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EXPRESSION OF DROSOPHILA'S 27 kDa HEAT SHOCK PROTEIN INTO RODENT CELLS CONFERS THERMAL RESISTANCE

Emmanuelle ROLLET[1], Josée N. LAVOIE[2], Jacques LANDRY[2] and Robert M. TANGUAY[1]*

[1]Laboratoire de génétique cellulaire et moléculaire, CHUL, Ste-Foy, Qc G1V 4G2, Canada

[2]Centre de recherche en cancérologie, L'Hôtel-Dieu de Québec, Québec G1R 2J6, Canada

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SUMMARY: The role of hsp27, one of *Drosophila melanogaster*' s small heat shock proteins, in the process of thermotolerance was investigated. The coding sequence of hsp27 was subcloned downstream of the human hsp27 promoter which has been shown to be constitutively expressed in Chinese hamster O23 cells. Cellular resistance to a thermal stress was measured two days after transfection by a survival assay following a 3.5 h heat treament at 44°C. Expression of *Drosophila* hsp27 was shown to confer thermal resistance to O23 cells in a manner which was dependent on the level of expression of this hsp. Immunoblot analysis confirmed that the thermal resistance was related to the expression of *Drosophila* hsp27 as none of the endogeneous hsps showed an increased level under these conditions.

Heat shock proteins are synthesized in response to exposure to supraoptimal temperatures or to various forms of physical and chemical aggressors (reviewed in 1,2). These proteins which are well conserved in evolution are generally divided in three major groups according to their size; hsp90, the hsp70 family and the small hsps. Induction of hsps by a mild heat shock confers a transitory state of thermotolerance in mammalian cells (3,4). It has recently been shown that expression of human hsp27 transfected in Chinese hamster or mouse cells confers protection from thermal killing (5). Interestingly, human hsp70 has also been reported to confer resistance to thermal stress in a similar study in rat cells (6). In yeast, hsp70 (7) and hsp104 (8) have also been reported to be involved in the development of thermotolerance.

In *Drosophila* cells, where the heat shock response has been extensively studied, there are four small heat shock proteins: hsp27, hsp26, hsp23 and hsp22. No function(s) has yet been clearly assigned for any of these hsps which show, in addition to induction by heat shock, a developmentally regulated pattern of expression (9). Treatment of *Drosophila* with the molting hormone, ecdysterone, which is known to induce the small hsps has been correlated with the acquisition of thermotolerance (10). However there is no direct evidence for a role of a single *Drosophila* hsp in cellular resistance to thermal stress. As the small hsps are only partially

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conserved through evolution, it was of interest to test whether hsps from invertebrates could be substituted to higher vertebrates hsps inferring that they have conserved function(s). The gene coding for *Drosophila*'s hsp27 was subcloned under the control of the constitutive human hsp27 promoter, expressed by transfection in Chinese hamster cells and the relative survival of cells following a strong heat shock was measured. We show that expression of this invertebrate hsp27 can confer a dose-dependent protection against thermal stress.

MATERIALS AND METHODS

Cells

The O23 rodent cell line is an anchorage-independent and tumorigenic subclone of CCL39 (ATCC), a Chinese hamster lung fibroblast cell line. The cells and their transfectants were grown in DMEM supplemented with glucose (4.5g/l), sodium bicarbonate (2.2g/l), sodium pyruvate (0.11 g/l) and 5% fetal bovine serum as previously described (5).

Plasmids

Construction of pHS2711 and pHS27B-A containing respectively the complete human hsp27 gene or only its promoter region (control) has been reported previously (11). pHS2727 consists of a 1.9 kb XbaI - Hind III fragment containing the complete *Drosophila melanogaster* hsp27 coding sequence with 40 bp of 5' end leader and 700 bp of 3' end flanking sequences cloned into the Hind III site of the human hsp27 promoter in plasmid pHS27B-A. Transcription of the inserted *Drosophila* hsp27 is thus driven by the human hsp27 promoter. The three plasmids used are represented schematically in figure 1.

Transfection and thermal resistance assay

Exponentially growing O23 cells plated the day before transfection at a density of 10^5 cells per cm^2 were transfected by the calcium phosphate precipitate method of Wigler et al (12) essentially as described previously (5). The precipitate obtained from 10 to 40 µg of DNA was left 16 hours on cells. Cells were then trypsinized and plated at a density of 7 x 10^5 cells/25 cm^2. Two days after transfection, they were assayed for thermal resistance to a 3.5 h treatment at 44°C. Cells were trypsinized after the heat shock and plated at various densities at 37°C. The relative survival level was determined from the number of cells capable of forming colonies (more than 50 cells), 10 days after the heat shock.The data was corrected for the plating efficiency of non-heat shocked cells.

Gel electrophoresis and immunoblotting

Proteins extracted 48 hours post-transfection were solubilized in SDS buffer, separated on 12% SDS-polyacrylamide gels (13), transferred electrophoretically to nitrocellulose membranes and reacted with the antibodies as previously described (14). The antibodies against human hsp27 and Chinese hamster hsp27 (both diluted 1:1000) have been described previously (5). The antibody to *Drosophila* hsp27 (dilution 1:1000) was elicited by immunizing a rabbit with a ß-galactosidase-hsp27 fusion protein (containing the Sma-Dra fragment of the *Drosophila* hsp27 gene) produced from the expression vector pUR 289. An antibody against human hsp70 and specific for the inducible form of hsp70 was also used at a dilution of 1:5000 (Tanguay et al, unpublished).

RESULTS AND DISCUSSION

Expression of the human hsp27 gene cloned into the plasmid pHS2711 has been shown to be constitutive after transfection in Chinese hamster O23 cells (5). The level of expression is very high since the protein can be detected by Coomassie blue staining. Because of this high expression level of the human hsp27 promoter, the *Drosophila* hsp27 gene was cloned downstream of this promoter, as indicated in figure 1. Since heat shock is unnecessary to produce the protein in this system, the function of hsp27 can be studied in absence of the other hsps. We therefore investigated whether *Drosophila* hsp27 could confer thermotolerance when

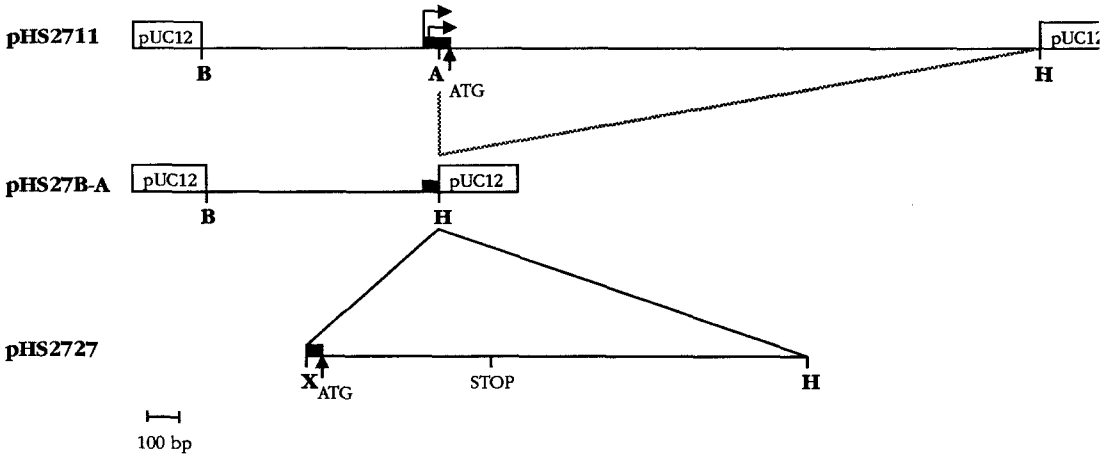

Figure 1. Schematic representations of the three plasmids used in this study. See Materials and Methods.

expressed in O23 cells, two days post-transfection. Table 1 indicates that after a treatment of three and half hours at 44°C, the survival level of control plasmid pHS27B-A-transfected cells falls dramatically. When compared to these control cells, the survival level in pHS2727-transfected cells was increased by more than 7 to 100 fold. Such variation was not unexpected since the transfection efficiency also varied in different experiments. The transfection efficiency under our conditions as determined by immunofluorescence was found to vary between 10% and 20% in different transfection assays. At any rate, the survival level of *Drosophila* hsp27-transfected cells was always higher than that of cells transfected by the plasmid without insert (pHS27B-A). The survival level of cells transfected with *Drosophila* hsp27 (pHS2727) was also lower than that of cells transfected with human hsp27 (pHS2711). Northern blot analysis of the RNA from transfected cells indicates that this difference is not due to a lower amount of *Drosophila* hsp27 mRNA than that of the human hsp27 mRNA (data not shown). However on Coomassie blue stained gels, the human hsp27 protein was clearly visible while the *Drosophila* hsp could not be seen indicating that the invertebrate hsp27 is expressed less efficiently in this system. This lower level of expression of *Drosophila* hsp27 may be a consequence of the 5'

Table 1. Survival levels of pHS2727-transfected cells. Two days after transfection with each of the three plasmids, cells were heat shocked at 44°C for 3.5 hours, then plated at three different dilutions (10^4, 10^5, 10^6) for colony formation at 37°C. Ten days later, surviving colonies were stained with a solution of 0.25% trypan blue in 5% acetic acid. Three independant experiments are shown. Values are given as the number of colonies/10^6 cells.

	Exp. 1	Exp. 2	Exp. 3
pHS 27BA	1 ± 1	6 ± 1	12 ± 2
pHS 2727	137 ± 2	173 ± 6	86 ± 3
pHS 2711	236 ± 3	371 ± 4	406 ± 6

leader or 3' regions of this invertebrate mRNA. These sequences are known to play a role in translation efficiency (15).

To demonstrate that this thermoprotection was the consequence of *Drosophila* hsp27 expression in O23 cells, the relative survival level was measured after transfection with increasing amounts of pHS2727 DNA . As shown in figure 2, the survival increases linearly with the amount of transfected pHS2727 DNA. The level of expression of *Drosophila* hsp27 was measured by immunoblot and the increase in the survival level correlates well with the increase in the amount of hsp27. Thus the thermal protection observed in the transfected cells seems directly related to the amount of *Drosophila* hsp27 present in these cells.

Since hsp70 has also been proposed to play a role in thermotolerance (7,16), we tested if this hsp was induced in cells transfected under our conditions. As shown in figure 3, hamster hsp70 is expressed in heat shocked O23 cells but not any of the transfected cells. The blot assay also shows that hamster hsp27 is not induced in transfected cells, but only in heat shocked O23 cells. Thus transfection *per se* does not seem to be a stress for O23 cells since two of the major inducible hsps are not detectable two days after transfection. We thus conclude that the thermoprotection effect observed in pHS2727 transfected cells is a consequence of *Drosophila* hsp27 expression.

In summary, we have shown that expression of *Drosophila* hsp27 is sufficient to confer thermal resistance to Chinese hamster cells. As in the case of human hsp27 (5), the efficiency of

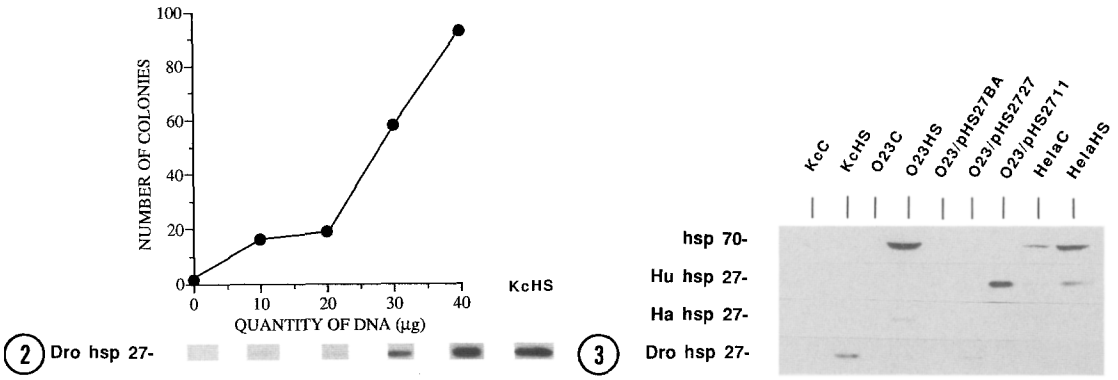

Figure 2. Dose-dependent effect of expression of *Drosophila* hsp27 on thermal protection. O23 cells were transfected with 0 to 40 µg of pHS2727 DNA. After a 3.5 hour heat shock at 44°C, 10^6 cells were plated for colony formation at 37°C (each point in triplicate). For each point, proteins were extracted two days after transfection and 20 µg was separated by SDS-PAGE, transferred onto nitrocellulose sheets and reacted with the antibody to *Drosophila* hsp27 .

Figure 3. Immunoblot analysis of hsps expression in tranfected O23 cells.
The presence of hamster hsp70 and of hamster, human and *Drosophila* hsp27 was analysed by immunoblot. *Drosophila* Kc cells were heat shocked at 35°C for one hour and recuperated at 23°C for four hours. They are the positive control for *Drosophila* hsp27 expression. Hamster 023 cells were heat shocked 20 minutes at 44°C and recuperated at 37°C for eight hours. They are the positive control for hamster hsp70 and hsp27 expression. HeLa cells were heat shocked at 44°C for 90 minutes, they are the positive control for human hsp70 and hsp27. Four identical gels were performed, electroblotted onto nitrocellulose sheets, then reacted with antibodies to human hsp70 and (Hu) hsp27, hamster (Ha) hsp27 and *Drosophila* (Dro) hsp27.

protection is dependent on the level of the hsp produced. The protection conferred to mammalian cells by an invertebrate hsp is of particular interest as human and *Drosophila* hsp27 only show a weak homology (35% identity at the amino acid level). These results thus suggest that this function of small hsps is conserved through evolution although the primary sequence is only partly conserved. The region which shows the highest conservation is that showing homology with α-crystallin in the carboxy terminal of the molecule. Interestingly, yeast hsp26 which only shows some 20% identity with either human or *Drosophila* hsp27 has been reported to have no major effect on thermotolerance (17). A detailed analysis of the regions of hsp27 conserved between *Drosophila* and human but lost in yeast may reveal domains which are likely to be involved in the protective function of this hsp.

How a cell becomes thermotolerant remains unknown at this point. Several hsps have been reported to be involved in this mechanism: human and mouse hsp27 (5,18) , *Drosophila* hsp27 (this study) , mammalian hsp 90 (19) and 70 (16). In *Drosophila*, the present study provides the first evidence of a role of one of the hsps, hsp27, in stress resistance. The three other low molecular weight hsps (26, 23 and 22) are presently being tested for their capacity to confer thermotolerance.

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