasts, which was calculated after 30 days of culture.

### *Heat Shock Treatment*

Rice cell cultures (in AA medium) or isolated protoplasts (in KPR medium) were incubated at 40°C for 2 h or at 45°C for 8 min, and then cooled in ice water for 10 seconds.

### *Protoplast Viability Test*

Protoplast viability was measured by absorption of fluorescein diacetate (FDA) and was scored by fluorescence microscopy on a glass slide (Larkin, 1976). One drop of protoplast culture was mixed with one drop of 0.2% FDA in CPWM13 medium, allowed to

remain at room temperature for 5 min and then examined by fluorescence microscopy. Protoplast viability was expressed as a percentage of the total cells examined:

$$\frac{\text{Fluorescing protoplast number}}{\text{Total intact protoplast number}} \times 100\%$$

#### Measurement of Protein Synthesis

After different temperature treatments, rice cells or protoplasts in culture medium were labeled with  $^3\text{H}$ -leucine (5.0 Ci/mole, NEN, Co. USA) or  $^{35}\text{S}$ -methionine (500 Ci/mole, NEN, Co. USA) for various periods at  $28^\circ\text{C}$  in a shaking water bath. At the end of the labeling periods, pelleted cells or protoplasts were washed with culture medium and total labeled proteins were extracted with protein extraction buffer (50 mM Tris-HCl, pH 8.5, 2% SDS, 2% 2-mercaptoethanol, and 1 mM PMSF) at room temperature. The extract was centrifuged at 20,000  $\times g$  for 30 min and filtered through a layer of miracloth (Calbiochem). The uptake of  $^{35}\text{S}$ -methionine by rice cells or protoplasts was determined by the measurement of total radioactivity in each filtrate in a Beckman Liquid Scintillation Spectrometer (Model 1801). The incorporation of  $^{35}\text{S}$ -methionine or  $^3\text{H}$ -leucine into proteins was determined by blotting an aliquot of each sample on 3-MM filter paper as described by Mans and Noveli (1961). The proteins in the supernatant were precipitated with 4 volumes of acetone and stored at  $-20^\circ\text{C}$ .

#### Gel Electrophoresis and Fluorography of Labeled Proteins

Proteins prepared as described above were dissolved in sample buffer for one-dimensional SDS-PAGE or two-dimensional gel electrophoresis. The SDS-PAGE followed the method of Laemmli (1970). The two-dimensional gels were run according to O'Farrell (1975) using urea and Ampholines (a 1:4 mixture of pH 3.5-10 and pH 5-7 resulting in a pH gradient of 4.5-8.5). Equal amounts of radioactivity were loaded in each experiment. Fluorography of the gels was accomplished using EN $^3$ HANCE (New England Nuclear) and Kodak film (XAR-5).

## Results

### Heat Shock Treatment Enhanced the Growth Rate of Rice Cells or Protoplasts

Rice cells were grown in R2 medium for 10 days, treated with a non-lethal heat shock, such as  $40^\circ\text{C}$  for 2 h or  $45^\circ\text{C}$  for 8 min, and then transferred to fresh culture medium at  $28^\circ\text{C}$ . After either heat shock treatment, cell growth rate, as measured by pelleted cell volume (Fig. 1) or by dry weight (Fig. 2), was significantly enhanced.

### Heat Shock Treatment Caused an Enhancement of the Incorporation of Amino Acids into Proteins and Changed the Protein Synthetic Patterns in Rice Cells

Rice suspension cells were preincubated at  $40^\circ\text{C}$  for 2 hours or at  $45^\circ\text{C}$  for 8 min and followed by an ice-water bath (10 sec) and then incubated at  $27^\circ\text{C}$  for various duration. These rice suspension cells were labeled with  $^3\text{H}$ -leucine at  $28^\circ\text{C}$  for 2 hours. The results in Fig. 3 indicate that the heat shock treatment enhanced amino acid incorporation into proteins about 1.5 to 2

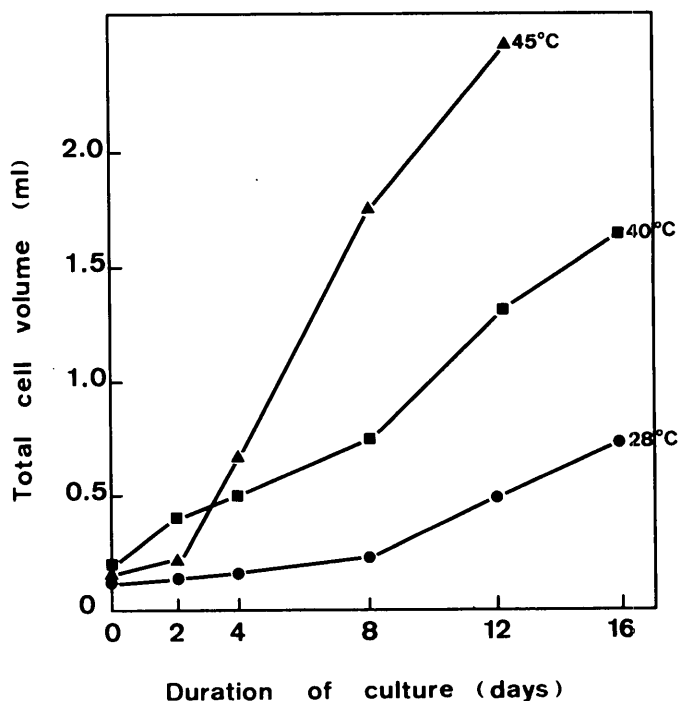


Fig. 1. The growth curve of rice suspension cells after heat shock treatment as measured by pelleted cell volume. The original concentration of cell suspension was 0.5 mg/100 ml. After  $40^\circ\text{C}$  (2 h) (■---■) or  $45^\circ\text{C}$  (8 min) (▲---▲) and ice-water (10 sec) treatment, all the cultures were incubated at  $28^\circ\text{C}$  in a rotary shaking water bath for different intervals. The cell volume was measured as described in Materials and Methods.

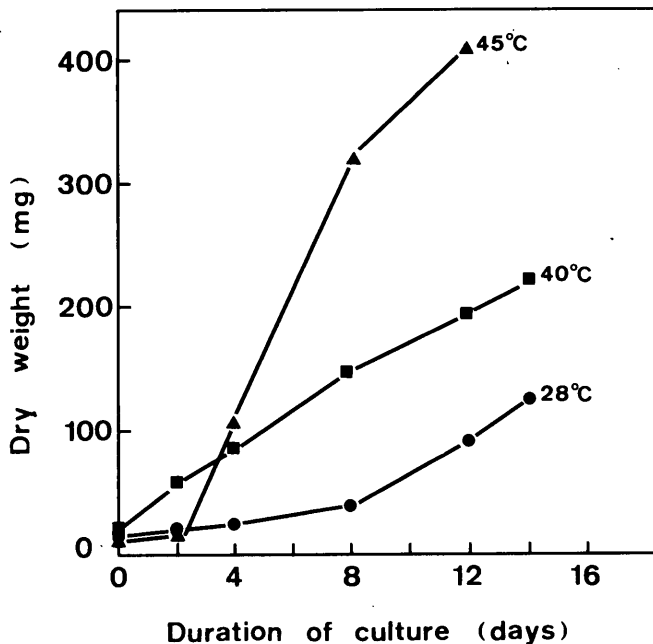


Fig. 2. The growth curve of rice suspension cells after heat shock treatment as measured by dry weight. Treatment conditions are described in Fig. 1. After different intervals of culture, samples were collected on filter paper and dried at 70°C for 4 h.

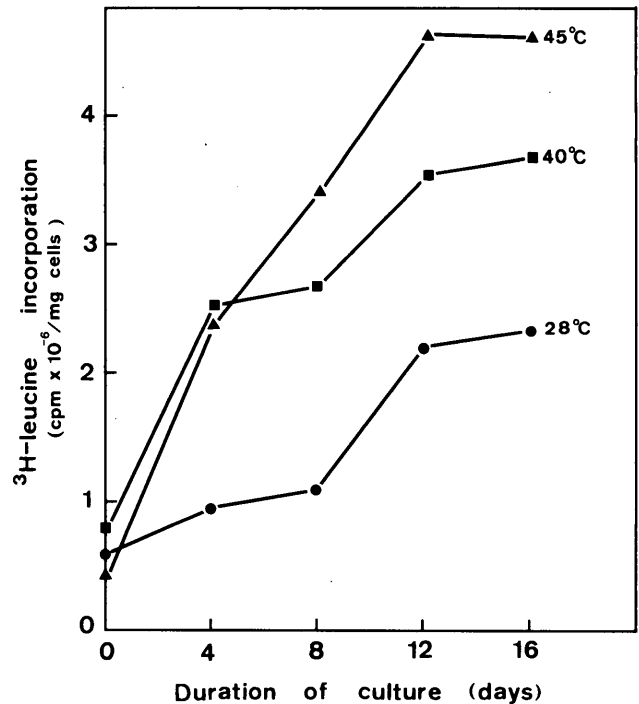


Fig. 3. Effects of heat shock treatment on the incorporation of <sup>3</sup>H-leucine into proteins in rice suspension cells. Rice suspension cells were pre-incubated at 40°C for 2 hours or at 45°C for 8 min followed by ice-water treatment (10 sec) and then incubated at 27°C for various duration. These rice suspension cells were labeled with <sup>3</sup>H-leucine at 28°C for 2 h.

fold compared with the 28°C control, non-heat-treated cells. Heat shock treatment also caused changes in protein synthetic patterns in rice cells. We detected eight newly synthesized proteins in the cells after heat shock treatment. The molecular weights of these heat shock proteins (HSPs) were 92, 84, 68-70, 56, 27, 22, and 15-18, respectively. The induced HSP synthetic patterns, as detected by SDS-PAGE, were similar to continuous HS at 40°C or a brief HS at 45°C followed by 28°C incubation (Fig. 4). However, three protein bands (70, 27, and 22 KD, shown with arrows in Fig. 4) were much more abundant in 40°C treated cells than in cells subjected to the brief 45°C HS. One additional protein band (6-8 KD) was present in cells subjected to the brief 45°C heat shock (Fig. 4).

#### Heat Shock Treatment Enhanced the Viability of Rice Protoplasts

When rice protoplasts were isolated from cell suspension cultures, incubated at 28°C for 12 hours, and then treated at 40°C for 2 h or 45°C for 8 min and fol-

owed by an ice-water bath water (10 sec), about 80% of the protoplasts remained intact. Comparison of the 40°C- or 45°C-heat shock treated protoplasts with 28°C control reveals that the viability of heat shock-treated protoplasts was slightly enhanced (Fig. 5). When viability was measured at 12 h after treatment, the percentages of viable protoplasts at 45°C-, 40°C-treated and the 28°C control were 94%, 84% and 78%, respectively. After three days of culture, the viability of 45°C-heat shock treated protoplasts was similar to that of at 12 h (Fig. 5).

Twelve-hour cultures of newly isolated protoplasts were treated under heat shock regimes at 40°C (2 h) or at 45°C (8 min), and cultured by the "agarose segment" culture method. After 5 days of culture, the intact protoplast, division frequency and plating efficiency were examined. Results are shown in Table 1. The plating efficiency of 45°C-treated protoplasts was 1.8-fold higher than that of the 28°C control, but there was no difference between that of the 40°C-treated protoplasts and the 28°C control protoplasts.

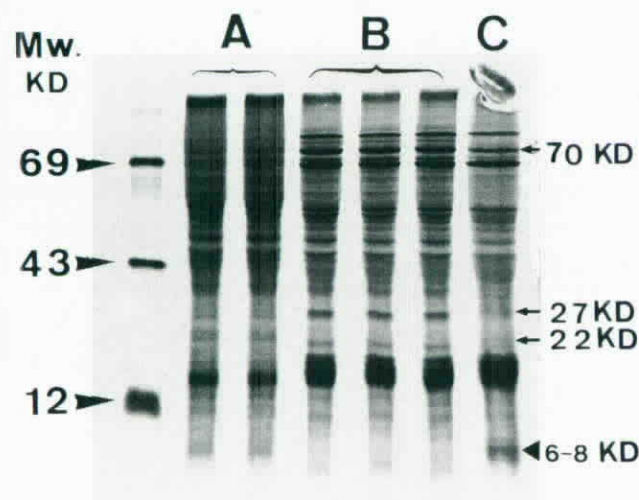


Fig. 4. Effects of heat shock treatment on protein synthetic patterns in rice cells analyzed by 12.5% SDS-PAGE and fluorography. A: Control: Cells were grown at 28°C and labeled with  $^3\text{H}$ -leucine at 28°C for 2 h; B: Cells were pre-treated at 40°C for 30 min and then labeled at 40°C for 2 h; C: Cells were pre-incubated at 45°C for 8 min and then placed in ice-water for 10 sec. The cells were then labeled with  $^3\text{H}$ -leucine at 28°C for 2 h;  $\rightarrow$  indicates decrease of protein synthesis in 45°C when compared with 40°C treated rice cells.  $\blacktriangleright$ , indicates increase of protein synthesis in 45°C when compared with 40°C treated rice cells.

#### Heat Shock Treatment Changed the Pattern of Protein Synthesis in Rice Protoplasts

To understand the relationship between metabolic changes and an enhancement of plating efficiency in 45°C heat shock treated protoplasts, radioactive  $^3\text{H}$ -leucine or  $^{35}\text{S}$ -methionine was used as a tracer to analyze quantitative and qualitative changes of protein synthesis. Since the uptake of ions or molecules in newly isolated protoplasts is usually very low (Liu, 1983), it was necessary to determine time courses of labeled amino acids incorporated into protein activity. Fig. 6 shows that isolated protoplasts which were cultured for 10–12 h showed a high rate of incorporation of  $^3\text{H}$ -leucine. In the following experiments, 12 h-cultured protoplasts were used to study the effect of heat shock treatment on quantitative or qualitative changes in protein synthesis. Table 2 shows the effect of heat shock treatment on  $^{35}\text{S}$ -methionine uptake and its incorporation into proteins. After heat shock treatment, the uptake of  $^{35}\text{S}$ -methionine in protoplasts decreased about 14% (40°C-treated) to 25% (45°C-treated). Total incorporation of  $^{35}\text{S}$ -methionine into protein

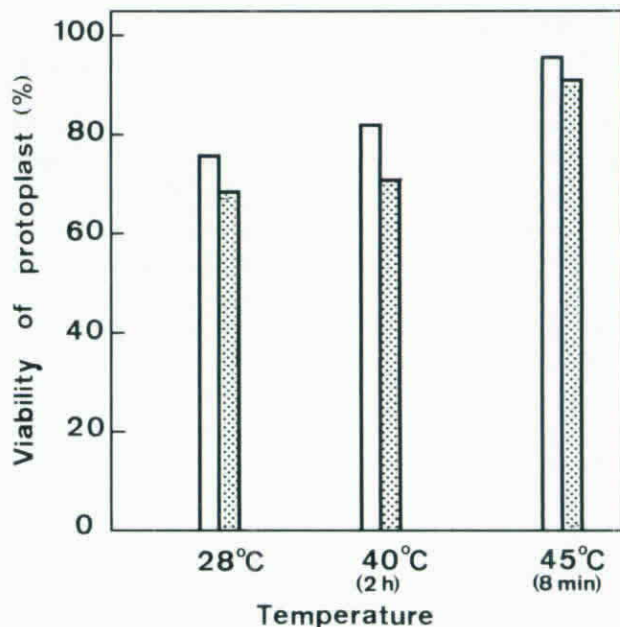


Fig. 5. Effects of heat shock treatment on the viability of rice protoplasts. The viability of protoplasts was measured by the absorption of fluorescein diacetate (FAD) into protoplasts as determined by fluorescence microscopy.  $\square$ , Examination after 12 h of protoplast culture;  $\square$  (stippled), Examination after 3 days of protoplast culture.

Table 1. Effects of heat shock treatment on the division frequency and plating efficiency of rice protoplasts

The 12-h cultures of newly isolated protoplasts were treated under heat shock regimes at 40°C (2 h) or at 45°C (8 min), and cultured using the "agarose segment" method. After 5 days of culture, the percentage of plated protoplasts remaining intact was determined using a haemocytometer under light microscope. The division frequency was calculated as the percentage of plated protoplast dividing after 14 days of culture. Plating efficiency was based on macroscopic colony formation from plated protoplasts, which was calculated after 30 days of culture. Each item of data was the means of three experiences.

Temperature (°C)	Intact protoplasts at day 5 (%)	Division frequency at day 14 (%)	Plating efficiency at day 30 (%)
28	42	6.5	0.20
40	35	6.2	0.21
45	55	10.8	0.35

of the control (28°C) protoplasts and 40°C-treated protoplasts was approximately the same, but the total incorporation of  $^{35}\text{S}$ -methionine into proteins of 45°C-treated protoplasts was only 45% that of the 28°C control. This may have been caused by an increased frequency of broken protoplasts after 45°C heat shock treatment.

Protein synthetic patterns in 40°C heat shock-treated and 45°C heat shock-treated protoplasts were very similar to those of control cells when analyzed by one dimensional SDS-PAGE and fluorography (Fig. 7). Comparison of protein synthetic patterns in the two different heat shock treatments showed that three protein bands (59, 51, and 34 KD) were unique under 45°C, and that three protein bands (70, 59 and 37 KD) were much more abundant than at 40°C. Only the 45°C HS treatment, however, could enhance the plating efficiency of rice protoplasts (Table 1). In order to further

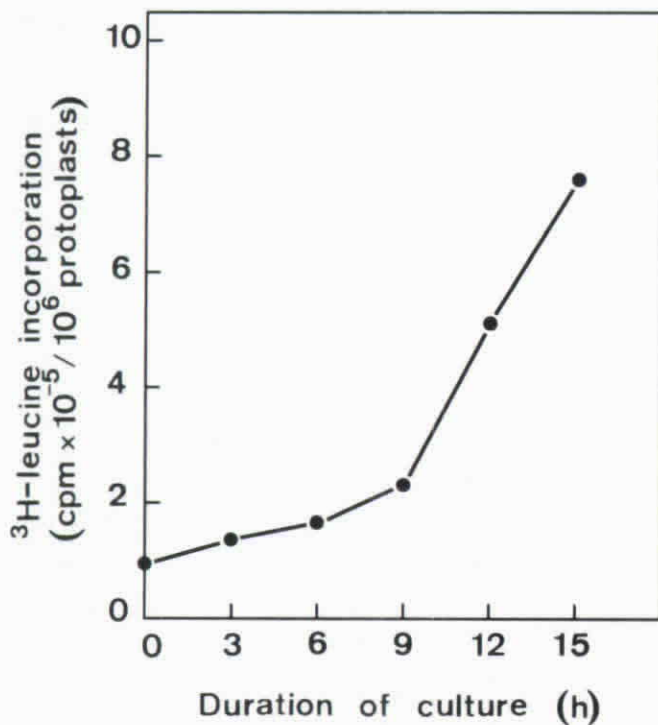


Fig. 6.  $^3\text{H}$ -leucine incorporation into proteins in rice protoplasts after different intervals of culture. Protoplasts were isolated from a cell suspension culture and then  $4 \times 10^5$  protoplast cells were cultured at 27°C in KPR medium for various durations. The protoplasts were labeled with  $^3\text{H}$ -leucine at 28°C for 2 h. Procedures are described in Materials and Methods.

**Table 2.** Effects of heat shock treatment on  $^{35}\text{S}$ -methionine incorporation into proteins in 12 h cultures of rice protoplasts

The 12-h cultured protoplasts ( $1 \times 10^6$ ) were incubated at 28°C, 40°C and 45°C, respectively, and labeled with  $^{35}\text{S}$ -methionine for 2 h. The measurement of uptake and incorporation into proteins is described in Materials and Methods.

Temperature (°C)	$^{35}\text{S}$ -Met uptake (cpm $\times 10^6$ )	$^{35}\text{S}$ -Met incorporation (cpm $\times 10^6$ )
28	45.10 (100%) <sup>a</sup>	8.15 (100%) <sup>a</sup>
40	38.75 (85.9%)	7.62 (93.4%)
45	33.75 (74.8%)	3.67 (45.0%)

<sup>a</sup>The uptake or incorporation rate at 28°C was taken as 100%.

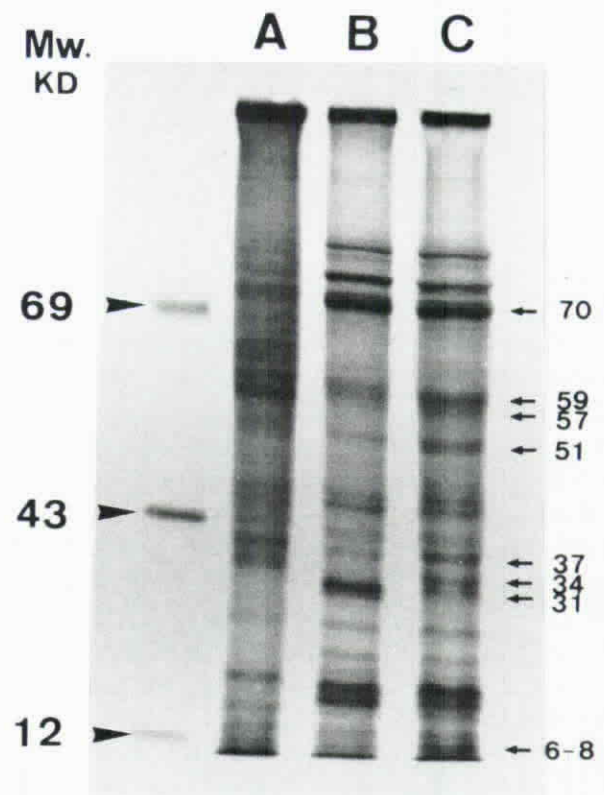


Fig. 7. Effects of heat shock at 40°C (B) or at 45°C (C) on protein synthetic patterns in rice protoplasts analyzed by 12.5% SDS-PAGE and fluorography. Rice protoplasts were labeled with  $^{35}\text{S}$ -methionine at 40°C for 2 h (B) or 45°C pre-treated protoplasts for 8 min, followed by ice-water treatment for 10 sec and then were labeled with  $^{35}\text{S}$ -methionine at 28°C for 2 h (C).

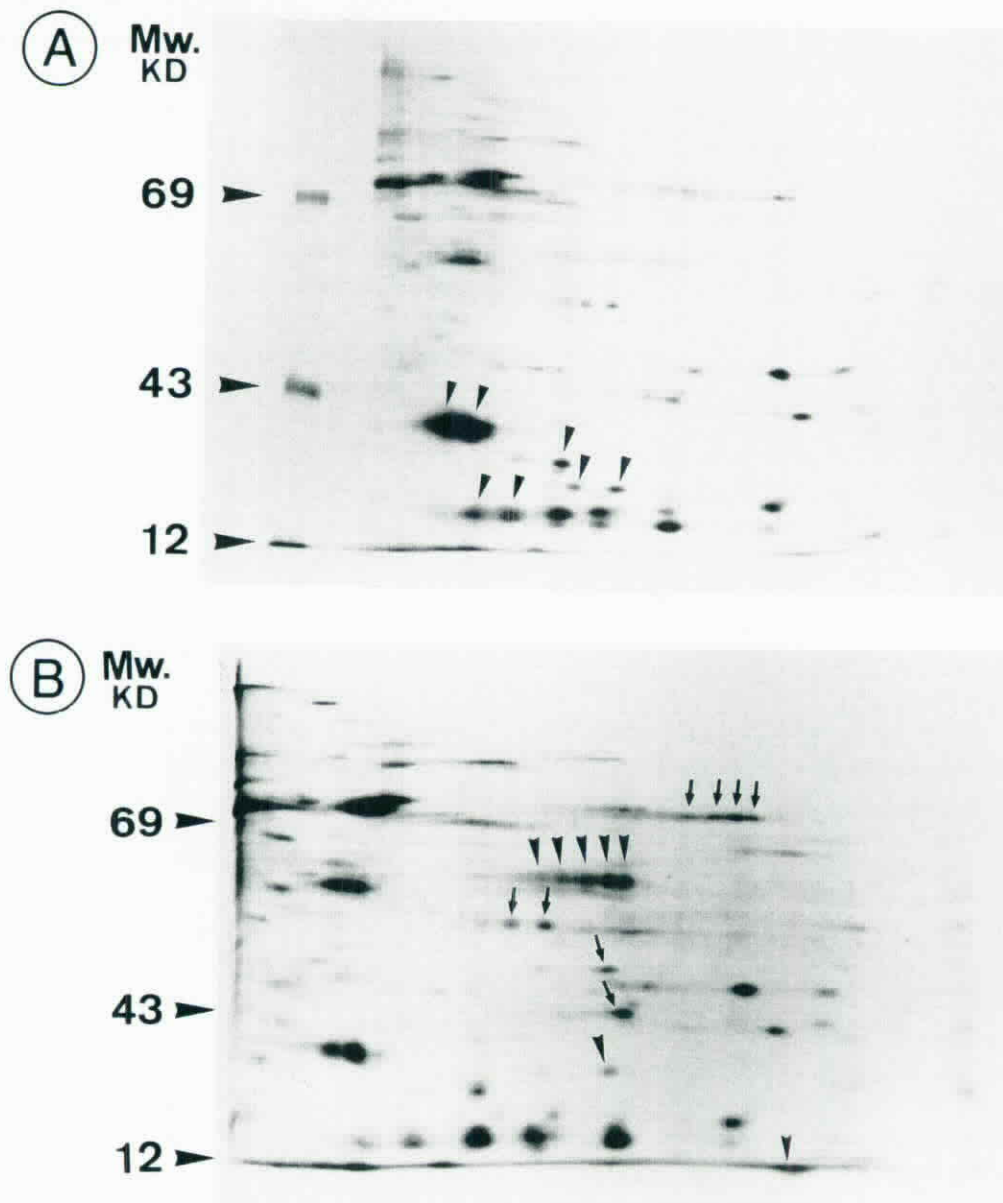


Fig. 8. 2-D gel electrophoresis of  $^{35}\text{S}$ -proteins isolated from rice protoplasts after 40°C (2 h) (A) or 45°C (8 min) (B) heat shock treatment. ▶, indicates decrease of protein synthesis in 45°C HS-treated compare to 40°C HS-treated protoplasts; ◀, indicates uniquely synthesized proteins in 45°C HS-treated protoplasts; →, indicates much more abundant protein synthesis in 45°C HS-treated than in 40°C HS-treated protoplasts.

compare the differences of protein synthetic patterns between 45°C HS-treated protoplasts and the 40°C HS-treated protoplasts, two-dimensional gel electrophoresis and fluorography were used to analyze  $^{35}\text{S}$ -labeled proteins. The results illustrated in Figs. 8A and 8B and indicate that 59, 51, and 34 KD class proteins were uniquely synthesized in 45°C HS-treated protoplasts and that 59 and 34 KD proteins could be resolved into 5

and 1 protein spots, respectively. These spots could not be found in 40°C HS-treated proteins (Fig. 8A and 8B). In addition to 70 KD, 51 KD, and 37 KD peptides were much more abundant in 45°C HS-treated than in 40°C HS-treated protoplasts. However, 7 protein spots in 40°C HS-treated protoplasts were much more abundant than in 45°C HS-treated protoplasts. 15–18 KD HSPs in rice could be separated into 15 protein spots.

When protoplasts were treated at 45°C for 5 min rather than 8 min, the plating efficiency was similar to that of the 28°C control. The protein synthetic patterns in 45°C HS-5 min-treated protoplasts were also similar to those of the 28°C control (data not shown). These experiments indicate that four groups of heat shock proteins (70, 59, 51, and 34 KD) may play a role in the enhancement of cells division and protoplast proliferation in rice.

### Discussion

A wide range of organisms respond to elevated temperature (heat shock, above 10°C higher than normal growth temperature) (Schlesinger *et al.*, 1982). When soybean seedlings are shifted from a normal growth temperature of 28°C to 40°C, there is a dramatic change in the protein synthetic patterns; a new set of heat shock proteins (HSPs) is rapidly synthesized, and the synthesis of normal proteins is greatly inhibited (Barnett *et al.*, 1980; Key *et al.*, 1981). The physiological function of HSPs has not been documented, but the induction of synthesis, accumulation and cellular localization of HSPs are an essential component of the protection process from heat injury (Schlesinger *et al.*, 1982; Lin *et al.*, 1984).

Our study indicates that proliferation of rice suspension cells or protoplasts can be enhanced by a heat shock treatment at 45°C for 8 min and followed by an ice-water bath (10 sec). This observation is similar to the report of Thompson *et al.* (1987). The enhancement of protoplast division may be attributed to the loss of those protoplasts less capable division during the heat shock treatment (Thompson *et al.*, 1987). But another possible cause of enhancement of rice protoplast stability and division may be a physiological response which is triggered by heat shock treatment. Heat shock treatment not only caused the enhancement of <sup>3</sup>H-leucine incorporation into proteins by about 1.5 to 2-fold (Fig. 3), but also induced seven newly synthesized proteins in rice protoplasts (Fig. 4). These heat shock proteins (HSPs) may play some important physiological roles in the enhancement of rice protoplast division. Heat shock treatment could improve protoplast division by association of HSPs with plasma membranes and other organelles, such as nuclei, mitochondria, and ribosomes. Plasma membrane integrity could be increased by this association with HSPs. Several investi-

gators have reported that HSPs can selectively localize in association with special organelles and plasma membrane. This special association can enhance protection from injury caused by high temperature (Lin *et al.*, 1984; Lin *et al.*, 1985; Chou *et al.*, 1989). The association of HSPs with the plasma membrane plays an important role in preventing leakage of solutes from cells of soybean seedlings (Lin *et al.*, 1985). Increased solute leakage is attributable to loss of membrane integrity through lipid phase transitions and to the effect on membrane-bound transport proteins (Levitt, 1980). The integrity of plasma membranes was enhanced by heat shock treatment (at 45°C for 8 min), and the viability of the protoplasts was also enhanced (Fig. 5). Altman *et al.* (1982) also reported that protoplasts are subjected to plasmolysis-induced osmotic stress during isolation and can be stabilized by the addition of polyamines. These compounds are able to maintain membrane functions normally impaired by various other stress conditions.

Only the 45°C heat shock treatment for 8 min could enhance the plating efficiency of rice protoplasts; the 40°C heat shock treatment would not induce this enhancement effect (Table 1). Our results were the same as those reported by Mensfield and Key (1987) and provided the first evidence of a possible role of HSPs in the enhancement of rice protoplast division after 45°C heat shock treatment. These proteins may be early gene products induced by 45°C heat shock temperature.

The function of HSPs has not been documented. Minton *et al.* (1982) proposed a model for the biological role of HSPs in stabilizing non-specifically other proteins which are highly susceptible to inactivation or denaturation by heat. Our previous report indicated that 15-18 KD proteins, 68-70 KD proteins and, perhaps, 24 KD and 22 KD proteins are important for providing protection from heat denaturation in soybean seedlings (Jinn *et al.*, 1989). Hemmingsen *et al.* (1988) also proposed that HSPs can stabilize proteins by maintaining them in a soluble, undenatured form, which indicates a function analogous to the chaperones (Hemmingsen *et al.*, 1988). Results of this paper indicate a positive correlation between the synthesis of HSPs and the enhancement of division in rice suspension cells or protoplasts. Elucidation of the physiological roles of HSPs in enhancement of protoplast division await further investigation.



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## 由生化觀點探討熱休克處理促進水稻細胞及原生質之增殖

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經 45°C 熱休克處理 8 分鐘，然後再以冰水處理 10 秒，會促進水稻 (*Oryza sativa* L.) 細胞或原生質之生長速率及存活性。45°C 處理過的原生質，其碟養效率 (Plating efficiency) 比 28°C 對照組高 1.8 倍，但是 40°C 對照組無顯著差異。水稻細胞經上述熱休克處理後，在 28°C 繼續培養，發現經熱休克處理的細胞，不僅會促進 <sup>35</sup>S-甲硫胺酸併入蛋白質，經膠體電泳分析後發現，亦會造成其蛋白質合成類型之改變，發現有三條蛋白質帶，59 KD，(在 2-D 膠體電泳上有 5 點)，51 KD 及 34 KD (在 2-D 上有一點) 是在 45°C 處理組中所僅有的，而 70 KD (在 2-D 上有 4 點) 及 37 KD 在 45°C 時比在 40°C 處理時之合成量較多些。原生質體若經 45°C 處理 5 分鐘，則其碟養效率與 28°C 對照組相似，其蛋白質合成類型亦與 28°C 對照組相似。由本研究得知四組熱休克蛋白質 (70, 59, 51 及 34 KD) 可能在水稻細胞或原生質之細胞分裂及原生質之增殖上扮演相當重要角色。