Enhanced Protein Homeostasis Mechanisms in Naked Mole Rats Cells

Harrison Pride[1], Alex Coles[2], Jillian Mochnick[3], Yiqiang Zhang[4,5], Rochelle Buffenstein[4,5], Peter J Hornsby[4, 5], Steve Austad[5,6], Arlan Richardson[5,6], and Viviana I. Pérez[3,7]

Departments of Microbiology[1] and Biochemistry and Biophysiscs[3], Oregon State University; Department of Chemistry and Biochemistry[2], University of Michigan, Flint, Flint, MI 48502; Departments of Physiology[4], Cell and Structural Biology[5], Barshop Institute for Longevity and Aging Studies[6], The University of Texas Health Science Center, San Antonio, Texas 78249; and Linus Pauling Institute[7], Oregon State University, Corvallis, Oregon 97331

Nobel Peace 1962 Nobel Chemistry 1954

Abstract

Phylogenic studies suggest that cells from long-lived species are more resistant to a variety of stressors than short-lived species. However, there is little information on the cellular mechanisms that give rise to increased resistance to stress. Our previous studies have shown that liver proteins from a long-lived species have lower levels of protein ubiquitination which is associated with increased proteasome activity, suggesting that mechanisms of protein quality control could play a critical role in assuring longevity of long-lived species. In this study, we evaluated whether autophagy and heat shock chaperones proteins (HSPs) are associated with longevity in rodents using skin fibroblasts isolated from mice and naked mole rats (NMR); two species that are similar in body size but differ almost 10 fold in longevity. Our results indicate that macroautophagy induced by serum-starvation is significantly enhanced in NMR compared to mouse which correlates with an inhibition of the mTOR pathway and increased LC3 conversion. We also found that several HSPs (e.g., Hsp90, Hsp70, Hsp40, Hsp 27) were significantly higher at both basal and after heat shock conditions. These observations suggest that NMR, a long-lived species, has increased mechanisms to ensure protein quality (autophagy and HSPs) and support the idea that protein homeostasis could play an important role in promoting longevity.

Introduction

Naked mole rats (MRs) are unique rodents that hold the record for the longest lifespan, over 28 years. It is believed that the reason for this long lifespan is how their cells handle stress. Our previous studies suggest that mechanisms that are important for protein quality control (protein homeostasis) could play a critical role in longevity of NMR. In this study, we evaluated autophagy and heat shock chaperones proteins (HSPs) and to determine how they may contribute to enhanced longevity, we used skin fibroblast from two clades, i.e., short- and long-lived rodents [mice and naked mole rats (MRs)], and short- and long-lived bats.

Methods

Cells: Rodents: Primary skin fibroblast from mice [*Mus musculus*, 35gr and 4 years (y)], and naked mole rat (NMRs) [*Heterocephalus glaber,* 30 gr and 30 y], were cultured at 35^{0}C and 5% CO2. **Bats:** evening bat (EB) [*Nycticeius humeralis,*11gr and 6y] shortest-lived bat known v.s little brown bat (LBB) [*Myotis lucifugus,*8gr and 34y] were cultured at 37^{0}C and 5% CO2.

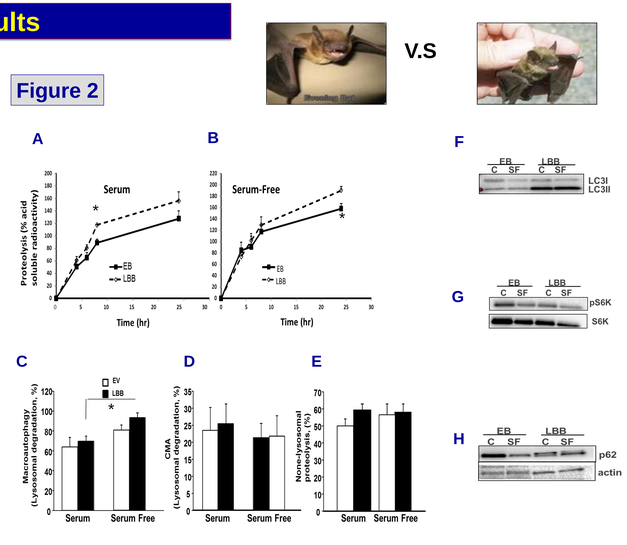
Autophagy measurement: Autophagy was measured by monitoring the degradation of radioactively-labeled long-lived proteins as described by Massey et al. (2010). Macroautophagy was measured in presence of 3-methyladenine (3-MA), and an estimate of the chaperone mediated autophagy (CMA) was obtained by measuring the difference in the rate of protein degradation between that observed in the presence of 3-MA and the rate in the presence of leupeptin plus ammonium chloride. Non-lysosomal protein degradation was measured as the residual value of protein degradation obtained in presence of all three inhibitors under serum or serum-free conditions. Furthermore, we measured of the LC3 ratio (LC3II/LC3I) and p62 level were measured.

Heat shock protein: Cell cultures were exposed to a 1 hour heat shock (41^{0}C), after which samples were placed at normal temperature (35 or 37^{0}C) and collected at 0, 2, 4, 6, 24 hrs thereafter. Cells were lysed, and cellular levels of Hsp 90, Hsp 70, Hsp 40, and Hsp 27, were determined by western blots analysis.

Results

Figure 1

Figure 2



Macroautophagy induced by serum-starvation is significantly enhanced in fibroblasts from the NMRs compared to mice (Figure 1) or fibroblasts from the LBB compared to EB (Figure2). Protein degradation in skin fibroblast from NMRS v.s mice or LBB vs. EB was determined by a pulse-chase technique using [^{3}H]-valine (2μCi/ml) at 24 hours. Total rates of protein degradation in fibroblast from long-lived species [NMRS (open circles) and mice (solid squares) in medium supplemented with 10% FBS (**A**) or in serum-free medium (**B**). Protein degradation measured by Macroautophagy (**C**), Chaperone-mediated autophagy (CMA) (**D**), and Non-lysosomal protein degradation (**E**). The ratio of LC3II to LC3I conversion (LC3II/LC3I) (**F**); mTOR signaling through the ratio of P-S6/S6 (**G**); and P62 levels (**H**), were measured by western blot analysis of total cellular homogenates. Long-lived species (NMRS or LBB) (solid bars) and short-lived specie (mouse and EB) (open bars) under serum and serum-free conditions. The data are the mean ± SEM of triplicate measurements obtained from 3 different animals and were analyzed by the non-parametric test of ANOVA. The asterisk (*) and pound (#) denote a statistically significant difference between NMRS and mouse at the p<0.05 and p<0.01

Figure 3

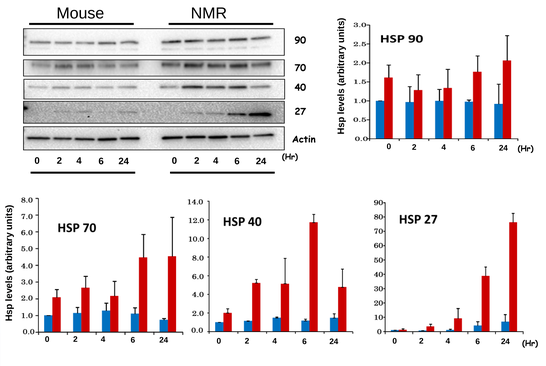


Figure 3: Heat Shock proteins levels are higher in NMRs than mice. Heat Shock proteins (Hsp) 90, Hsp 70, Hsp40, Hsp 27, were measured by western blotting in whole cell extract. Significant differences in all heat shock proteins were observed in NMRs compared to mice. A big difference was specially observed for those small molecular weight Hsp (e.g., Hsp 27 and Hsp40). This data confirm that NMRs have a better machinery for proteome homeostasis. The data are the mean ± SEM from 3 different animals and were analyzed by the non-parametric test of ANOVA. The asterisk denote a statistically significant difference p<0.05.

Figure 4

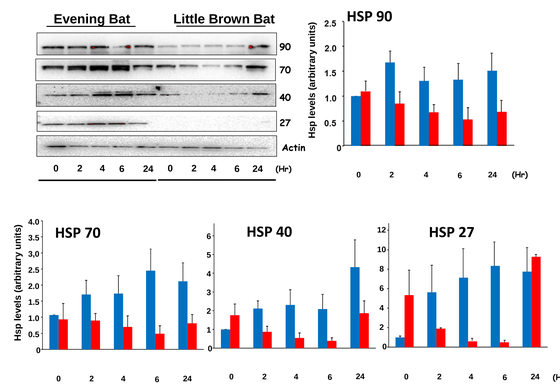


Figure 4: Heat Shock proteins levels are similar or lower in LBB (long-lived bat) than EB (short-lived bat). Heat Shock proteins (Hsp) 90, Hsp 70, Hsp40, Hsp 27, were measured by western blotting in whole cell extract. Our data suggest that heat shock proteins are not elevated in the long-lived bat compared to the short-lived one (even suggest that EB has higher heat shock response). The data are the mean ± SEM from 3 different animals and were analyzed by the non-parametric test of ANOVA. The asterisk denote a statistically significant difference p<0.05.

Conclusions

- **Macroautophagy**. We find that serum starvation induction of macroautophagy is considerably more pronounced in long-lived species (NMR and LBB) compared to their shorter-lived relatives (mice and EB, respectively). At least in the case of the NMR/Mouse pair, this correlates with a stronger inhibition of mTOR.
- **Heat shock chaperones**. Induction of these proteins by heat shock did not show a consistent pattern: induction was stronger in the long lived species of the NMR/mouse pair, but in contrast, it was slightly stronger in the shorter lived member of the bats pair.

Collectively these data provide further evidence that naked mole-rats have better protein homeostasis mechanisms than mice and including bats. Furthermore our data also suggest that enhanced autophagy may be a longevity determinant since is the common mechanism among these two long-lived species, NMRs and LBB. Our current study where we are measuring these mechanisms in a third clade, the marsupials, will allow us to test this hypothesis; we will expect that the long-lived marsupial will have enhanced autophagy compared to the short-lived one.

Acknowledgements

Financial support was provided by Ellison Medical foundation (to both V.P and RB), American Federation for Aging Research and The Glenn Foundation (RB).