



# Heat shock induces variably the major heat shock proteins of CV1 clones

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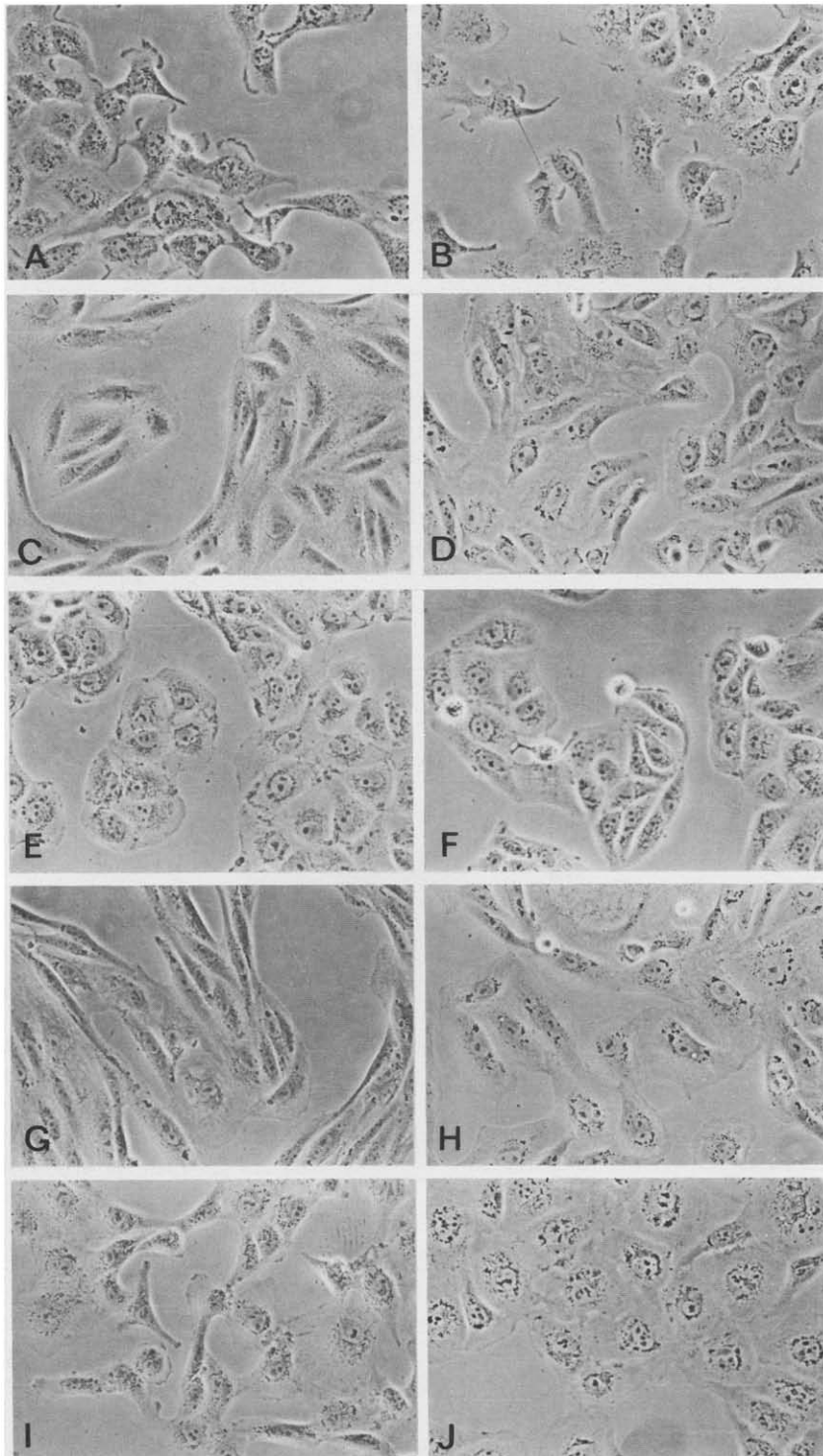
CV1 cells have been subcloned several times. Five of these clones were studied for the induction of the major heat shock proteins. These CV1 clones exhibit morphological differences as well as differences in SDS-PAGE protein profiles. These clones responded to heat shock variably as judged from the induction of the major heat shock proteins, 70, 72 and 92 kDa. Variable expression of the heat shock proteins suggests that the selective pressure for isolation of cell clones may affect gene expression differently.

*Cell clone      Heat shock      Protein synthesis induction*

## 1. INTRODUCTION

The response of cells to different stress factors is a matter of intense investigation [1]. The overall response to stress differs in different cell types [1,2], although there is a more or less common response of all cell types from bacteria to man [1]. The variation that exists in different cell types accounts for the expression of several polypeptides. All cell types do not respond to stress in the same manner. Cells may respond variably in a certain stress condition depending on the severity of the stress. Thus, HeLa cells at different elevated temperatures express variably the 70 and 72 kDa heat shock proteins [3]. It is well documented that the severity of the stress plays a dominant role in the corresponding cellular response [1,2]. Viral infections constitute a stressing factor that has been extensively studied. Several oncogenic viruses have been found to induce heat shock proteins in their permissive cell types [5–10]. The responsible viral genes for this induction have been identified in two cases [7,8] and their stressing ability could be mimicked by using high infective doses of mutant viruses lacking these genes [8,11]. Recently it was reported that teratocarcinoma cells, depending on their differentiated status, express the heat shock proteins upon thermal treatment [11,12]. However

there are contradictory reports of the induction of such heat shock proteins [11–14]. Meanwhile it has been reported that embryonic carcinoma cells spontaneously express heat shock proteins in the absence of stress and independently of their origin and culture conditions [13]. Such spontaneous expression of heat shock proteins by different cell types or the level of induced expression of the 70 kDa heat shock gene suggests a variation in the degree of control of the genes depending on cell type [11]. Differences between cell types do not appear simply to depend on the tissue or species from which they were derived. The selective pressure for isolation of cellular clones *in vitro* could well be another means for obtaining cell types with altered characteristics. African green monkey kidney cells CV1, that are used for the study of the SV40 virus, have apparently been subcloned several times. Different clones of CV1 cells exhibit morphological differences that correspond to several biochemical ones. For example, electrophoretograms of several CV1 clones exhibit differences in newly synthesized protein profiles. Polyacrylamide gel electrophoresis was used to study whether these CV1 clones would respond variably to heat shock. It was found that the main heat shock proteins, 70, 72 and 92 kDa, were variably induced in different CV1 clones.



**Fig.1.** Photomicrographs of the different CV1 clones before and after 1½ h heat shock. CV1 MA2 cells before (A) and after heat shock (B); CV1 USA3 cells before (C) and after heat shock (D); CV1 P3 cells before (E) and after heat shock (F); CV1 TR7 cells before (G) and after heat shock (H); CV1 V4 cells before (I) and after heat shock (J); × 115.

## 2. MATERIALS AND METHODS

CV1 cells were grown in glass petri dishes in Dulbecco's modified minimal essential medium supplemented with 7% newborn calf serum. Cells growing in 7 cm petri dishes were subjected to heat shock at 43.5°C for 1½ h. After heat treatment the cultures were washed with Tris-Dulbecco's, pH 7.4, and fed with low-methionine growth medium. At that time, 20 µCi [<sup>35</sup>S]methionine (800 Ci/mmol, New England Nuclear) was added and the cultures were transferred to 37°C for another 1½ h. Control cultures were identically labelled but kept continuously at 37°C. After radioactive labelling the cultures were washed twice with Earle's BBS, the cells scraped off the petri dishes with a rubber policeman and collected by centrifugation. Collected cells were resuspended in reticulocyte standard buffer (RSB) and were used either as a whole cell extract after sonication to disrupt cellular integrity or following NP40 lysis. NP40 was added to a final concentration of 0.5% and, following vigorous agitation, the lysate was centrifuged at 2000 rpm for 2 min to remove nuclei. The collected supernatant will be referred to as the cytoplasmic extract. Whole cell and/or cytoplasmic extracts were mixed with Laemmli PAGE sample buffer and polyacrylamide gel electrophoresis [15] was performed on 15% acrylamide gels. The same amount of radioactivity was loaded in each gel slot. Electrophoretograms were finally processed for fluorography [16] and the dried gels exposed at -70°C to Kodak XAR-5 film for the appropriate time.

## 3. RESULTS

CV1 clones, MA2, USA3, TR7, V4 and P3 grown on glass substrate show microscopical differences as both sparse and confluent cultures. All 5 CV1 clones tend to acquire an epithelial morphology (fig.1) but with discrete differences. Polyacrylamide gel electrophoretograms of whole cell extracts (fig.2) reveal differences between the 5 CV1 clones mainly in the major protein bands in the molecular mass range 30-100 kDa. These differences also reflect the morphological differences observed by microscopical examination. According to their electrophoretograms the above CV1 clones can be separated in 3 groups, the first group

being of USA3 and TR7, the second of V4 and P3 and the third only of MA2 CV1 clone. A more detailed analysis of the electrophoretograms (fig.2) reveals many more minor differences between the 5 CV1 clones at the quantitative and qualitative level.

The CV1 cells respond variably to elevated temperatures depending on the temperature and the time of maintenance at the elevated temperature (Agelidis, personal communication). HeLa cells and other cell types [1,3] behave similarly. The 5 CV1 clones were brought to 43.5°C for 1½ h and labelled afterwards at 37°C for another 1½ h. Under these conditions the electrophoretograms (fig.3) revealed differences in the degree of synthesis of the main cytoplasmic heat shock proteins expressed by CV1 cells [9]. Fluorograms were scanned with a Hoefer gel scanner and the scans were normalized to actin which is the major protein band in the fluorograms. The degree of synthesis of the 70 kDa heat shock protein was measured as the ratio of the transmittance of the 70 kDa heat shock protein over actin, while that of

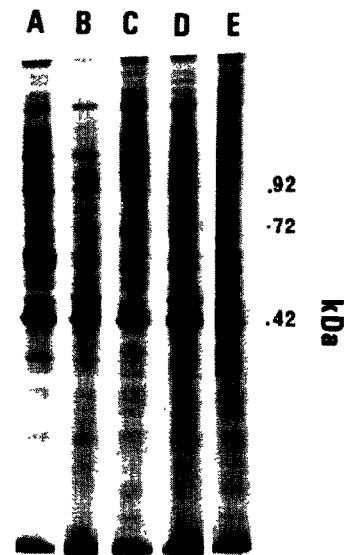


Fig.2. SDS-PAGE profiles of [<sup>35</sup>S]methionine-labelled proteins from whole cell extracts of CV1 clones. All cultures were labelled at 37°C. Proteins were analyzed on 15% acrylamide gels. The same amount of acid-precipitable radioactive extracts were loaded in each lane. (A) MA2, (B) USA3, (C) P3, (D) TR7 and (E) V4 CV1 clones.

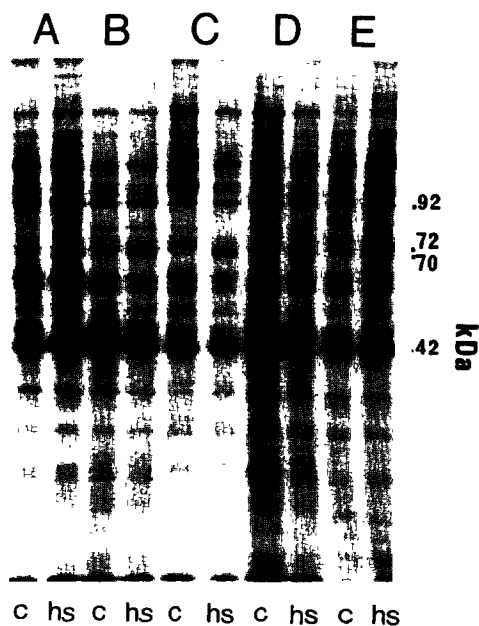


Fig.3. SDS-PAGE profiles of [ $^{35}$ S]methionine-labelled proteins from cytoplasmic extracts of CV1 clones. Cultures were first incubated either at 43.5 or 37°C and then labelled at 37°C. Proteins were analyzed on 15% acrylamide gels. The same amounts of acid-precipitable radioactive extracts were loaded in each lane. (A) MA2, (B) USA3, (C) P3, (D) TR7 and (E) V4 CV1 clones. c, control cultures; hs, thermally treated cultures.

the 72 and 92 kDa proteins was measured as the ratio of induced over non-induced synthesis. Calculated values for all 5 CV1 clones and for the 3 heat-induced proteins are listed in table 1. It is obvious that from the 5 CV1 clones P3 is the one that responds with highest degree of synthesis of the 70 kDa heat shock protein, while the 72 and 92 kDa proteins appear not to be induced in this CV1 clone. In the MA2 clone, where the 70 kDa heat shock protein appears with an average degree of synthesis, both the 72 and 92 kDa heat-inducible proteins appear with a high degree of synthesis. In general we can conclude from electrophoretograms of the cytoplasmic extracts that the main heat shock proteins are variably induced in these 5 CV1 clones. Using whole cell extracts instead of cytoplasmic ones we obtain similar fluorograms for the synthesis of the 70, 72 and 92 kDa heat shock proteins. We observed no differences in the fluorograms if instead of exponentially growing cultures, confluent cultures were used. The

Table 1

Relative induction of the synthesis of the heat shock proteins

CV1 clones	Heat shock proteins (in kDa)		
	70 <sup>a</sup>	72 <sup>b</sup>	92 <sup>c</sup>
P3	0.61	0.91	1.08
V4	0.54	1.10	1.00
USA3	0.51	1.07	1.25
MA2	0.48	1.23	1.30
TR7	0.46	1.06	1.18

<sup>a</sup> 70/actin

<sup>b</sup> 72 induced/non-induced

<sup>c</sup> 92 induced/non-induced

Values were calculated from scans of fig.2, after normalization to actin

morphological differences observed in control exponentially growing cultures were also observed in the heat-treated cultures (fig.1). Before being subjected to the elevated temperature non-attached cells were removed by washing and no further cell detachment was observed. In other experiments the same CV1 clones, when subjected to 43.5°C for up to 8 h, did not show any significant cell detachment.

#### 4. DISCUSSION

The response of cells under stressing conditions is a universal phenomenon. Independent of their evolutionary status, different cell systems respond to heat shock with the expression of the heat shock proteins. It appears that, of these proteins, the 70 kDa heat shock protein is the most conserved in nature. Its role has not yet been characterized but it seems that at least in several cases the 70 kDa heat shock protein is spontaneously or continuously expressed in non-stressing conditions [11,13]. This may soon help in finding some physiological role in non-stressing conditions that will probably reflect on its role in stressing conditions.

It was recently considered that cell regulatory systems vary in cells of the same origin and that this may be a result of the action of cell transcriptional factors [11]. If such factors can be variably expressed, or act in cells of the same organism but with different tissue origins or with different differentiation status, then this would suggest that

variable expression of heat shock proteins in cell clones would also reflect variable differentiation of the cloned cells from their parental cell type. Selective pressures applied during cell cloning presumably account for the observed differences in heat shock protein expression in the CV1 clones studied. Such constraints should be taken into consideration when considering the effects of environmental or other pressures, such as virus infection, on heat shock protein induction.

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