Proteasome activation during cardiac hypertrophy by the chaperone H11 Kinase/Hsp22

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Aims The regulation of protein degradation by the proteasome during cardiac hypertrophy remains largely unknown. Also, the proteasome translocates to the nuclear periphery in response to cellular stress in yeast, which remains unexplored in mammals. The purpose of this study was to determine the quantitative and qualitative adaptation of the proteasome during stable cardiac hypertrophy.

Methods and results We measured proteasome activity, expression and sub-cellular distribution in a model of chronic cardiac hypertrophy induced by the stress-response chaperone H11 Kinase/Hsp22 (Hsp22). Over-expression of Hsp22 in a transgenic (TG) mouse leads to a 30% increase in myocyte cross-sectional area compared to wild-type (WT) mice (P < 0.01). Characterization of the proteasome in hearts from TG mice vs. WT revealed an increased expression of both 19S and 20S subunits (P < 0.05), a doubling in 20S catalytic activity (P < 0.01), a redistribution of both subunits from the cytosol to the nuclear periphery, and a four-fold increase in nuclear-associated 20S catalytic activity (P < 0.001). The perinuclear proteasome co-localized and interacted with Hsp22. Inhibition of proteasome activity by epoxomicin reduced hypertrophy in TG by 50% (P < 0.05). Adeno-mediated over-expression of Hsp22 in isolated cardiac myocytes increased both cell growth and proteasome activity, and both were prevented upon inhibition of the proteasome. Similarly, stimulation of cardiac cell growth by pro-hypertrophic stimuli increased Hsp22 expression and proteasome activity, and proteasome inhibition in that setting prevented hypertrophy. Proteasome inhibitors also prevented the increase in rate of protein synthesis observed after over-expression of Hsp22 or upon addition of pro-hypertrophic stimuli.

Conclusions Hsp22-mediated cardiac hypertrophy promotes an increased expression and activity, and a subcellular redistribution of the proteasome. Inhibition of the proteasome reverses cardiac hypertrophy upon Hsp22 over-expression or upon stimulation by pro-hypertrophic hormones, and also blocks the stimulation of protein synthesis in these conditions.

KEYWORDS Growth factors; Hypertrophy; Transgenic animal models

1. Introduction

A change in cardiac cell size results from an adaptation in the rates of protein synthesis and degradation,1 and the proteasome represents the main proteolytic pathway for intracellular proteins.2 The emerging roles of the proteasome in cardiac tissue have been reviewed recently.3,4 A potential role for the proteasome in cardiac hypertrophy remains largely unknown. However, the activation of the proteasome during stress is necessary to maintain a pool of amino acids for the synthesis of nascent proteins.5 In addition, a recent study also demonstrated that cellular stress in yeast promotes a translocation of the proteasome from the cytosol to the nuclear periphery, where it participates in cell survival by maintaining DNA integrity.6

We showed that proteasome activity increases in response to pressure overload, and that blocking such activation prevents the development of cardiac hypertrophy.7 In the present study, we hypothesized that proteasome inhibition might as well reverse pre-existing hypertrophy. For that purpose, we investigated the regulation of proteasome expression, activity, composition, and subcellular location in a model of stable cardiac hypertrophy triggered by increased expression of the stress-responsive heat shock protein H11 Kinase/Hsp22 (Hsp22), which is mainly expressed in heart and skeletal muscle.8 We previously found that Hsp22 is involved in the response to the two...
most common forms of cardiac cell stress, i.e. overload and hypoxia. Cardiac expression of Hsp22 increases in a canine model of cardiac hypertrophy, suggesting that it might participate in mechanisms of cardiac cell growth. A transgenic (TG) mouse with cardiac-specific expression of Hsp22 was generated that reproduces the same level of overexpression found in the canine model of hypertrophy. This TG mouse shows a pattern of myocardial hypertrophy with normal cardiac function, which results from an activation of Akt and the mammalian target of rapamycin. This model is particularly suitable to examine the steady state expression found in the canine model of hypertrophy. This TG mouse shows a pattern of myocardial hypertrophy with chronic and stable hypertrophy. In particular, overexpression of Hsp22 does not induce a transition into heart failure, at the opposite of the more frequently used models of cardiac overload by aortic constriction. To better define the mechanisms by which Hsp22 promotes cardiac hypertrophy, we characterized its effects on the proteasome, both in vivo and in vitro.

2. Methods

2.1 Animal model

We used TG mice (1- to 9-months old) expressing the coding sequence of human Hsp22 and a C-terminal haemagglutinin (HA) tag, and their wild-type (WT) littermates. The construct was introduced by pronuclear injection in zygotes of FVB mice. TG mice were genotyped by polymerase chain reaction (PCR) using a forward primer specific of the αMHC promoter and a reverse primer corresponding to the HA-tagged C-terminus. Epoxomicin and lactacystin were diluted in saline/10%Ethanol and injected daily (0.5 mg/kg i.p. for 7 days), based on our previous experience with the inhibitor, and on our observation that proteasome activity recovers 2 days after a single injection of epoxomicin (data not shown). Shams were injected with the vehicle only. Haemodynamics and echocardiography were performed as described. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2 Cross-sectional area

Samples from TG mice and WT littermates were fixed in 10% formalin. Myocyte cross-section area was determined on digitized images of TRITC-labelled wheat germ agglutinin-stained sections. The myocyte outlines were traced and the cell areas measured using Image-ProPlus Software System (Silver Springs, MD, USA). At least 100 myocytes were measured in each region.

2.3 Protein extraction

Total protein extraction was performed at 4°C in a lysis buffer (25 mmol/L Tris–HCl-pH 8.0, 150 mmol/L NaCl, 15 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L DTT, 0.5% Triton X-100 and 5% glycerol) supplemented with protease, kinase and phosphatase inhibitors, followed by centrifugation at 12 000 × g for 20 min at 4°C. Proteasome purification was performed on an affinity column with the ubiquitin-like (UBL) domain of the Rad23 human homologue bound to glutathione S-transferase (GST) (Calbiochem, San Diego, CA, USA). Subcellular fractions were prepared by differential centrifugation of tissue homogenized manually in hypotonic buffer. After an initial spin at 100 g to discard the cellular debris and unbroken cells, the nuclear fraction was obtained from low-speed centrifugation (500 g), followed by three washes with phosphate-buffered saline. The cytosolic fraction was collected as the supernatant resulting from the ultracentrifugation (100 000 g) of the homogenates. Proteins were denatured by boiling, resolved on SDS-PAGE gels, and transferred to membranes. Correct separation of the fractions was verified by western blotting for glucose-6-phosphate dehydrogenase (G6-PDH, cytosol), and histone 1 (nucleus). For immunoprecipitation, 30 μL PBS-washed protein A-Sepharose was incubated overnight at 4°C with 1 μg of antibody. Precipitation was performed at 4°C after incubating 100 μg of cellular extract with the beads for 2 h, and the complex was washed three times with 150 mM NaCl, 20 mM Tris–HCl, pH 7.5. Antibodies against the proteasome components were from Biomol (Plymouth, PA, USA). Other antibodies were from Santa Cruz (Santa Cruz, CA, USA), except the custom-made anti-Hsp22 antibody described previously. Antibodies were added at the recommended dilution. Detection was performed by chemiluminescence.

2.4 Immunofluorescence

Frozen tissue sections of hearts from both WT and TG mice were fixed in 2% paraformaldehyde/methanol, and incubated overnight with specific antibodies. After washing, slides were incubated for 3 h with 2% rabbit or goat serum labelled with Alexa 488, and mounted with DAPI medium.

2.5 Protease assays

The chymotrypsin-like activity of the proteasome was measured from 25 μg of total protein extracts added to 1 mL assay buffer [25 mmol/L HEPES (pH 7.5), 0.5 mmol/L EDTA] containing 40 μmol/L of the fluorogenic substrate Suc-LVY-AMC (Boston Biochem, Cambridge, MA, USA) and incubated at 37°C for 2 h, followed by fluorescence measurement (Turner Designs, Sunnyvale, CA, USA). Cathepsin B activity was measured by fluorometry using the substrate Z-Arg-Arg-AMC (Calbiochem, San Diego, CA, USA).

2.6 Quantitative polymerase chain reaction

Total RNA was extracted by the phenol/chloroform method. Primers were designed from mouse sequences corresponding to the genes of interest. After reverse transcription (RT), the cDNA was used for quantitative PCR (40 cycles of a 10 s-step at 95°C and a 1 min-step at 60°C) with SybrGreen (Applied Biosystems, Foster City, CA, USA) on a 7300 Sequence Detector (Applied Biosystems, Foster City, CA, USA). Values are reported per level of cyclophilin, used as a housekeeping gene.

2.7 Cell culture

Cardiac myocytes were prepared from 1-day-old Wistar rats as before and plated at a density of 106 cells per well. The culture medium was changed to a serum-free medium after 24 h. Cell growth was measured by the protein/DNA ratio. Protein synthesis was measured by the rate of [3H]phenylalanine incorporation. The adenovirus harbouring Hsp22 was described before.

2.8 Statistical analysis

Results are presented as the mean ± SEM for the number of samples indicated in the legends. Statistical comparison was performed using the Student’s t test, with a significance of P < 0.05.

3. Results

3.1 Characterization of the transgenic mouse

Compared to WT mice, hearts from TG mice show a significant increase in heart weight/body weight and in heart weight/tibial length, which was already observed in 1 month-old mice and which remained stable thereafter (Figure 1). This phenotype was accompanied by the induction of a “foetal gene program” including an increased expression
of the atrial natriuretic factor (ANF) and a decreased expression of the Ca\(^{2+}\) ATPase Serca 2a (Figure 1). We showed before\(^{10}\) that the contractile performance of the hearts from TG mice, including ejection fraction, developed pressure and heart rate, is comparable to the WT.

3.2 Activation of proteasome activity in the transgenic mouse

To characterize the potential regulation of the proteasome in our model, we first determined whether proteasome abundance is affected by the phenotype of hypertrophy in the TG mouse. The proteasome contains 19S or 11S regulatory subunits, capping the 20S catalytic subunit.\(^{4}\) The 19S subunit is composed of proteins with (Rpt) or without (Rpn) ATPase activity.\(^{20}\) The 11S particle is made of three proteins (PA28\(\alpha\), \(\beta\), and \(\gamma\)).\(^{21}\) The 20S core subunit is made of four stacked rings, including \(\alpha\) proteins in the outer rings and \(\beta\) proteins in the inner rings.\(^{22}\) The expression of representative examples of these protein families was increased in the TG mouse heart compared to WT (Figure 2A). Quantitation of these changes showed a one-and-a-half to three-fold increase in TG vs. WT (\(P\), 0.01) for the components of the three subunits (Figure 2B).

To determine whether this increased abundance results in increased catalytic activity, we used a fluorescent assay that measures the chymotryptic activity of the proteasome.\(^{7}\) Figure 2C shows the linearity of this assay in a mouse heart extract from WT and TG, based on both the incubation time and the amount of protein added. Using this assay, the increase in abundance of proteasome subunits found in TG hearts resulted in a two-fold increase in chymotryptic activity. This activity was decreased by 90% after addition to the assay buffer of the inhibitor epoxomicin, which specifically blocks the chymotryptic activity of the proteasome,\(^{23}\) confirming that it results from an activation of the 20S proteasome catalytic subunit (Figure 2D). Because the activity of the 19S regulatory subunit and the assembly of the 20S catalytic subunit both require energy,\(^{4}\) the assay was repeated in presence of different concentrations of ATP. As expected,\(^{24}\) addition of ATP progressively increased the proteasome activity until reaching a plateau (Figure 2E). At all concentrations tested, the proteasome activity was significantly higher in TG compared to WT (Figure 2E). Time course measurement of proteasome activity showed a significant difference between WT and TG at each age point (Figure 2F). Interestingly, a progressive increase in proteasome activity with age was found in both WT and TG (Figure 2F), which parallels the corresponding increase in heart weight.

3.3 Interaction of the proteasome with H11 Kinase/Hsp22

We determined next whether the mechanism of increased proteasome expression in the TG mice results from a transcriptional activation. Quantitative RT–PCR was performed on total RNA from WT and TG mice at three different age points (1, 3, and 9 months) to measure the transcripts encoding the proteins \(\alpha5\), \(\alpha6\) and Rpn2, which are representative examples of proteins indispensable for the normal function of the 19S (Rpn2) and the 20S (\(\alpha5\) and \(\alpha6\)) subunits. Interestingly, WT mice but not TG mice showed a significant developmental up-regulation of the 20S proteins (Figure 3A). At no point, the transcript expression of any of the three subunits was higher in TG mice compared to WT, showing that the increased expression of proteasome proteins in the TG mice more likely results from a post-transcriptional mechanism of regulation.

Increased stability and activation of the proteasome may result from its interaction with heat shock proteins.\(^{15,26}\) We determined by immunoprecipitation whether proteasome
subunits interact with Hsp22 itself, or with Hsp70 and Hsp27, two other major Hsps in the heart. As shown in Figure 3B, the proteins α5, α6 and Rpn2 co-purified with Hsp22, and with both Hsp70 and Hsp27. This co-purification was markedly increased in TG compared to WT. To improve the specificity of this observation, the proteasome was purified from total protein extracts of WT and TG hearts using an affinity column containing the UBL domain of the Rad23 human homologue bound to GST. Under these conditions, the interaction with Hsp22, Hsp70 or Hsp27 was still detected (Figure 3C). A column containing the GST without the Rad23 UBL domain was used as negative control (Figure 3C).

We showed before that increased expression of Hsp22 in vivo is accompanied by a subcellular redistribution of the protein around the nucleus.14 Interestingly, the yeast proteasome shows a similar pattern of redistribution upon cellular stress.6 Therefore, we tested whether the proteasome subunits interacting with Hsp22 would show a cellular translocation. The localization of the proteasome proteins and of Hsp22 was measured by subcellular fractionation from freshly homogenized cardiac tissue of WT and TG mice, separately in the nuclear and cytosolic fractions. Western blotting of representative examples of the 19S and 20S subunits was repeated in these fractions. The proteasome proteins were detected only in the cytosolic fraction of WT mice (Figure 4A and B). In TG mice, a significant amount of protein could also be detected in the nuclear fraction (Figure 4A and B).

To determine whether the proteasome detected in the nuclear fraction is catalytically active, measurement of the chymotryptic activity of the 20S subunit was repeated specifically in this fraction from both WT and TG mice, in the absence or presence of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C). Reciprocally, the chymotryptic activity was increased by four-fold in TG vs. WT (P < 0.001), which was abolished after addition of epoxomicin (Figure 4C). In WT, this activity was not significantly different with or without the inhibitor (Figure 4C).

To confirm these results, one example of proteins from the 19S subunit (Rpt1) and from the 20S subunit (α5) of the proteasome were detected by immunofluorescence in Figure 2 Increased expression of the proteasome in the transgenic mouse. (A) Immunoblots of representative examples of proteasome proteins from 19S, 20S, and 11S subunits in samples from WT and TG. Glucose 6-phosphate dehydrogenase (G6PDH) is a loading control. (B) Quantification of the corresponding changes (n = 5/group). (C) Linearity of the chymotryptic assay (n = 3/group) in hearts from WT vs. TG, as a function of the incubation time and the protein amount. (D) Proteasome activity in WT vs. TG, with or without epoxomicin (n = 5/group). (E) Proteasome activity in WT vs. TG with different concentrations of ATP (n = 3/group). (F) Time course of proteasome activity in TG vs. WT. **P < 0.01; *P < 0.05 vs. WT. #P < 0.05 vs. corresponding group without epoxomicin.
frozen sections of hearts from both WT and TG mice. Counter-staining with DAPI was performed to identify the nuclei. The TG mouse showed a perinuclear accumulation of these proteins (Figure 4D). As expected, Hsp22 was found at the same location in the TG mouse (Figure 4D), which further supports the results obtained by co-immunoprecipitation. This perinuclear pattern was not found in WT mice (Figure 4D).

3.4 Proteasome inhibition reduces cardiac hypertrophy by H11 Kinase/Hsp22

To test the physiological significance of proteasome activation in the phenotype of cardiac hypertrophy by Hsp22, mice from both WT and TG groups were treated for 1 week with epoxomicin, and compared to vehicle-treated mice. At the dose chosen (0.5 mg/kg), epoxomicin treatment in vivo suppressed the increase in chymotryptic activity found in the TG mouse (Figure 5A). The drug did not significantly affect heart mass in WT mice but it reduced by about 50% the increase in the heart weight/body weight ratio and in the heart weight/tibial length ratio (Figure 5A) when administered in TG mice. Myocyte cross-sectional area was measured in the different groups. A 40% decrease in cardiac cell cross-sectional area was found in treated TG mice compared to the vehicle (**P < 0.01), whereas WT mice showed no significant difference (Figure 5A). Haemodynamic measurements were performed in all groups, and show that the effect of epoxomicin on heart mass cannot be attributed to variations in ventricular or aortic pressures, a parameter that could possibly attenuate wall stress (Table 1).

Epoxomicin, which binds the β5 subunit of the proteasome and therefore inhibits only the chymotryptic activity, is considered a very specific inhibitor of the proteasome. To verify that the action of the inhibitor is specific for the proteasome, we performed additional experiments with lactacystin, which, at the opposite of epoxomicin, targets the three catalytic activities of the organelle, and which belongs to a different family of chemical compounds. Lactacystin inhibited proteasome activity and decreased heart size in TG mice to a similar extent as epoxomicin (Figure 5B). The specificity of epoxomicin on the proteasome protease activity was further tested by measuring the activity of another protease, cathepsin B. Although this activity was also increased in TG compared to WT, it was not affected by epoxomicin (Figure 5C).

3.5 Cardiac cell growth requires an activation of the proteasome

We tested whether acute, adeno-mediated expression of Hsp22 in isolated cardiac myocytes would be sufficient to activate the proteasome. This model also allows investigating more mechanistically the cause-consequence relationship between Hsp22 over-expression, proteasome activation and cardiac cell growth. Isolated neonatal rat cardiac myocytes were incubated for 48 h with 5 or 10 multiples of infection (moi) of adenovirus harbouring the Hsp22 sequence, or with the bGal control. An increase in Hsp22 expression was observed in myocytes, which was proportional to the amount of adenovirus (Figure 6A). Similarly, both proteasome activity and cell growth (as measured by the protein/DNA ratio) increased proportionately to Hsp22 expression (Figure 6A).
can be reproduced in vitro upon short-term over-expression of Hsp22, and is proportional to the extent of hypertrophy. Twenty-four hours after infection with the adenovirus harbouring βGal or Hsp22, cardiac myocytes were treated for 24 h with 5 μM lactacystin or epoxomicin, or with the vehicle. Proteasome inhibitors did not affect the increased expression of Hsp22 following adenovirus infection (Figure 6A) but they prevented proteasome activation in response to Hsp22 over-expression, and remarkably they also blocked hypertrophy (Figure 6A).

We tested whether these findings could be reproduced with well characterized pro-hypertrophic stimuli. Cardiac myocytes were treated with either 1 nM insulin or 1 μM angiotensin-II (AT-II). Both hormones stimulated cell growth, and increased both Hsp22 expression and proteasome activity (Figure 6B). Addition of proteasome inhibitors in these conditions prevented the increase in cell growth as measured by the protein/DNA ratio (Figure 6B). Interestingly, proteasome inhibitors in that setting further increased Hsp22 expression (Figure 6B), which was not observed when these inhibitors were added in absence of pro-hypertrophic stimuli (Figure 6B), suggesting that proteasome inhibition affects the cardiac phenotype only when cell growth is stimulated.

Because proteasome inhibitors block cardiac hypertrophy in cells exposed to Hsp22, insulin or AT-II, they might interfere with the increased rate of protein synthesis observed in these conditions. This hypothesis was tested by measuring the rate of [3H]phenylalanine incorporation in our different experimental groups. In control, non-stimulated myocytes, the protein synthesis rate was not affected by the addition of proteasome inhibitors (Figure 6C). The incorporation rate of phenylalanine increased by up to 10-fold in myocytes after exposure to Hsp22, insulin or AT-II (Figure 6C), which was totally prevented by epoxomicin and lactacystin (Figure 6C). These results are consistent with the interpretation that blocking proteasome activation upon stimulation of cardiac cell growth will also block the activation of protein synthesis, thereby maintaining the myocytes in a steady-state of protein turnover. Proteasome inhibitors did not affect the activity of cathepsin B, although the activity of this enzyme was increased upon Hsp22 over-expression, in agreement with our data in vivo (Figure 6D).
4. Discussion

We demonstrate an increased expression, increased activity, and subcellular redistribution of the proteasome in a model of stable and chronic myocardial hypertrophy by overexpression of the stress protein Hsp22. Inhibition of the proteasome reverses the extent of hypertrophy. In addition, we show that the subcellular distribution of the proteasome is altered, as significant accumulation was observed in the nuclear periphery, similarly to findings described in yeast. An activation of the proteasome was reproduced in vitro upon acute adeno-mediated over-expression of Hsp22 in cardiac myocytes, or upon exposure of myocytes to prohypertrophic stimuli. Using that model, we show that proteasome activation and increased protein synthesis are interdependent.

We previously showed that proteasome inhibition before the initiation of pressure overload prevents the development of cardiac hypertrophy without affecting ventricular contractile function.\(^7\) The present study extends these findings by showing that proteasome inhibition can also reverse stable and chronic cardiac hypertrophy by Hsp22. Our data in vitro also show that proteasome inhibition prevents the growth response of cardiac myocytes to AT-II or insulin. Taken together, these results show that the proteasome plays a role in the cardiac growth response to various stimuli, including pressure overload, compensated hypertrophy, neurohormones and growth factors.

The current investigation raises several questions. First, why is proteasome activation required for cardiac growth? It is our hypothesis that regulatory proteins that might otherwise prevent cardiac hypertrophy are targeted to the proteasome during overload by the activation of specific ubiquitin ligases. This hypothesis is supported by the observation that proteasome inhibitors prevent the increase in protein synthesis that is expected when cardiac cell growth is stimulated, whereas they have no noticeable effect in non-stimulated cells (Figure 6C). For example, in cardiac myocytes, Foxo3a blocks hypertrophy by transactivating the expression of atrogin-1,\(^29\) which targets for degradation several components of the sarcomere. The insulin-like growth factor-1 promotes hypertrophy in part by increasing the ubiquitination of Foxo via Akt.\(^30\) Another mechanism of specific substrate degradation involves the inducible cyclic AMP early repressor (ICER), which inhibits the transactivation of genes involved in cardiac cell growth during stimulation of the β-adrenergic signalling pathway.\(^31\)

A second question relates to the subcellular translocation of the proteasome to the nuclear compartment. A recent
The proteasome can be tethered by specific adaptor proteins to the outer nuclear membrane in the yeast, which is stimulated upon cellular stress. The degradation of specific DNA binding elements that shuttle between the nucleus and the cytosol (such as Foxo or ICER) would be more efficient upon nuclear accumulation of the proteasome. Another role for the nuclear proteasome is a quality control mechanism by which aberrant or denatured nuclear proteins can be promptly destroyed in order to maintain DNA integrity. Also, this translocation places the proteasome in the vicinity of the endoplasmic reticulum (ER) and may relate to the unfolded protein response, by which denatured proteins in the ER are destroyed. In addition to its role in cell growth, the unfolded protein response is essential for cell survival because the accumulation of denatured proteins triggers pro-apoptotic signals. It is also possible that a perinuclear localization of the proteasome and its strong interaction with Hsps could protect the proteasome itself against proteolytic degradation, which would offer a mechanism for the post-transcriptional increase in expression of proteasome in the TG mouse. In conclusion, the present study demonstrates a regulation of the proteasome during cardiac hypertrophy mediated by Hsp22, which involves a change in expression, associated with the 20S core. Therefore, proteasome composition and substrate specificity may vary according to the subcellular distribution.

A third question concerns the relation between proteasome activity and Hsps. A modulation of proteasome activity by Hsp27 has already been shown before in other models, and our data indicate that Hsp22 may play a similar role in a context of cardiac cell growth. We found an increase in Hsp22 mRNA and protein expression in a canine model of prolonged left ventricular hypertrophy, suggesting that Hsp22 might participate in mechanisms of cell growth, which was confirmed upon generation and characterization of the TG mouse. Although this protein was first coined 'H11 Kinase', the human genome project now refers to it as Hsp22, and the mouse genome annotates this protein as C-crystallin. It is likely that the most important biological activity of Hsp22 results from its chaperone function because the enzymatic activity of the protein is an auto-kinase activity. It is unlikely that Hsp22 phosphorylates other molecules considering that the sequence of Hsp22 is too small to express all the domains required for a full serine/threonine kinase activity.

In conclusion, the present study demonstrates a regulation of the proteasome during cardiac hypertrophy mediated by Hsp22, which involves a change in expression.
activity, composition and subcellular distribution. This study shows that proteasome inhibition can reduce pre-existing hypertrophy, which is complementary to our previous observation that proteasome inhibitors can also prevent the development of hypertrophy when administered before the onset of pressure overload. Importantly, the data collected in vitro also show that the inhibition of the proteasome directly prevents cardiac cell growth induced by Hsp22 overexpression or by well-known pro-hypertrophic hormones, which at least partially results from the prevention of a stimulation of protein synthesis in such conditions. Future studies will be needed to determine exactly how proteasome inhibition interferes with protein synthesis.

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