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Heat Shock and Cell Cycle Dependence of Cell Surface Proteins in Mouse Tumor Cells

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溫熱處理와 細胞週期에 따른 생쥐 腫瘍細胞의 膜表面蛋白質의 變化

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摘 要

溫熱處理에 대해서 細胞가 反應하여 適應하는 過程을 細胞膜水準에서 연구하기 위해서 培養中인 纖維芽細胞性 腫瘍細胞를 이용하여 細胞週期, 細胞密度 및 溫熱處理에 따른 膜表面蛋白質의 變化를 lactoperoxidase를 이용한 iodination과 galactose oxidase를 이용한 tritiation 方法을 써서 分析하였다.

細胞週期와 細胞密度에 따라 膜表面蛋白質은 定量的 變化를 보였는데 G_1期에서는 LETS 蛋白質과 高分子蛋白質이 크게 增加하였고 細胞密度의 증가에 따라서는 125K 蛋白質의 增加와 130K 및 100K 蛋白質의 減少가 特異하게 나타났다. 溫熱處理후의 시간경과에 따른 변화를 보면 處理직후 80K이상의 蛋白質은 모두 사라지고, 24시간 지나면 70K 蛋白質이 현저한 增加를 보였지만 48시간이 경과하면 다시 減少하고 高分子蛋白質들이 原狀으로 回復되었다. 또한, 溫度를 39°~45°C까지 증가시켜 보았을때 70K 蛋白質이 41°C에서 가장 큰 폭으로 增加하는 特異한 현상이 관찰되었다.

아울러 이 70K 蛋白質은 trypsin을 처리하면 사라졌는데 galactose oxidase로 tritiation하였을 때도 iodination하였을 때와 동일한 變化樣相을 보였다. 이러한 결과와 細胞質蛋白質과 比較한 結果로 미루어 볼 때, 70K 蛋白質은 膜表面蛋白質이며 糖蛋白質이고 또한 HSP 70과 같은 蛋白質인 것으로 推定되었다.

이 蛋白質의 細胞膜에 있어서의 가능한 機能과 腫瘍細胞가 正常細胞에 비해서 溫熱處理에 敏感한 原因등에 관해서 考察하였다.

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INTRODUCTION

Recently, the processes of the response and the adaptation of cells to various stimulations have been reported in eukaryotes and prokaryotes. The cellular response to heat shock, in particular, has become the focus of many investigations. Exposure of avian and mammalian cells grown in tissue culture to elevated temperature or to several chemicals induced a profound change in their pattern of protein synthesis. In this process, the translation of normal cellular protein is suppressed and the synthesis of a new class of proteins, known as "heat shock proteins" (HSP's) is greatly enhanced (Ashburner and Bonner, 1979). The major heat shock proteins fall into several size classes with molecular weights of 20K to 35K, 65K to 70K and 80K to 100K.

The heat induced responses have been extensively studied in *Drosophila melanogaster*, where the appearance of newly synthesized six specific proteins has been correlated with the puffing of polytene chromosomes and initiation of gene transcription. While, at the translational level, preexisting polysomes disappear, leaving intact messages for cell recovery. A similar set of proteins is induced when cells are incubated with various other agents, including chelators, certain transition metal ions, sodium arsenite, as well as amino acid analogues(Kelley and Schlesinger, 1978; Wang *et al.*, 1981). Three proteins with molecular weight of 24K, 70K, 83K, were synthesized in unusually large amounts after heat shock. The 70K protein(s) induced has closely similar electrophoretic mobilities with a protein at 68K~80K, being the most reproducible and commonly elicited species. Similar responses have been reported for *Dictyostelium* (Loomis and Wheeler, 1980), *Xenopus* oocytes (Bienz and Gurdon, 1982), *Tetrahymena pyriformis* (Guttman *et al.*, 1980), chicken embryo fibroblasts (Kelley and Schlesinger, 1978) and Chinese hamster ovary cells (Oleinick, 1979).

Since heat-shocked cells are less susceptible to the effect of further heat treatment, it has been suggested that the function of these proteins is to confer resistance to further lethal heat shock. But their induction mechanism and function remain as yet unknown (Landry *et al.*, 1982).

Under normal growth conditions, however, the HSP 68 and HSP 83 of avian cells are methylated but HSP 25 is not. Induction of the synthesis of these proteins results in a dramatic increase in the methylation of HSP 68. Although the functional significance of heat shock protein methylation remains elusive, methylation of protein has very important roles in signal processing in procaryotes. Rubin *et al.* (1982) reported that several specific proteins increased phosphorylation, while phosphorylation of other proteins decreased in mouse fibroblast cell line clone 1D by hyperthermia. They suggested that heat treatment induces or activates one or more specific phosphokinase(s) with the ability to phosphorylate HSP's with approximate molecular weights of 37K, 36K, 23K and 16K. These altered

phosphokinase activities were similar if not equivalent to those observed with interferon and transformed cells.

Sinibaldi and Morris (1981) reported that HSP 83 was limited to the detergent extracts, while the remaining HSP's probably were structural elements of the nucleus rather than the chromatin. HSP 68 is resolved by isoelectric focusing into a major acidic (68Ka) and a more basic (68Kb) component. HSP 68Ka is indistinguishable from a protein that copurified with brain microtubules and also a component of intermediate filament enriched cytoskeletons prepared from a number of avian or mammalian cell types (Wang *et al.*, 1981).

The intracellular distribution of these proteins has been investigated through autoradiographic analysis of *D. melanogaster* cells. Velazquez *et al.* (1980) concluded that little heat shock proteins become associated with mitochondria, despite the many lines of evidence linking the response to respiratory stress and HSP's are very highly concentrated in nuclei and plasma membrane. They quantitatively associated with chromosomes in a non-random manner, their concentration in highly condensed chromatin being very low relative to that of other chromosomal loci. The finding that some HSP's accumulated at the plasma membrane has led to the hypothesis that they may play some role in ion balance or be involved in adjusting the fluidity of the cell membrane. Others have proposed an association of some HSP's with a hexose transport system (Kelley and Schlesinger, 1978).

The development and decay of thermotolerance in tumor cell line MH-777 occurred concomitantly with an elevated synthesis of a small set of HSP's. Exposure of mammalian cells to supraoptimal temperatures induces a transient, increased tolerance to subsequent thermal treatment. Interestingly, there are several reports suggesting that in mammalian cells plasma membranes might be the primary target of hyperthermia and that changes in its structure might be an important step in the development of thermotolerance (Landry *et al.*, 1982).

In this paper, a marked alteration in the surface proteins of mouse fibroblast SCK cells after heat shock is presented, using lactoperoxidase-catalyzed radioiodination method. Exposure of SCK cells to heat shock results in the decrease in high molecular proteins and in dramatic increase in 70K protein. Because 70K protein was also labeled increasingly by galactose oxidase-sodium (^{3}H-)borohydride method after heat shock, this surface protein was concluded to be the glycoprotein and it might be the HSP 70.

MATERIALS AND METHODS

Cell Culture

The SCK tumor cells used were originally prepared from a mammary carcinoma of female A/J mice which arose spontaneously in 1974 and was adapted to grow both *in vivo* and *in vitro* by Dr. Clement in the Radiobiology Laboratory, University of Minnesota

Medical School (Kang, *et al.*, 1980). Exponentially growing cells were subcultured with 0.25% trypsin and inoculated at an appropriate number of cells in RPMI 1640 medium supplemented with 10% fetal calf serum and penicillin-streptomycin (100 units/ml), and were incubated in a CO_2-incubator at 37°C.

Heat Treatment

SCK cells in culture were treated with 0.25% trypsin at 37°C for 15 min. The viable cells were counted using the trypan blue exclusion method and an appropriate number of cells were plated in T-type glass culture flasks or Falcon flasks. After 2-day culture, culture flasks inserted to plastic shelves were immersed horizontally in a constant-temperature circulating water bath. After heat treatment for various lengths of time, the flasks were removed and the cells were cultured.

Cell Synchronization

Cell synchronization was done using a modified method of Hynes and Bye (1974). Briefly, three days prior to experiments, 2×10^5 cells were seeded, and then transferred to 0.5% serum media and left for 48 hr arrest in the early G_1 phase of the cell cycle. Five hr after transferring to 10% serum media, an addition of 1.5mM hydroxyurea (HU) for 15 hr led to arrest at the beginning of the S period. When replaced to fresh 10% serum media, all the cells resumed growth, commencing DNA synthesis almost immediately. After 6 hr, an addition of 10 μg/ml colchicine for 3 hr led to arrest in mitosis. The cell number doubled by 10 hr after released from a HU block, ie., all the cells are released from the block synchronously. These procedures were checked by DNA determination per cell using fluorescent dye, 3,5-diaminobenzoic acid (DABA).

Labeling of Surface Proteins with ^{131}I

Lactoperoxidase used in the present study was purchased from Sigma Chemical Co. and Na ^{131}I was obtained from the Radiation Biology Laboratory, Korea Energy Research Institute. Surface protein labeling with ^{131}I was essentially based on the method of Hubbared *et al.* (1972). Briefly, culture media were removed and cells were washed with 0.9% saline 3 times. Cells were scraped with a rubber policeman and collected by centrifugation. Cell pellet was resuspended in 0.5ml phosphate-buffered saline (PBS) and added by 100 μl of lactoperoxidase (500 μg/ml) and 150 μCi Na ^{131}I (1 mCi/ml). Iodination reaction was initiated by the addition of 20 μl of 90 mM H_2O_2 and another 20 μl of H_2O_2 was added 10 min later.

After 10 min of incubation, reaction was stopped by the addition of 10ml PBS containing 0.5 mM Na_2SO_3 and 5mM phenylmethylsulfonyl fluoride (PMSF). Cells were washed 3 times by centrifugation with phosphate buffered-sodium iodide to remove excess radioiodine. The cells collected were suspended in 0.1 M Tris-HCl buffer, pH 6.8.

Labeling of Glycoproteins with KB^3H_4

SCK cells in monolayer were incubated in 0.5 ml of PBS, pH 7.0 containing 10 Sigma units of galactose for 90 min at room temperature. The monolayer cells were washed twice

with PBS, pH 7.4, scraped into the same buffer and centrifuged. The pellet was resuspended in 0.5 ml PBS and labeled for 30 min at room temperature by adding 50 μl of KB $^3H_4$ in 0.001 N NaOH (nominally 1 mCi, specific activity 1.1 Ci/mmole, New England Nuclear Corp.). All buffers contained 2 mM PMSF, a protease inhibitor. Samples were homogenized by hand homogenizer. Homogenate was stored in a refrigerator until required for analysis (Critchley, 1974; Gahmberg and Hakomori, 1973).

Gel Electrophoresis

The discontinuous SDS-polyacrylamide gel electrophoresis was performed essentially according to the method of Laemmli (1970). Protein in homogenate was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard and an equal amount of protein was applied to separate slots of 7.5% slab gels. Samples were dissolved in 0.65 M Tris-HCl pH 6.8, containing 2% SDS, 5% mercaptoethanol, and 0.01% bromophenol blue. Samples were boiled for 3 min. Electrophoresis was carried out with a constant current of 50-70 mA for 4-6 hr.

Upon completion of electrophoresis, gels were stained in 0.2% Coomassie brilliant blue in 40% methanol and 10% acetic acid for 3 hr, and destained in 35% methanol and 7% acetic acid until the background became clear.

Autoradiography and Fluorography

Destained gels were dried under gentle vacuum for autoradiography. Autoradiograms were prepared by exposing the dried gels on X-ray film for a few days. Developed X-ray films were scanned in a Transidyne densitometer.

Tritiated proteins were detected on slab gels by fluorography. Gels were fixed and stained, incubated with 20 volumes of dimethylsulfoxide (DMSO) (two changes, 1 hr each), then with 4 volumes of 22.2% 2,5-diphenyloxazole (PPO) in DMSO for 3 hr. The gels were washed with 20 volumes of water, dried and exposed for 2-7 days at −70°C.

RESULTS

Cell Cycle and Density Dependence of Cell Surface Proteins

Autoradiograms of ^{131}I-labeled surface proteins at different cell cycle stages are shown in Fig. 1. When the SCK cells were transferred from serum deprivation to 10% serum plus 1.5 mM HU, the cells were arrested at the margin of DNA synthetic period (G_1/S), and when returned to 10% serum without HU, they progressed through S (iodinated 4 hr after release from HU), G_2 (iodinated 8 hr after release from HU), and mitosis in fairly synchronous manner. The cells in mitosis were iodinated within the next 2-3 hr. Similarly, the cells in early G_1 phase were iodinated 14 hr after release from HU. In each case an equal amount of 120 μg protein was applied to the gel for analysis. Molecular weight markers used were 200K chicken myoblast myosin, 84K bovine lactoperoxidase, 68K bovine serum albumin, and 43K ovalbumin.

Numerals 1-8 on the left side of the figure refer to labeled protein bands found in normal mouse fibroblast by Hynes and Humphryes (1974) and those 9-12 additional bands by Critchley (1974). As shown in the figure, there was no qualitative change during the cell cycle, all membrane proteins varied in its accessibility at the cell surface depending on the stages of the cell cycle.

A large external transformation-sensitive (LETS) protein (band 1 in the figure) present at the surface of cells, which is absent or much reduced in amount in transformed cells and has apparent molecular weight of 200K (band 2 in the figure), has been demonstrated as a transformation-sensitive protein by Gahmberg and Hakomori in 1973. As expected, the LETS protein detected in the SCK tumor cells was found likely much reduced in amount. Cells arrested in G_1 showed a densely-labeled band 1 compared to the cells passing through cell cycle after release from HU. Similar pattern of difference was also found in other high molecular proteins above band 8. The cells in S phase, on the contrary, showed specifically enhanced labeling in band 8a as well as in low molecular proteins. The cells in mitosis exhibited a low accessibility for high molecular proteins whose iodination profile was the same as the cells in G_1.

As is shown in Fig. 2, the densitometric scan of the autoradiogram reveals more clearly the labeled protein profiles. In order to present a quantitative assessment of iodinated surface protein changes, the area under each band was counted and normalized to the percentage of total area in each lane as shown in Table 1. Bands 7 and 8, with molecular weight of 100K and 85K were the most densely labeled, despite they were scarcely labeled in normal confluent cells (Gahmberg and Hakomori, 1973).

As shown in Fig. 3, surface proteins of SCK cells also exhibited a quantitative difference in labeling with change in cell density. The growth of the cell was found to slow down

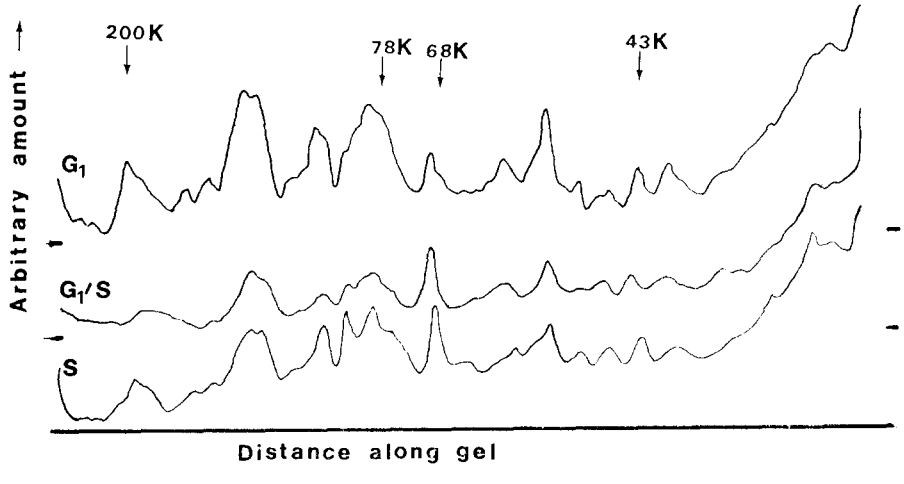


Fig. 2. Densitometric scanning patterns of the autoradiograms shown in Fig. 1. ^{131}I-labeled surface protein profiles at different phases are G_1, G_1/S, and S. The arrows indicate specific protein markers used.

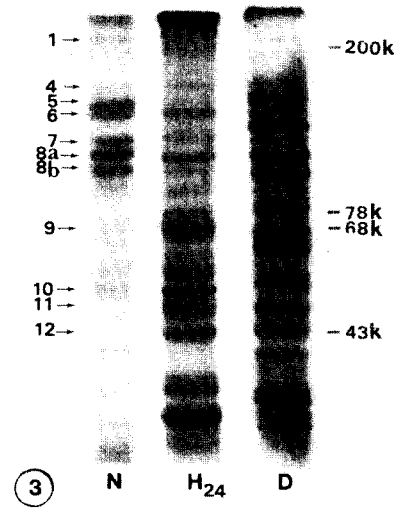


Fig. 1. Autoradiograms of SCK surface proteins at different phases of cell cycle. Membrane proteins were labeled by lactoperoxidase catalyzed iodination and then analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

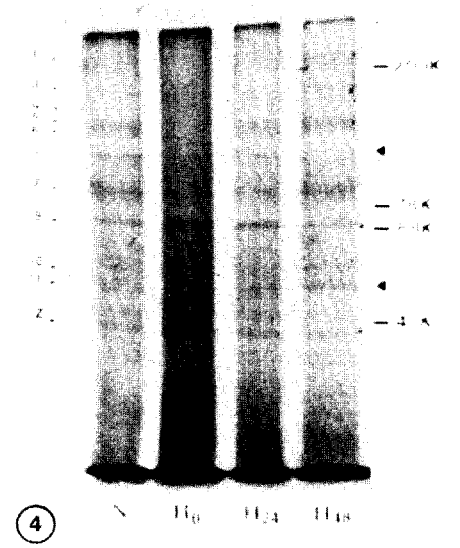
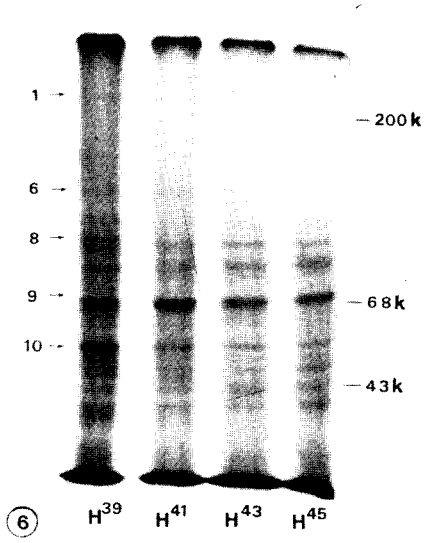
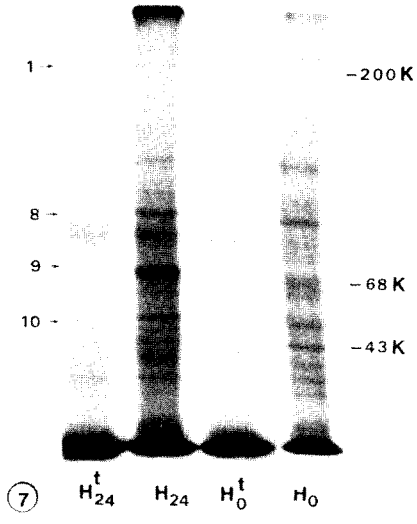


Fig. 3. ^{131}I labeled surface protein changes by heat shock or by cell density. N, surface proteins of untreated cells; H_{24}, those of cells iodinated 24 hr after heat shock at 43 C for 1 hr; D, those of confluent cells.

Fig. 4. Autoradiograms of surface protein changes labeled at different times after heat shock. N, labeled proteins of untreated control cells; H_0, those of cells iodinated immediately after heat shock; H_{24}, iodinated after 24 hr; H_{48}, iodinated after 48 hr.





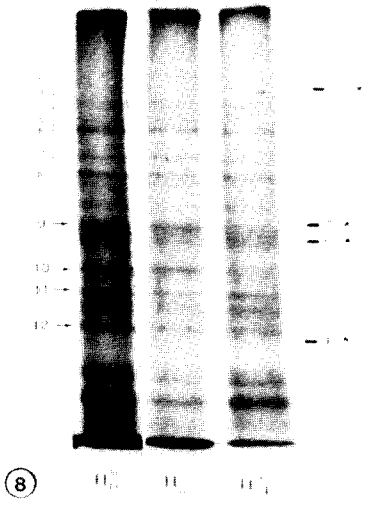


Fig. 6. Surface protein changes by various heat shocks. Superscript numerals refer to the temperature at which heat shock is given.

Fig. 7. Trypsin-sensitivity of the surface proteins after heat shock. Superscript t refers to mild trypsin treatment and subscript numerals refer to the time at which iodination was made.

Fig. 8. Fluorograms of surface glycoproteins labeled by galactose oxidase-sodium (^{3}H-) borohydride were shown along with surface proteins labeled by lactoperoxidase catalyzed iodination for comparison. Superscript G refers to tritium labeling and subscript numerals refer to the time at which labeling was made.

Table 1. Changes in cell surface proteins during cell cycle as measured by the peak area of densitometric scanning pattern.

Band No.	Approx. mol. wt.	Percent of total peak area				
		G_1	G_1/S	S	G_2	M
1	250K	3.78	1.74	2.30	2.93	2.39
2	200K	1.24	0.87	0.79	0.83	1.06
3	185K	1.49	0.09	0.79	0.75	0.60
4	160K	2.19	0.70	1.04	0.45	1.00
5	130K	9.47	3.92	4.32	6.31	6.84
6	125K	9.24	7.14	5.00	5.03	7.78
7	100K	7.45	3.22	4.89	4.58	4.32
8	85K	18.33	10.46	11.76	10.51	12.03
9	70K	4.42	7.49	5.18	2.58	3.19
10	60K	4.87	3.75	3.88	2.85	3.66
11	55K	7.45	7.84	5.65	6.23	4.26
12	45K	3.78	5.40	4.39	3.90	2.86

at a concentration of $4\sim5 \times 10^6/7$ ml and the saturation was reached at $7\sim8 \times 10^6/7$ ml under the conditions used. Significant changes in iodination pattern occurred only when cell growth began to slow down. In growth arrested dense cultures, band 6 is by far the most prominently labeled, whereas the two major bands 5 and 7 were significantly reduced in intensity.

Heat Shock Effects on Cell Surface Proteins

Fig. 4 shows the heat-induced changes in surface proteins labeled at different times after heat shock. Iodination was done at 0, 24, or 48 hr after the heat shock at 43°C for 1 hr and are designated as H_0, H_{24} and H_{48}, respectively. In the H_{24}, band 1 and other high molecular proteins decreased markedly, whereas most of low molecular proteins increased. At the same time, the major bands, 5 and 7 seemed to be completely disappeared.

Three closely spaced proteins, molecular weight of which ranged around 85~95K (bands 7, 8a, and 8b), were found interesting in that specific decrease was observed in bands 7 and 8b after heat shock, in bands 7 and 8a after mild trypsin, and in band 7 in high density cells. The band 9, apparent molecular weight of 68~70K increased significantly along with other low molecular proteins after heat shock.

Fig. 5 clearly shows the appearance of adaptation of SCK cells to heat shock within 48 hr. The H_0 exhibited the loss of high molecular proteins above band 8 and increase of low molecular proteins. After 24 hr, bands 5~8 increased gradually and most of the high molecular proteins returned to normal level by 48 hr. In the meantime, the enhancement of LETS protein was suggestive of the fact that the cells were highly synchronized. After

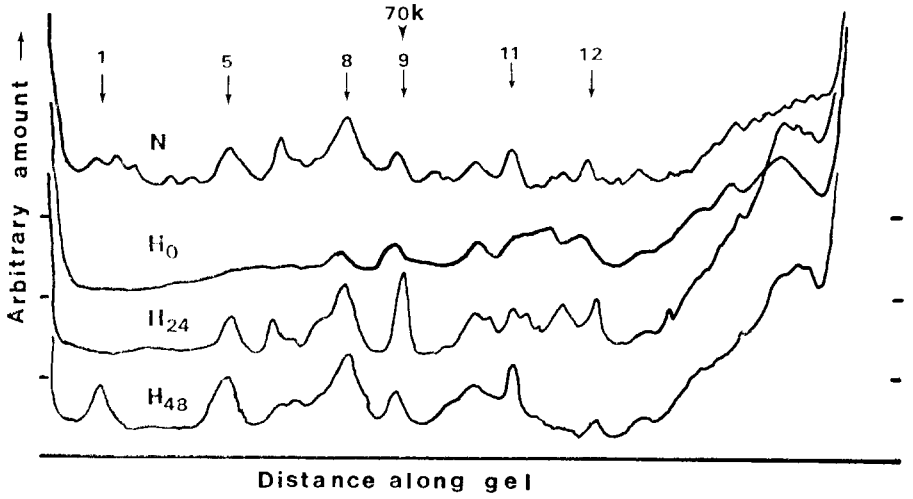


Fig. 5. Densitometric scanning patterns of the autoradiograms shown in Fig. 4. Abbreviations are the same as in Fig. 4. The arrows and numerals indicate the position and number of the bands.

Table 2. Changes in cell surface proteins after heat shock as measured by the peak area of densitometric scanning pattern. Abbreviations are the same as shown in Fig. 4.

Band No.	Approx. mol. wt.	Percent of total peak area			
		N	H_0	H_{24}	H_{48}
1	250K	2.51	0.49	1.06	3.56
2	200K	1.60	0.49	0.77	0.78
3	185K	1.46	0.59	1.15	1.49
4	160K	1.34	0.74	0.90	0.89
5	130K	3.74	1.71	1.28	3.27
6	125K	4.80	2.45	3.07	3.91
7	100K	4.80	2.20	2.98	3.27
8	85K	8.09	3.42	7.45	9.96
9	70K	4.43	5.00	6.73	2.92
10	60K	6.31	6.61	5.54	5.94
11	55K	6.86	6.12	3.88	4.80
12	45K	3.43	5.88	5.20	2.66

heat shock, the 70K protein increased specifically in H_{24}, followed by a decrease to normal level in H_{48}. Densitometric scanning of H_{24} exhibited about 70% increase in 70K protein compared to the control.

The heat-induced surface protein changes at temperature range of 39~45°C are shown in Fig. 6. A significant change was observed in 69~70K protein and the most notable

increase of this protein was found at 41°C.

Fig. 7 represents the trypsin-sensitivity of the heat shocked surface proteins. H_0^T denotes the sample that was prepared by treating a mild trypsin (20 μg/ml for 10 min) immediately after heat shock and H_{24}^T that prepared 24 hr after heat shock. Most of the cell surface proteins were relatively sensitive to trypsin and reorganized protein pattern was found in the range of 80~90K and 40K proteins. The protein which appeared in H_{24} was almost completely lost by mild trypsin treatment. This might serve as a clue for this protein to be on the membrane surface.

Fig. 8 shows the fluorograms of surface proteins labeled by galactose oxidase-sodium (^{3}H-)borohydride at 0 and 24 hr after heat shock and by lactoperoxidase for comparison. The tritium labeling pattern for glycoprotein was quite similar to radioiodine labeling pattern for surface protein, indicating the major surface proteins observed were glycoproteins.

DISCUSSION

Proteins exposed at the surfaces of cells are likely to be involved in intercellular communication and recognition of external stimulation. Their functions are associated with alterations in surface molecules.

The surface protein pattern of SCK fibroblast was confirmed here by two labeling methods, iodination and galactose oxidase-sodium (^{3}H-)borohydride. These methods label only those proteins exposed at the surface of cells. Band profiles were consistent qualitively, although the proteins were shown to vary in its accessibility at the cell surface, depending on the factors such as the phase in cell cycle and cell density.

Cells arrested in G_1 had the highest levels of high molecular proteins and LETS protein, whereas cells continuing through the cell cycle or arrested at other phases had lower levels. Various evidences suggest that high molecular proteins could play a role in growth control. It could fulfill this role either as a receptor for external signals or in a structural role affecting the activity of membrane proteins, or both (Hynes, 1974).

No function is yet attributable to LETS glycoprotein (fibronectin) with molecular weights of 250K that migrates as band 1. Fibronectin is a high molecular glycoprotein found in soluble form in the plasma and other body fluids. The soluble form of fibronectin is known to be composed of two disulfide-bonded polypeptides of 220K. Several types of adherent cells synthesize and secrete insoluble fibronectin in cell culture. Recent studies have implicated a role for fibronectin in cell adhesion, in cell motility *in vitro* and in cell migration *in vivo*. Fibronectin interacts with different types of collagen and particularly strongly with gelatin (Vartio and Vaheri, 1981). This protein, which has also been found in a number of other cells is thought to be identical with a fibroblast-specefic antigen (Critchley, 1974).

The radioactivity profile of ^{131}I-labeled surface proteins was also consistent when the radioactivity of sliced gels was counted in a Packard Liquid Scintillation Spectrometer (unpublished data).

All the proteins labeled by the iodination were relatively trypsin-sensitive, being concentration dependent. And comparison of Coomassie brilliant blue staining pattern with iodinated one suggested that major labeled bands are the surface proteins (unpublished data).

Heat-shock effect on membrane surface was generally grouped to the marked decrease of the high molecular proteins and the increase of the low molecular proteins. This is in sharp contrast to the finding that several low molecular proteins of cytoplasmic proteins as well as tubulin (55K) were completely repressed at the same temperature as we used (Rubin *et al.*, 1982). In particular, LETS protein and proteins above band 8 were correlated with their roles in membrane structure and cell attachment. This phenomenon has also been implicated cytologically, where cell morphology has been observed by inverted microscopy to change into round form after heat shock.

The 70K protein increased much higher in cell membrane iodinated 24 hr after heat-shock than that iodinated immediately. The decrease to low level of this protein was shown 48 hr after heat-shock. Landry *et al.* (1982) reported that heat shock-induced proteins were synthesized during the first 4 hr following the conditioning treatment and maintained in the cells at a high level for several hours but became undetectable by 82 hr. At the same time, the synthesis of these heat shock proteins were involved in the development and decay of thermotolerance. *Xenopus* oocytes respond to high temperature by the synthesis of one major 70K protein and the translation of preformed heat-shock mRNA continues for more than a day after a shift back to a normal temperature, but within 2 days (Bienz and Gurdon, 1982).

When temperature was increased from the control level of 37°C to values ranging from 39° to 45°C, 70K protein induced markedly at temperatures above 41°C. Rubin *et al.* (1982) reported that 95K and 70K heat shock proteins appeared at 41°C and higher in mouse fibroblast cell line clone 1D. Thus, 41°C is apparently a significant transition temperature.

By various cultured adherent cells, 70K glycoprotein is synthesized and secreted. This protein is gelatin-binding glycoprotein like fibronectin but is synthesized separately from fibronectin and processed from a 68K precursor polypeptide (Vartio and Vaheri, 1981).

In investigating the cellular distribution of heat shock proteins by electron microscopy in cells labeled with ^{3}H-leucine, there appeared to be a tendency for these proteins to accumulate at membranes as well as the nuclei and the cytoplasm. Since heat-induced proteins do not appear in vacuoles whereas control glands continue to contribute proteins to these structures, it might be argued that this accumulation is illusory, due simply to the exclusion of heat shock proteins from large regions of the cytoplasmic field. However, specific incorporation into membranes is strongly suggested by the high density of grains

observed over the lumen of heat-shocked glands. This region is very rich in membranes due to the dense microvilli which line the lumen.

The results presented here and various evidences provide that the 70K protein is a membrane surface protein induced by heat shock and may be the glycoprotein. The two-dimensional gels of Mirault *et al.* (1978) provided the evidence that there are several polypeptides in the 70K region, ranging in from 70K to 72K daltons. The evidence, both genetic and biochemical, indicates that these are very similar proteins. One interpretation is that of post-translational modification of a parent polypeptide. Alternatively, since there is a good evidence that there are multiple copies of the coding sequence at HSP 70 gene sites, it may be that each of these proteins is the product of a particular gene copy.

The function of the major heat-shock protein is as yet unknown. The primary targets of heat-shock could be an enzyme of a surface receptor whose malfunction indirectly signals the actual transcriptional and translational events. A possible candidate for this protein is a receptor protein that recycles from the cell surface in a manner of modulation of surface receptors. Heat-shock could change the location of this kind of protein as well as affect its basic structure or interaction with membranes. An additional feature of a surface receptor is the requirement for glycosylation, and it is noteworthy that alteration in cellular glycosylation can induce the 76K and 95K proteins (Pouyyssegur and Yamada, 1978: Shiu *et al.*, 1977).

Chicken fibroblast heat-shock porteins HSP 68 and HSP 83 are methylated. The predominant methylation site of the 83K are lysyl residues and that both lysyl and arginyl residues are methylated in the 68Ka and 68Kb. Each of the three polypeptides contain one to three methylated lysine residues per molecule. In addition there are one to two N^G-monomethyl arginines per molecule of both 68Ka and 68Kb. Methylation reactions occur during or soon after polypeptide synthesis and the extent of methylation appears to vary after heat shocks (Wang and Lazarides, 1982).

The function of protein methylation in eukaryotic cells remains largely unknown. However, aspartic acid has been identified as an *in vivo* site of methylation in human erythrocyte cytoskeletal and membrane proteins. In bacterial chemotaxis, methylation and demethylation of glutamyl residue of membrane receptor has been shown to be a key factor in the process of adaptation to new chemical environment or heat-shock (Springer *et al.*, 1979; Wang and Koshland, 1980; Engstrom and Haselbauer, 1980).

Heat treated cells possessed altered phosphokinase activity and several proteins exhibited increased phosphorylation. Other studies have associated heat induced differences in nuclear phosphoproteins with the control of transcription (Glover *et al.*, 1981). Whitlock *et al.* (1980) suggested a possible mechanism whereby stimuli at the cell surface may influence biochemical events at the nuclear level; changes in the intracellular Ca^{2+}-dependent kinases in the nucleus, cytoplasm or membrane. The regulation of HSP's may

be mediated by second messenger as Ca^{2+}, because cell surface alteration by heat shock may change intracellular Ca^{2+} concentration.

On the other hands, heat-shock may be only one way of influencing a complex interacting network or cascade of cell reactions associated with mechanisms for homeostasis and cell survival. This concept is supported by the fact that heat shock proteins are known to be induced under conditions of environmental stress such as viral infection, anaerobic treatment and glucose depletion.

Anaerobic treatment, heat-shock and deciliation result in similar alterations in the pattern of protein synthesis in *T. pyriformis*. These stress-induced proteins are very similar to the HSP's of *D. melanogaster* (Guttman *et al.*, 1980; Sachs *et al.*, 1980). The proteins induced by glucose starvation were associated with cell surface membrane (Pouyssegur and Pastan, 1977). Trosper and Levy (1977) have synthesized a photoreactive radiolabeled glucose derivative that inhibited D-glucose uptake, labeled four proteins of the plasma membrane fraction of rat adipocyte. The pattern of these proteins is strikingly similar to that of proteins induced in the chemical and heat-shock treated cells. Transport of glucose and of other metabolites would become increasingly important for the survival of the cells.

Interferon has been shown recently to induce a specific set of proteins, 120K, 80K and 67K in interferon treated mouse and human fibroblast cells. Heat induced altered protein sysnthesis and post-translational modification of certain proteins are similar to those observed with interferon. The appearance of the induced proteins correlates with the antiviral effect of interferon, thus suggesting that these proteins might be involved in the antiviral mechanism (Gupta *et al.*, 1979).

Certain neoplastic cells have been found to be selectively killed by hyperthermia in both the cancer patient and tissue culture. Studies on mammalian cells have implicated among many possibilities significant changes in membrane permeability and patterns of translation. The survival of tumor cells *in vivo* seems to be influenced greatly by occlusion of vasculature and subsequent environmental changes unfavorable for the repair of thermal damage (Kang *et al.*, 1980).

Because the optimal temperatures of HSP induction are different from normal and malignant cells, they may have the differential heat susceptibilities, corresponding to the function of HSP.

ABSTRACT

The primary concern has been focused on the response and adaptation of mouse fibroblast tumor cells to heat-shock in the level of membrane surface proteins, using two labeling techniques, lactoperoxidase-catalyzed iodination and galactose oxidase-sodium borohydride.

Cells arrested in G_1 phase exhibited the highest level of LETS protein and high

molecular proteins than did cells passing through G_1/S, S, G_2 and M, and unsynchronized cells.

Confluent cells were found to show an increase in 125K proteins and a decrease in 130K and 100K proteins selectively.

The adaptation processes of tumor cells after heat-shock were observed. All the proteins above 80K were reduced immediately after heat-shock, whereas 70K protein increased markedly 24 hours after heat-shock. The 70K protein and high molecular proteins returned to normal level in 48 hours.

The 70K protein was found to be trypsin-sensitive and was similarly labeled by galactose-oxidase as well as by lactoperoxidase. It was, therefore, concluded that 70K protein is glycoprotein located on the surface membrane and might be the HSP 70.

Possible function of heat-shock protein on the surface membrane and the relation of this protein to differential heat-sensitivity of tumor cells are discussed.

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