Proteasomal Oscillation during Mild Heat Shock in Aging Human Skin Fibroblasts

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ABSTRACT: Augmentation of proteasome machinery is emerging as a significant gerontomodulatory consequence of hormetic stimulation, such as mild heat stress. This study describes the phenomenon we term *hormetic proteasomal oscillation*, wherein mildly heat-stressed human fibroblasts (41°C, 1 h) display an adaptation response pattern in proteasome activity. Remarkably, such response appears to be diverse in severely heat-stressed or senescent fibroblasts. This proteasomal oscillation, as an innate cellular reaction to heat and aging, however, is independent of 20S proteasome protein levels and nuclear factor-E2-related factor 2 (Nrf2) transactivation.

KEYWORDS: proteasome; heat stress; fibroblast; hormesis; Nrf2; aging

INTRODUCTION

The proteasome is an essential protein quality-control mechanism responsible for the removal of abnormal or damaged proteins.[1] It is present in three forms with distinguishing functions: the 20S, the immuno-proteasome (20S + 11S), and the 26S (20S + 19S). The amount of the different forms of proteasome is dynamic relative to cell type, cell cycle, and physiologic environment.[2] Moreover, the proteasome is involved in signal transduction, cell cycle regulation, gene expression, apoptosis, and antigen presentation.[1] Hence, problems associated with proteasome function are bound to perturb cellular homeostasis and could lead to the accumulation of protein aggregates, deregulation of survival pathways, and senescence.[3]

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Various stressors, such as heat, UV light, and oxidative agents, have been reported to inhibit proteasome function.[4–7] Imai *et al*., however, have shown that this stress-associated decline in proteasome function is transient, and in fact, it was noted that proteasome activity even increases after exposure to heat shock.[5] The loss of 26S proteasome is the main reason for the downregulation of the proteasome activity, resulting in an increase in the relative amounts of the 20S proteasome.[5] After several hours, the level of the 20S proteasome is depressed as a result of the reassembly of 19S and 20S into the 26S proteasome.[5] From these findings, it has been proposed that the physical dynamics of the 19S regulatory units of the proteasome could lead to the oscillatory nature of proteasome activity. As this phenomenon has been elucidated in a yeast model and at nonphysiologic temperatures, we have investigated whether proteasome oscillation exists in normal human cells and within a hormetic framework.[9]

EXPERIMENTAL METHODS

Proteasome activity and Western analysis were performed as previously described.[10] Human adult skin fibroblast cells (ASF-2) were derived from the breast biopsy specimen of a consenting young healthy Danish woman (aged 28 years).

RESULTS AND DISCUSSION

After heat treatment of early-passage young ASF-2 cells at 41°C for 1 h, we describe the characteristic hormetic proteasomal oscillation as follows: phase I, the damage-response phase in which there is an initial decline of proteasome activity by 14%, remaining at low levels for 5 h; phase II, the hyperactivation phase in which there is a sudden burst of proteasome activity by about 25%; and phase III, the stabilization phase, in which there is gradual waning of proteasome activity until returning to the baseline levels in 2 h. Interestingly, the lag time prior to phase II was much extended when cells had become senescent and upon exposure to higher temperatures (FIG. 1A). Higher temperature caused proteasomal activity to decrease progressively during phase I (FIG. 1B). Proteasomal protein level was checked by measuring the level of the 20S α3 subunits, but there was no significant change found (data not shown). Furthermore, we also investigated the involvement of the transcription factor Nrf2, which is known to induce several proteasomal genes in response to oxidative stress, but no induction of Nrf2 was found in heat-shock-treated cells (data not shown).[11]

Consistent with the reported findings from mouse and yeast models, observations in human fibroblasts imply that the proteasomal oscillation as a heat stress response may be conserved.[5,6] In addition, preliminary data from our laboratory from *D. melanogaster* also confirm the proteasomal oscillation notion (data not shown).

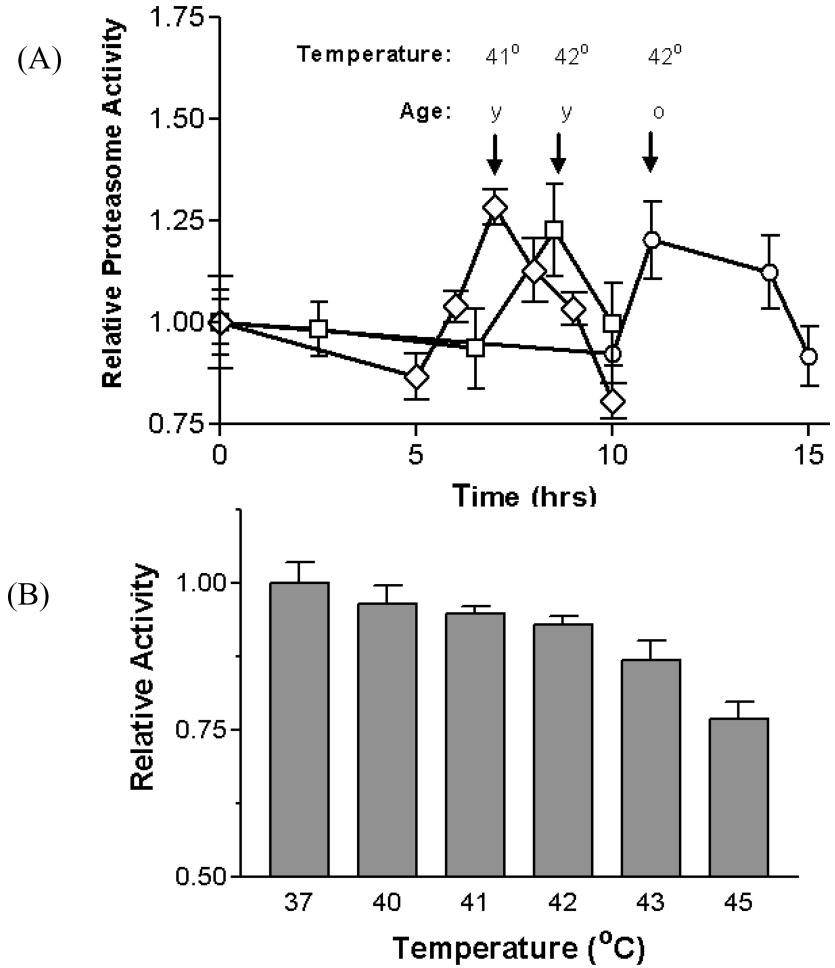

FIGURE 1. Chymotrypsin-like proteasome activity of ASF-2 cells. (**A**) Proteasome levels were measured within 15 h after heat shock (at 41 and 42°C) in young (Y) and old (O) cells, as indicated. (**B**) The effect of different temperatures on proteasome activity after 5 h post-heat treatment. Proteasome activity was measured as arbitrary fluorescence units of 7-amido-4-methylcoumarin (AMC) per mg protein per min liberated from the test substrate suc-LLVY-AMC.

More importantly, the data presented here show that such oscillation is induced at hormetically favorable temperatures. We surmise that this apparent downregulation of proteasomal activity may likely be a regulatory event more than a result of direct thermodynamic damage inflicted on the proteasome subunits. Bose *et al*. implicate the stress signaling pathways in altering the phosphorylation status of the proteasome that could lead to the detachment/reattachment of the 29S cap.[12] Further investigations into the role of stress

kinases in proteasome regulation during hormetic stress are under way. Understanding its regulatory pathways is important in manipulating the proteasome machinery for developing new anti-aging strategies and treatment of protein conformational disorders such as Alzheimer's disease.

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