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HEAT SHOCK-RELATED DECLINE IN ACTIVITY AND AMOUNTS OF ACTIVE ELONGATION FACTOR 1α IN AGEING AND IMMORTAL HUMAN CELLS

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A significant decline in amount of active elongation factor, EF-1α, and in its catalytic activity was observed in cell-free extracts prepared from normal human diploid fibroblasts (MRC-5) and their SV40-transformed counterparts, after subjecting the cells to 60 min heat shock at different temperatures. Old MRC-5 cells which had become senescent on serial passaging were more sensitive to heat shock-related changes in activity and amounts of active EF-1α than were rapidly proliferating normal and transformed cells.

Mammalian cells respond to elevated temperatures first by inhibiting the synthesis of normal proteins and then by synthesizing preferentially the so-called heat shock proteins (1-3). However, it is still unclear which steps of protein synthesis are affected during heat shock, and to what extent. There is some evidence that both initiation (4-7), and elongation (8-10) of normal proteins slow down during heat shock. Although changes in activities and extent of phosphorylation of initiation factors during heat shock have been reported (4-7), estimation of amounts and activities of elongation factors has not been made. We have therefore studied heat shock-related changes in activity and amounts of active elongation factor, EF-1α, in normal and transformed human cells. For this purpose, we have used normal diploid human fibroblasts, MRC-5, which have limited lifespan *in vitro*, and its SV40-transformed immortal cell line, MRC-5V2.

The amounts of active EF-1α were estimated in cell-free extracts by stoichiometric binding of [^{14}C]Phe-tRNA to ribosomes in the presence of GDPNP, a non-hydrolysable analogue of GTP, whereas the catalytic activity of EF-1α was measured in the presence of excess GTP in order

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that EF-1α could function in multiple rounds of aminoacyl-tRNA binding to ribosomes (11). Our results show a decline both in amounts of active EF-1α and its specific activity during heat shock of human cells at different temperatures. The extent of decline was more pronounced in senescent MRC-5 cells than rapidly proliferating young MRC-5 and MRC-5V2 cells.

MATERIALS AND METHODS

Cell culture Cultures of normal human embryonic lung fibroblasts, strain MRC-5, and its SV40-transformed cell line MRC-5V2 (12), were maintained routinely at 37°C using DMEM containing 10% foetal calf serum, as described previously (11). In the present series of experiments, MRC-5 cells on serial passaging reached a cumulative population doubling level 49, which is here taken as 100% lifespan. As described by Hayflick (13), cultures are considered to be in Phase II until slowing-down of their growth during the last few population doublings (Phase III) before they cease growth completely. However, we have not observed such changes in growth characteristics in MRC-5V2 cells on serial passaging for more than a thousand times now.

Heat shock Cells were plated at a density of about 5 $\times 10^4$ cells/cm^2 either into culture flasks for assaying EF-1α or into Leighton tubes for measuring protein synthesis. After 20 h, cells in exponential phase of growth were transferred to a thermostatted gyratory water bath for heat shock that lasted for various lengths of time depending upon the nature of the experiment.

EF-1α assay Methods for estimating the amounts of active EF-1α and its catalytic activity were as described previously (11). Briefly, after the completion of the period of heat shock, cell-free extracts from about 4 $\times 10^6$ cells were prepared by sonication in 0.5 ml EF-1α protective buffer (25 mM Tris/HCl pH 8, 50 mM KCl, 2 mM benzamidine, 1 mM DTT, 1 mM PMSF, 0.1 mM EDTA and 25% glycerol). The sonicates were centrifuged (23,000 g for 45 min) and EF-1α assays were performed using the supernatants. The reaction mixture for the binding assays contained in 50 µl: 60 mM Tris/HCl pH 7.5, 6 mM magnesium acetate, 20 mM NH_4Cl, 100 mM KCl, 0.7 mM DTT, 13 mM sucrose, 0.5 mM ß-mercaptoethanol, 0.6 mM PMSF, 1.1 mM benzamidine, 14% glycerol, 0.06 mM EDTA, 0.01% w/v BSA, 10 pmol [^{14}C]Phe-tRNA^{Phe} (1118 dpm/ pmol), 2 A_{260} O.D. of salt-washed ribosomes from *Artemia salina* and finally 0.1 mM GDPNP for stoichiometric assay or 2 mM GTP for catalytic assay. Numerous experiments in our laboratories have established that these assays for EF-1α are accurate to more than 95% limit (11). The extent of protein syntheis in cells was determined by measuring the incorporation of [^{35}S]methionine into TCA-insoluble material (14), whereas the total

protein content was determined by the BioRad method (BioRad, W. Germany).

RESULTS

The rates of protein synthesis in normal diploid fibroblasts MRC-5 and their SV40-transformed counterparts MRC-5V2 were reduced at temperatures higher than the normal 37°C. Results are presented relative to the values for Phase II MRC-5 cells at 37°C. Incorporation of [^{35}S]methionine into TCA-insoluble material in 60 min at 37°C was 53% more in MRC-5V2 cells and 30% less in Phase III MRC-5 cells than in young cells (Fig. 1). Between 37° and 45°C, there was an inverse relationship between temperature and the extent of protein synthesis during 60 min heat shock in young (Phase II; about 50% lifespan completed), old (Phase III; >98% lifespan completed) and transformed cells (Fig. 1). The inhibition of protein synthesis during 60 min heat shock at different temperatures, however, was similar in young MRC-5 and transformed cells, but was greater in old MRC-5 cells (Fig. 1).

Both the amounts of active EF-1α and its catalytic activity were affected in normal and transformed cells during heat shock at different temperatures (Figs. 2-5). Results are presented relative to the mean values for young MRC-5 cells at 37°C. Figure 2 shows that amounts of active EF-1α declined significantly in all cell types during 60 min heat

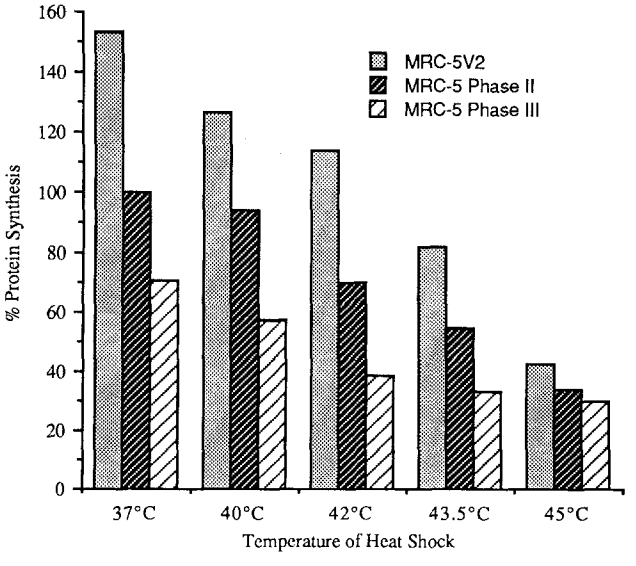

Fig. 1. Relative extent of protein synthesis in MRC-5 and MRC-5V2 cells during 60 min heat shock at different temperatures. Results are presnted relative to [^{35}S]methionine incorporation into TCA-insoluble material of Phase II young MRC-5 cells at 37°C in 60 min (100% = 740 dpm/μg protein).

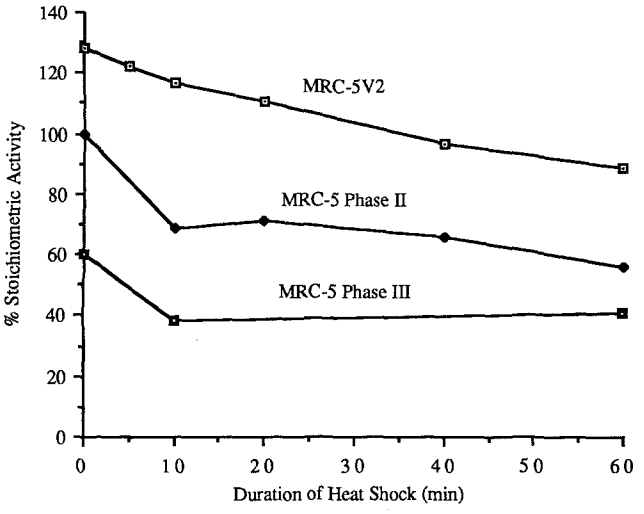

Fig. 2. Amounts of active EF-1α during heat shock of MRC-5 and MRC-5V2 cells, at 42°C. Results are presented relative to the amount of EF-1α in young MRC-5 cells at time zero (100% = 0.28 pmol/μg protein).

shock at 42°C. Within 10 min of heat shock, amounts of active EF-1α were reduced from 0.28 pmol and 0.17 pmol/μg protein in young and old MRC-5 cells, respectively, to 0.19 pmol and 0.11 pmol/μg protein, a decline of 31% and 37%, respectively (Fig. 2). There was another 10% decrease in amounts of active EF-1α in young cells during the remaining period of heat shock. It is important to note that at 37°C old cells already had 40% less amount of active EF-1α than young cells, and it was further reduced by 37% at 42°C.

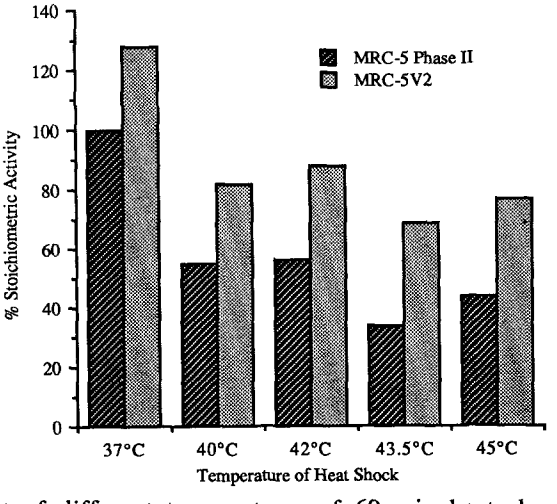

Fig. 3. Effect of different temperatures of 60 min heat shock on amounts of active EF-1α in MRC-5 and MRC-5V2 cells. Results are presented relative to the amount of EF-1α in normal cells at 37°C (100% = 0.28 pmol/μg protein).

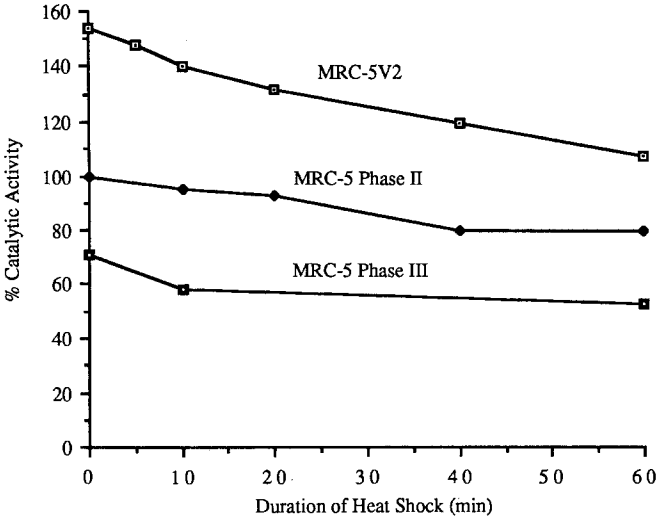

Fig. 4. Catalytic activity of EF-1α during heat shock of MRC-5 and MRC-5V2 cells, at 42°C. Results are presented relative to the catalytic activity of EF-1α in young MRC-5 cells at time zero (100% = 2.02 pmol Phe/μg protein).

In comparison to this, MRC-5V2 cells at 37°C had 28% more amount of active EF-1α than young MRC-5 cells. During 60 min heat shock at 42°C, there was a progressive decline in amount of active EF-1α in MRC-5V2 cells (from 0.36 to 0.25 pmol/μg protein; Fig. 2). This decrease, however, was to a lesser extent (31%) in transformed cells than in young MRC-5 cells (41%). At different temperatures of heat shock (40°, 42° and 43.5°C), the decrease in amounts of active EF-1α was inversely

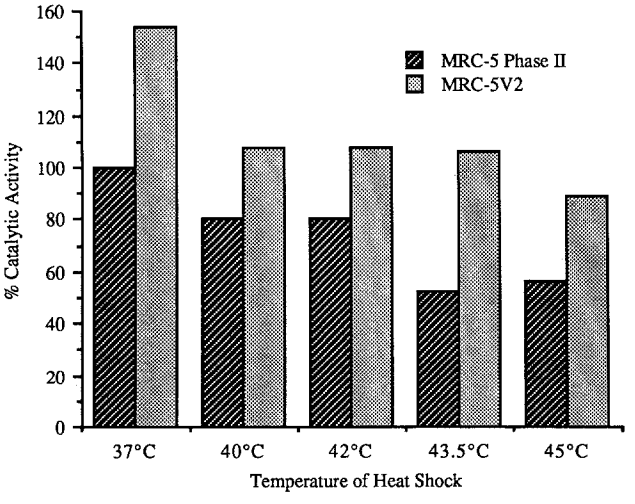

Fig. 5. Effect of different temperatures of 60 min heat shock on the catalytic activity of EF-1α in MRC-5 and MRC-5V2 cells. Results are presented relative to the catalytic activity of EF-1α in normal cells at 37°C (100% = 2.02 pmol Phe/μg protein).

related to the temperature (Fig. 3). For example, at 40° and 42°C the decrease was 45% and 36% in normal and transformed cells, respectively. At 43.5°C, there was a further decline of 22% and 14% in amounts of active EF-1α in the two cell types, respectively, which remained unaltered at 45°C (Fig. 3).

The catalytic activity of EF-1α also declined during heat shock. In contrast to the pattern of decline in amounts of active EF-1α, the decrease in catalytic activity during heat shock at 42°C was progressive in all cell types and was to a relatively lesser extent (Fig. 4). For example, after 60 min heat shock at 42°C, catalytic activities of EF-1α in young and old MRC-5 cells were reduced from 2.02 and 1.43 pmol Phe/μg protein to 1.62 and 1.07 pmol Phe/μg protein, a decrease of 20% and 25%, respectively (Fig. 4). In this case also, old cells, which already had 30% less catalytic activity of EF-1α than young cells, showed a further decrease of 25% during heat shock. In comparison, catalytic activity of EF-1α in transformed cells at 37°C was 54% higher than young cells, and was reduced by 30% during 60 min heat shock at 42°C (from 3.11 to 2.17 pmol Phe/μg protein; Fig. 4). At different temperatures both kinds of cells showed a decline in EF-1α activity to similar extent that was related inversely to the temperature of heat shock (Fig. 5). At 40° and 42°C, the decrease in catalytic activity of EF-1α was 20% and 30% in normal and transformed cells, respectively. There was a further decline of 24% and 10% in the catalytic activity of EF-1α in the two cell types at 43.5° and 45°C, respectively (Fig. 5).

DISCUSSION

Our results show that in human cells the slowing-down of protein synthesis during heat shock is accompanied by a decline in amounts of active EF-1α and in its catalytic activity. Although the role of elongation factors in heat shock-related inhibition of protein synthesis has been speculated (8-10), estimation of amounts and activities of elongation factors during heat shock have not been made earlier. We have observed a significant decline in amounts of active EF-1α and its catalytic activity in cell-free extracts prepared from normal and transformed human cells that have been given heat shock at different temperatures. Since the extent of heat shock-related inhibition of protein synthesis is more than the decline in activity and amount of active EF-1α, this factor is only one of the components that might be responsible for the inhibition of protein synthesis.

The rapidity of heat shock-related changes in activity and amounts of active EF-1α indicates post-translational regulation rather than

transcriptional and/or translational control as possible reasons for this. The possibility that transcription of EF-1α might be affected during heat shock is ruled out, because it has been observed that levels of EF-1α mRNA remain unaltered during heat shock of mouse cells (15) and HeLa cells (our unpublished observations). The regulation of EF-1α at translational level during heat shock is also very unlikely. This is because EF-1α is an abundant and long-lived protein in human cells, and its synthesis is not affected during different phases of the cell cycle, ageing and transformation (11,16-17). On the other hand, it is known that the catalytic activity of EF-1α is affected by post-translational methylation of its lysine residues (18). Therefore, the possibility that changes in methylation levels of EF-1α may account for heat shock-related changes warrants further investigation.

As regards the differential response of normal and transformed cells to heat shock, the increased death rate of the latter at elevated temperatures is well known and forms the basis of hyperthermia as a treatment against cancer (1-3,19-21). However, we have not observed any significant differences between the sensitivities of normal and transformed cells, in terms of decline in activity and amounts of active EF-1α during heat shock.

In relation to ageing, not much is known about the differences in response of young and old cells to heat shock. It has been reported that Phase III senescent human fibroblast cultures retained the capacity to synthesize heat shock proteins to the same extent as the young cells (21). We have observed differences between young and old MRC-5 cells with respect to the changes in activity and amounts of active EF-1α during heat shock. For example, young cells showed a biphasic decline in amounts of active EF-1α during heat shock at 42°C. At first, there was a reduction of 31% in amounts of active EF-1α followed by another 10% decline in the next 50 min. Old cells, on the other hand, had an immediate reduction of 37% in amounts of active EF-1α without any further decline. Similar differences between young and old cells were observed in case of heat shock-related decline in catalytic activities of EF-1α. These differences may be explained by a possible age-related increase in the ratio of heavy and light forms of EF-1α in senescent MRC-5 cells (11), which makes old cells more sensitive to heat. Further studies on differential response of normal ageing cells and immortal transformed cells to external stresses will be useful for understanding the molecular basis of such differences in order to develop new approaches towards treatment of cancer and other age-related conditions.

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